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L'implication des suppresseurs de tumeur, TET2 et TP53, dans l'évolution et le traitement de la Leucémie myélomonocytaire Chronique

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Aygun Imanci. L'implication des suppresseurs de tumeur, TET2 et TP53, dans l'évolution et le traitement de la Leucémie myélomonocytaire Chronique. Cancer. Université Paris-Saclay, 2023. Français. NNT: 2023UPASL113 . tel-04485463

HAL Id: tel-04485463

<https://hal.science/tel-04485463v1>

Submitted on 2 Dec 2024

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L'implication des suppresseurs de tumeur,
TET2 et TP53,
dans l'évolution et le traitement de la
leucémie myélomonocytaire chronique

*Involvement of Tumor Suppressors,
TET2 and TP53, in the Evolution and Treatment of
Chronic Myelomonocytic Leukemia*

Thèse de doctorat de l'université Paris-Saclay

École doctorale n° 582, Cancérologie : biologie – médecine – santé (CBMS)
Spécialité de doctorat : Sciences du cancer
Graduate School : Life Sciences and Health
Réfèrent : Faculté de Médecine

Thèse préparée dans l'unité de recherche U1287
Cellules souches hématopoïétiques et développement des hémopathies myéloïdes
(Université Paris-Saclay, Institut Gustave Roussy, INSERM),
Sous la direction de **Nathalie DROIN**, PhD-HDR.

Thèse soutenue à Villejuif, le 01 décembre 2023, par

Aygün IMANCI

Composition du Jury

Membres du jury avec voix délibérative

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|---|---------------------------|
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Titre : Implication des suppresseurs de tumeur, TET2 et TP53, dans l'évolution et le traitement de la Leucémie MyéloMonocytaire Chronique

Mots clés : Monocytes, TET2, TP53, LMMC

Résumé : La Leucémie myélomonocytaire chronique (LMMC) est une pathologie clonale de la cellule souche hématopoïétique qui présente des caractéristiques à la fois myéloproliférative et myélodysplasique. Elle est définie par une monocytose persistante supérieure à 0.5 G/L représentant plus de 10% des globules blancs. C'est une maladie rare des sujets âgés (médiane 72 ans) avec une survie de 2 à 3 ans. Les 3 gènes les plus souvent mutés sont TET2 (60%), SRSF2 (50%) et ASXL1 (40%). Des mutations des gènes de la voie RAS (NRAS, KRAS, CBL), identifiées chez 30% des patients, sont associées aux formes prolifératives.

Mon projet de thèse porte sur le rôle des suppresseurs de tumeurs TET2 et TP53 dans la LMMC. Dans cette pathologie, TET2 est fréquemment muté alors que les mutations de TP53 sont très rares. La première partie de ma thèse met en évidence une sur-expression de MIF (Macrophage migration inhibitory factor) dans les monocytes avec des mutations tronquantes de TET2 chez les patients LMMC. Nous avons montré dans différents modèles que MIF est sur-exprimé lorsque TET2 est moins exprimé. Des expériences de CHIP-PCR ont montré que TET2 se fixe directement au niveau du site d'initiation de la transcription (TSS) de MIF. Dans les monocytes avec des mutations tronquantes de TET2, une diminution du recrutement de HDAC1 et HDAC2 au niveau du promoteur de MIF a été identifiée. Cette modification de la structure chromatinienne favorise la transcription de MIF par l'intermédiaire du facteur de transcription EGR1 (Early Growth Response Protein 1). J'ai mis en évidence par co-immunoprécipitation une interaction entre EGR1 et TET2. Enfin, MIF est suffisant en soi pour induire un biais vers la différenciation monocyttaire au cours de la différenciation granulomonocytaire des cellules souches hématopoïétiques (CSH) de sang de cordon.

A l'opposé de TET2, TP53 est très peu muté dans la LMMC. L'expression du transcrit et de la protéine TP53 est très hétérogènes dans les monocytes des patients LMMC. J'ai mis en

évidence un sous-groupe de patients avec une diminution de l'expression de TP53 et de la protéine correspondante. Les analyses de séquençage d'ARN (RNAseq) suggèrent que les patients présentant une expression réduite de TP53 sont plus enclins à évoluer vers une leucémie aiguë myéloïde (LAM). Les monocytes des patients atteints de LMMC exprimant faiblement TP53 ont également une diminution de l'expression de CSF1R, suggérant un défaut de maturation. Des expériences de sh-SCRB et sh-TP53 dans les CSH au cours de la monocytose en présence de CSF1 ont mis en évidence un blocage de différenciation à un stade pré-monocytaire quand TP53 est moins exprimé, démontrant un rôle de TP53 dans la maturation terminale des monocytes. Nous avons mis en place des analyses de cytométrie en flux pour étudier l'étape finale de maturation des monocytes, impliquant la molécule de surface CD300E. Nos données indiquent que les patients présentant une faible expression de TP53 ont une expression plus faible du marqueur CD300E à la surface des monocytes et sont plus immatures. Ce défaut de maturation terminale, lorsque TP53 est moins exprimé a également été validé à l'échelle unicellulaire.

En recherchant les mécanismes impliqués dans la sous-expression de TP53, nous avons identifié une corrélation entre TP53 et EGR1 dans les monocytes de patients LMMC. La diminution de l'expression de EGR1 par des siARN diminue l'expression de TP53 dans les monocytes de patients. Enfin, en utilisant la cohorte DACOTA, nous avons mis en évidence que les patients exprimant faiblement TP53 avaient une moins bonne survie lorsqu'ils étaient traités par l'hydroxyurée.

Mes travaux permettent de mettre en évidence un intérêt pour la stratification des patients et suggèrent des approches thérapeutiques pour restaurer l'expression de TP53 dans le clone leucémique pour réduire le risque de transformation de la maladie chronique en leucémie aiguë.

Titel : Involvement of Tumor Suppressors, TET2 and TP53, in the Evolution and Treatment of Chronic Myelomonocytic Leukemia

Keywords : Monocytes, TET2, TP53, CMML

Abstract : Chronic myelomonocytic leukemia (CMML) is a clonal pathology of the hematopoietic stem cell that presents both myeloproliferative and myelodysplastic characteristics. It is defined by persistent monocytosis greater than 0.5 G/L representing more than 10% of white blood cells. It is a rare disease of elderly people (median 72 years) with a survival of 2 to 3 years. The 3 most frequently mutated genes are TET2 (60%), SRSF2 (50%) and ASXL1 (40%). Mutations in RAS pathway genes (NRAS, KRAS, CBL), identified in 30% of patients, are associated with proliferative forms.

My thesis project focuses on the role of tumor suppressors TET2 and TP53 in CMML. In this pathology, TET2 is frequently mutated while TP53 mutations are very rare. The first part of my thesis highlights an over-expression of MIF (Macrophage migration inhibitory factor) in monocytes with truncating mutations of TET2 in CMML patients. We have shown in different models that MIF is over-expressed when TET2 is less expressed. CHIP-PCR experiments showed that TET2 binds directly to the transcription start site (TSS) of MIF. In monocytes with TET2 truncating mutations, decreased recruitment of HDAC1 and HDAC2 to the MIF promoter was identified. This modification of the chromatin structure promotes the transcription of MIF via the transcription factor EGR1 (Early Growth Response Protein 1). I demonstrated by co-immunoprecipitation an interaction between EGR1 and TET2. Finally, MIF is sufficient by itself to induce a bias toward monocytic differentiation during granulomonocytic differentiation of cord blood hematopoietic stem cells (HSCs).

Unlike TET2, TP53 is very rarely mutated in CMML. The expression of the TP53 transcript

and protein is very heterogeneous in the monocytes of CMML patients. I highlighted a subgroup of patients with a decrease in the expression of TP53 and the corresponding protein. RNA sequencing (RNAseq) analyzes suggest that patients with reduced TP53 expression are more likely to progress to acute myeloid leukemia (AML). Monocytes from CMML patients weakly expressing TP53 also have decreased CSF1R expression, suggesting a maturation defect. Experiments with sh-SCRB and sh-TP53 in HSCs during monocytopoiesis in the presence of CSF1 demonstrated a block of differentiation at a pre-monocytic stage when TP53 is less expressed, showing a role for TP53 in monocyte terminal maturation. We implemented flow cytometry analyzes to study the final step of monocyte maturation by adding the surface molecule CD300E. Our data indicate that patients with low TP53 expression have lower expression of the monocyte surface marker CD300E and are more immature. This terminal maturation defect, when TP53 is less expressed, has also been validated at the single-cell level.

By investigating the mechanisms involved in TP53 underexpression, we identified a correlation between TP53 and EGR1 in monocytes from CMML patients. Decreasing EGR1 expression by siRNA decreases TP53 expression in patient monocytes. Finally, using the DACOTA cohort, we demonstrated that patients expressing low TP53 had poorer survival when treated with hydroxyurea.

My work highlights an interest in patient stratification and suggests therapeutic approaches to restore the expression of TP53 in the leukemic clone to reduce the risk of transformation of chronic disease into acute leukemia.

Remerciements

Je remercie les membres de mon jury de thèse, Pr Hélène CAVE et le Dr Laurent DELVA, d'avoir accepté d'être rapporteurs, Dr Françoise PFLUMIO et le Pr Jean SOULIER d'être examinateurs à cette thèse. Je tiens à exprimer ma profonde gratitude envers les membres de mon jury de thèse pour leur temps, leur expertise et leurs précieux commentaires.

Je souhaite exprimer ma reconnaissance envers les membres de mon comité de suivi de thèse, Vanessa PETIT (CEA), Dr Camille LOBRY et le Dr Jean-Luc PERFETTINI, pour leurs conseils qui ont contribué à l'avancement et à la réussite de ma thèse.

Je remercie la Ligue Contre le Cancer de m'avoir accordé une bourse couvrant les 4 années de ma thèse.

Je tiens à exprimer mon immense reconnaissance envers les médecins et les patients qui consentent au prélèvement de matériel biologique, ainsi qu'aux différents centres (EFS, Hôpital Cochin et l'Hôpital Henri Mondor) pour leur précieuse collaboration.

Un énorme remerciement aux personnes travaillant dans l'administration, plus particulièrement Mehdi, Paule, Rahma, Léa Poisot et le personnel de Paris-Saclay, pour leur précieux soutien et leur dévouement essentiel qui contribuent au bon déroulement de nos activités quotidiennes.

Je tiens à adresser mes plus sincères remerciements à ma directrice de thèse Dr Nathalie DROIN, pour son soutien constant, son expertise et ses précieux conseils qui ont contribué grandement à mon développement professionnel. Je te remercie de m'avoir accompagné tout au long de cette aventure et de m'avoir impliquée dans les différentes collaborations.

Je vous remercie Eric SOLARY de m'avoir permis d'intégrer l'équipe LMMC. Je vous remercie pour toutes les fois où vous m'avez aidée dans ma réflexion scientifique et pour votre participation à la correction de mon manuscrit.

J'adresse mes remerciements aux différentes plateformes de l'IGR qui ont participé à ce projet, la plateforme d'Imagerie et de Cytométrie avec Tudor, Philippe et Cyril, la plateforme génomique notamment Audrey, Maëla et Betty et également la plateforme de bioinformatique, en particulier Marine AGLAVE.

Je remercie également toutes les personnes qui ont contribué au projet, notamment Lucie Laplane, pour ses précieuses analyses bioinformatiques et ses réflexions, qui ont enrichi le projet. Dorothée Selimoglu-Buet, pour son expertise en cytométrie en flux et

sa contribution au projet, notamment pour le marquage des échantillons de patients en routine. Margot Morabito, celle qui m'a appris à « techniquer » rapidement, je te remercie pour ta pédagogie, nos discussions enrichissantes et ta contribution précieuse dans la gestion des échantillons de patients.

Je tiens à remercier l'équipe 1, Françoise, Emilie, Aline, Lucie, Dorothée, Eric Solary, Christophe, Richard, Anaïs, Dana, Valentine, Anne, Louis, Margaux, Guglielmo, Audrey, Mathieu, pour leur soutien dans l'avancement de mon projet, ainsi que pour leur participation active lors des réunions d'équipes.

Merci à Alexia, Lisa et Laure, que j'ai encadrée pour leur motivation et leur précieuse contribution au projet.

Un énorme remerciement à l'équipe 2, Hana, Isabelle, William, Caroline, Iléana, Najet, Monica pour votre aide, vos conseils pendant les réunions d'unité et votre soutien tout au long de cette thèse.

Je souhaite remercier tous les étudiants, post-docs, ingénieurs et techniciens de l'unité U1287, d'avoir participé de près ou de loin à l'avancement de ce projet. Je vous remercie pour votre bonne humeur et l'ambiance positive que vous créez qui contribue à rendre l'environnement de travail agréable pour tous 😊 😊.

Merci à Maxime (alias Sammy, j'espère que tu as la ref) pour ta bonne humeur et pour...m'avoir réservé quelques frayeurs tout au long de ces années !!!

Merci à Brahim (le nouveau papa du labo 😊), pour nos discussions.

Merci à Nasrine, celle qui est passionnée par les séries turques et possède une excellente maîtrise de la langue turque. Senin sayende türkçe konusabilirim, benimle çok dedikodu yapabiliriz. Hersey gönlünce olsun.

Un énorme remerciement à mon trio préféré, Donia, Amandine et Camélia :

- ❖ Donia, la fille la plus forte que je puisse connaître (tu vois de quoi je parle). Nous avons partagé des moments incroyables depuis notre rencontre. Notre lien perdure et j'espère sincèrement rester en contact avec toi pour les années à venir. Tu es une personne formidable, gentille, toujours présente ! Ne change surtout pas.
- ❖ Amandine, ahhh ma coupineeeee ça c'est ma coupinnneee ! Je tiens à m'excuser pour les fois où mes plaisanteries t'ont fait peur 😊 (même si ça me faisait bien rire). Notre partage de bureau a été ponctué de moments incroyables, de rires incessants et de discussions interminables. Ta présence au laboratoire me manque toujours !

- ❖ Camélia, notre future chercheuse, nos moments partagés resteront gravés dans ma mémoire. J'étais honorée de participer à ton mariage, de t'aider pour cette étape importante de ta vie. Bien que le transport du gâteau ait créé du stress !!! Qui sait, peut-être nous retrouverons-nous un jour dans le même laboratoire.

Je souhaite remercier :

- ❖ Valentine, à la fois amie et voisine de bureau, j'ai énormément apprécié partager 3 ans de ma thèse à tes côtés. Ta présence constante a été inestimable pour les analyses bioinformatiques, sans lesquelles je n'aurais pu générer ces données. J'espère rester toujours en contact avec toi et je te souhaite le meilleur pour ton avenir, pour tes futures post-docs 😊 (pour tes futurs projets, évite d'utiliser nos prénoms (celui de mon mari et le mien) pour désigner tes souris 😊😊).
- ❖ Dana, ma petite sœur du laboratoire, celle avec qui je joue le plus souvent pour te faire peur 😊 Tu es une personne extraordinaire, toujours joyeuse (parfois énervée aussi comme tootie). Je suis tellement contente de pouvoir travailler avec toi pour la fin de ta thèse. N'hésite surtout pas à venir voir Simba, tu lui manques 😊
- ❖ Anaïs, ma philosophee d'amour !!! tu es une personne incroyable, j'aime beaucoup discuter et passer du temps avec toi. Merci pour tes conseils précieux et pour ton implication dans mon projet. Ta réflexion et ta contribution ont été d'une valeur inestimable. Je n'ai aucun doute quant à ton avenir, je sais que tu vas réussir brillamment.
- ❖ Louis, l'ingénieur le plus aimable que je connaisse. Même si je sais que tu n'es pas passionné par les génotypages, je te souhaite bon courage pour l'analyses génétiques de tes souris jusqu'à la fin de la thèse de Val ! Merci infiniment pour les corrections que tu as apportées à mon manuscrit de thèse. Ton aide a été extrêmement précieuse, et tu as été d'une rapidité et d'une efficacité remarquables.
- ❖ Jean-Edouard Martin, mon JE, mon médecin préféré 😊 je te remercie pour ta motivation et ton enthousiasme constants envers mon projet. Ton soutien dès le départ a été une source d'encouragement et m'a permis d'avancer avec confiance. Pour des raisons de confidentialité, je ne préfère pas aborder certains sujets ici (j'espère que tu vois de quoi je parle Monsieur !). Ce qui m'impressionne chez toi, c'est ton talent pour motiver toutes les personnes avec qui tu interagis (« on va faire une qPCR », « on va faire un western blot » ...).

Je souhaite bon courage aux futures docteurs, Valentine, Anne, Dana, Anaïs, JE, Jean, Agathe et Nasrine.

Je suis infiniment reconnaissante envers mes proches qui ont toujours cru en moi et m'ont soutenu. Merci à BegBeg, pour son soutien constant et ses encouragements.

Merci à mes copines d'enfances Gülsüm et Nese d'avoir toujours répondu présent quand j'en avais besoin. Merci pour toutes les soirées et week-ends passés ensemble, qui m'ont procuré un réel bien-être. Merci également pour le moment de détente que vous m'avez apporté pour mon anniversaire, Seviyorum Sizi. Küçüklüğümden beri beni « doktor » diye çağıran ve doktor olabileceğime inanan Nazmiye tatama teşekkür ederim.

Un remerciement spécial à ma belle-sœur Emine pour sa capacité à me remonter le moral. Merci à toi et Ahmet pour la croisière offerte, une parenthèse formidable pour décompresser.

Merci à mes frères, Ogün et Eren (alias Elchapo) de leur soutien durant les moments de stress, votre présence a été d'un grand réconfort. Je vous remercie pour le temps que vous m'avez consacré lors de mes répétitions, même si le sujet pouvait parfois sembler complexe. Bienvenue dans la famille et merci pour ta présence ma belle-sœur Merve.

Sükrüye Anne ve Yüksel babama çok teşekkür ediyorum, bana güvendiğiniz için ve hep beni desteklediğiniz için.

Un immense remerciement à mon mari Ferdi, pour sa bravoure et son soutien face à mes variations d'humeur. Sa présence constante a été d'une aide inestimable. Je t'aime.

Annem ve babama, kosulsuz destekleriniz ve eğitimimde gösterdikleriniz çaba için size çok teşekkür ediyorum. Benim için yaptığınız her şey için teşekkür ederim.

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II. Liste des abréviations

A

- ARN : Acide ribonucléique
- ASXL : ASXL transcriptional regulator 1
- ADN : Acide désocytiribonucléique
- ASE : Agent stimulant l'érythropoïétine
- AZA : Azacitidine
- ARID4A : AT-rich interaction domain 4A
- ARCH : Aging-related clonal hematopoiesis
- APACHE II : Acute physiology and chronic health evaluation II
- ATACseq : assay for transposase-accessible chromatin with sequencing

B

- BAX : Bcl-2 associated X protein
- BID : BH3 interacting domain

C

- CFU : Colony-forming units
- CSH : Cellule souche hématopoïétique
- CMP : Common myeloid progenitor
- CLP : Common lymphoid progenitor
- CSF-1R : Colony stimulating factor 1 receptor
- CX3CR1 : C-X3-C motif chemokine receptor 1
- cMoP : Common monocyte progenitor
- CLEC12A : C-type lectin domain family 12 member A
- CEBP α/β : CCAAT/enhancer-binding protein alpha/beta
- CCR : Cancer colorectal
- CBL : Cbl proto oncogene
- CSPH : cellule souche progénitrice et hématopoïétique
- CHIP : Clonal hematopoiesis of indeterminate potential
- CDKN1A : Cyclin dependent kinase inhibitor 1A
- CREB : C-AMP response element-binding protein
- CXCR2 : CXC motif chemokine receptor 2
- CXCR4 : CXC motif chemokine receptor 4
- COP1 : E3 Ubiquitin ligase
- Cut&Tag : Cleavage Under Targets and Tagmentation

D

- DC : dendritic cell
- DAMP : Damaged associated molecular pattern
- DNMT : DNA methyltransferase
- DLBCL : Diffuse large B cell lymphoma
- DAC : Décitabine
- D-DT : D-dopachrome tautomérase

E

- ETS : E-twenty-six
- EGR : Early growth response
- EZH2 : Enhancer of zeste 2 Polycomb repressive complex 2 subunit

F

- FACS: Fluorescence activated cell sorting
- FIRE: fms-intronic regulatory element
- FAS : Fas cell surface receptor
- FISH : fluorescence in situ hybridization

G

- GMP : Granulocyte-monocyte progenitor
- GATA 1 : GATA binding protein 1
- GB : Globule blanc
- GM-CSF : Granulocyte macrophage colony stimulating factor

H

- HMA : DNA hypomethylating agent
- HAT : Histone acetyltransferase
- HDAC : Histone deacetylase
- HAUSP : Herpesvirus-associated ubiquitin-specific protease

I

- IRF8 : Interferon regulatory factor 8
- IREM2 : Immune receptor expressed in monocytic derived cells
- IL-1 β : Interleukine 1 beta
- IL-3 : Interleukine 3
- iPSC : Induced pluripotent stem cell
- IL-6 : interleukine 6

K

- KLF4 : Krüppel-like factor 4
- KDM6B : Lysine demethylase 6B

\mathcal{L}

- LT : Long term
- LRR : Leucin-rich repeats
- LMMC : Leucémie myélomonocytaire chronique
- LAM : Leucémie aiguë myéloïde
- LMC : Leucémie myéloïde chronique
- LPS : Lipopolysaccharide
- LLC : Leucémie lymphoïde chronique

 \mathcal{M}

- Mk : mégacaryocyte
- MPP : Multipotent progenitor
- MEP : Megakaryocytic-erythroid progenitor
- MDP : Monocyte-dendritic progenitor
- MO1 (cMO) : Monocyte classique
- MO2 (iMO) : Monocyte intermédiaire
- MO3 (ncMO) : Monocyte non classique
- MPP : Matrix metalloproteinases
- MDAPS : MD anderson prognostic score
- MIR150 : MicroRNA 150
- MDM2 : Mouse double minute 2
- MDM4 : Mouse double minute 4
- MCK : Muscle creatine kinase
- MDR-1 : Multidrug resistance
- MSC : Mesenchymal stem cell
- MIF : Macrophage migration inhibitory factor

 \mathcal{N}

- NOXA : BH3-only protein
- NF-kB : Nuclear Factor Kappa B Subunit 1

 \mathcal{O}

- OMS : Organisation mondiale de la santé

 \mathcal{P}

- PRR : Pattern recognition receptor
- PAMP : Pathogen associated molecular patterns
- PDGFRA/B : Platelet derived growth factor receptor alpha/beta
- PHF6 : PHD finger protein 6
- PV : Polycythanemia vera
- PUMA : p53 upregulated modulator of apoptosis
- PTEN : Phosphatase and TENsin homolog
- PARN : Poly(A)-specific ribonuclease

 \mathcal{R}

- RUNX1 : Runt-related transcription factor 1
- RAS : Rat sarcoma virus
- ROS : ROS proto-oncogene
- RPL26 : Ribosomal protein L26

 \mathcal{S}

- ST : short term
- SMD : Syndrome myélodysplasique
- SMP : Syndrome myéloprolifératif
- SRSF2 : Serine and arginine rich splicing factor 2
- SF3BA : Splicing factor 3b subunit 1
- SETBP1 : SET binding protein 1
- STAT5 : Signal transducer and activator of transcription 5
- SLAN : Sulfo LacNAc
- SCF : Stem cell factor
- SV40 : Polyomavirus
- Sp1 : Specificity protein 1
- Stat3 : Signal Transducer And Activator Of Transcription 3

 \mathcal{T}

- TNF α : Tumor necrosis factor alpha
- TLR : Toll like receptor
- TRAIL : Tumor-necrosis factor related apoptosis inducing ligand
- Tie2 : Angiopoietin-1 receptor
- TET2 : Ten-eleven-translocation 2
- TP53 : tumor suppressor protein 53
- TET3 : Ten-eleven-translocation 3
- TAD : transactivation domain
- TRIM28 : Tripartite motif-containing 28

 \mathcal{U}

- U2AF1 : U2 small nuclear RNA auxiliary factor 1

 \mathcal{V}

- VEGF : Vascular endothelial growth factor
- VIH -1 : Virus de l'immunodéficience humaine 1

 \mathcal{W}

- WT-1 :Wilms'tumor protein 1
- WGS : Whole genome sequencing
- WES : Whole exome sequencing

III. INTRODUCTION

1. Origine et formation des cellules sanguines : Les monocytes

A. L'hématopoïèse

L'hématopoïèse est le processus qui permet le renouvellement quotidien de 400 milliards de cellules sanguines. Ces cellules sont schématiquement de trois types :

- Globules rouges ou érythrocytes, les plus nombreuses (5 milliards/ml) dont le rôle principal est de transporter l'oxygène des poumons vers les tissus.
- Globules blancs ou leucocytes, impliqués dans la réponse immunitaire ou inflammatoire et la défense de l'organisme, au sein desquels on distingue les polynucléaires (neutrophiles, éosinophiles, basophiles), les lymphocytes et les monocytes.
- Les plaquettes ou thrombocytes qui sont des fragments cellulaires participant à l'hémostase, à la coagulation et à la cicatrisation.

Toutes ces cellules du sang proviennent d'un seul type cellulaire, la cellule souche hématopoïétique (CSH).

i. Siège de l'hématopoïèse

L'hématopoïèse fœtale est un processus conservé au cours de l'évolution. Le premier stade est une hématopoïèse primitive transitoire et extra-embryonnaire puisqu'elle émerge du mésoderme dans le sac vitellin au cours de la troisième semaine de l'embryogenèse humaine, donnant naissance, à des progéniteurs myéloïdes capables de se différencier en macrophage sans passer par le stade monocyte et à des mégacaryocytes. L'hématopoïèse se poursuit autour de la huitième semaine de gestation au niveau de l'endothélium intra-embryonnaire de la paroi ventrale de l'aorte descendante, dans la région dite « AGM » (Aorte, Gonade, Mesonephros) [1], [2]. Dans une troisième phase, les cellules souches hématopoïétiques à l'origine de l'hématopoïèse définitive colonisent le foie fœtal jusqu'au septième mois de gestation, date à laquelle l'hématopoïèse s'installe définitivement dans la moelle osseuse qui se trouve à l'intérieur des cavités osseuses (**Figure 1**). L'hématopoïèse est responsable de la production quotidienne de milliards de globules rouges, globules blancs et plaquettes [3], [4].

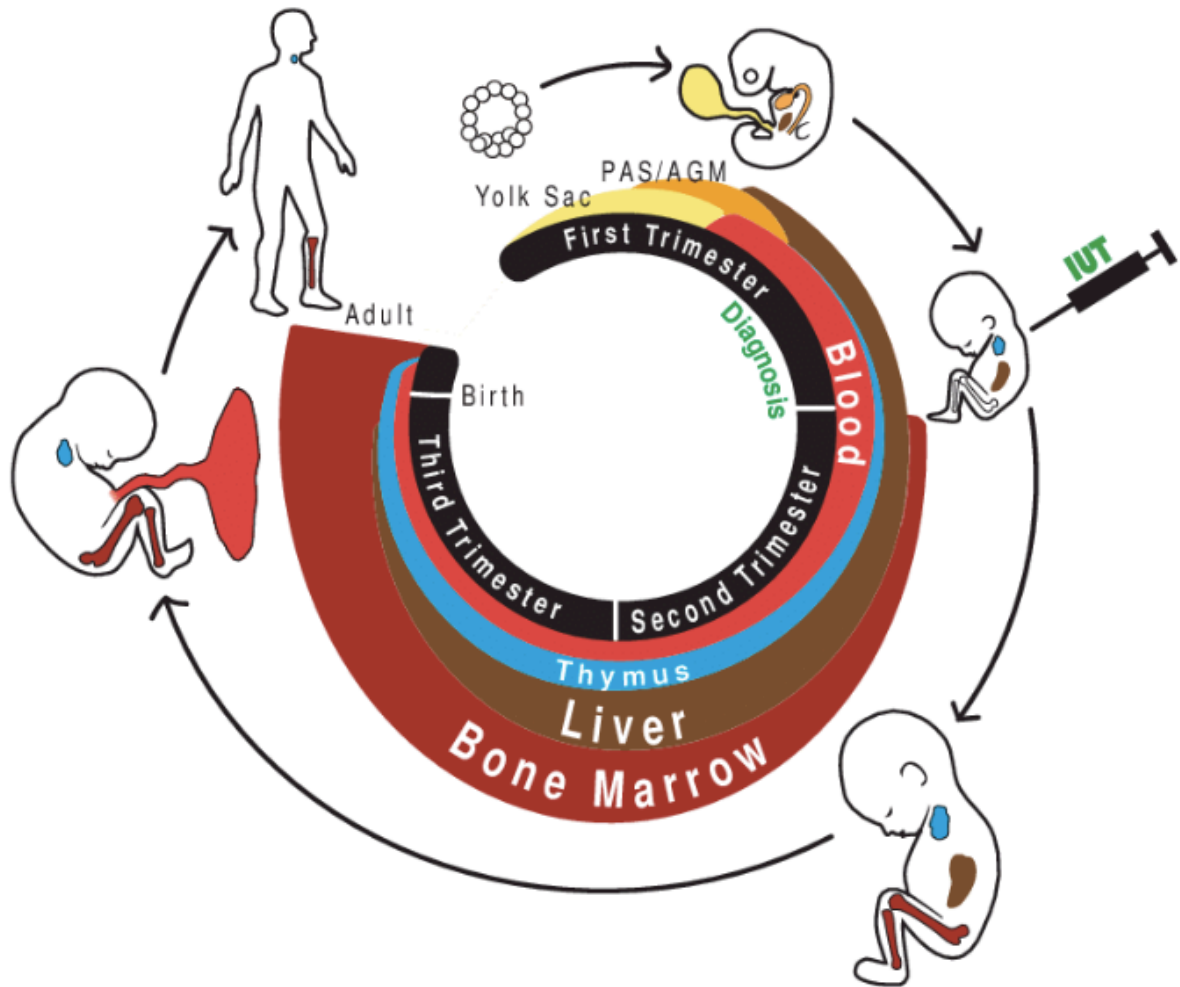


Figure 1 : Hématopoïèse chez l'embryon

(Tiré de Marcus O. Muench et al ; 2004)

Changement séquentiel des sites d'hématopoïèse au cours du développement humain.

L'hématopoïèse a longtemps été décrite comme un processus très hiérarchisé dans lequel toutes les cellules hématopoïétiques spécialisées sont générées à partir d'un petit nombre de cellules souches hématopoïétiques multipotentes. La structure générale de l'hématopoïèse est un processus par étapes, de la cellule souche hématopoïétique aux progéniteurs multipotents, puis des précurseurs aux cellules hématopoïétiques matures. Ce modèle est aujourd'hui controversé à partir de nombreux travaux de traçage cellulaire (lineage tracing) révélant une très grande plasticité au sein du système [5] **(Figure 2)**.

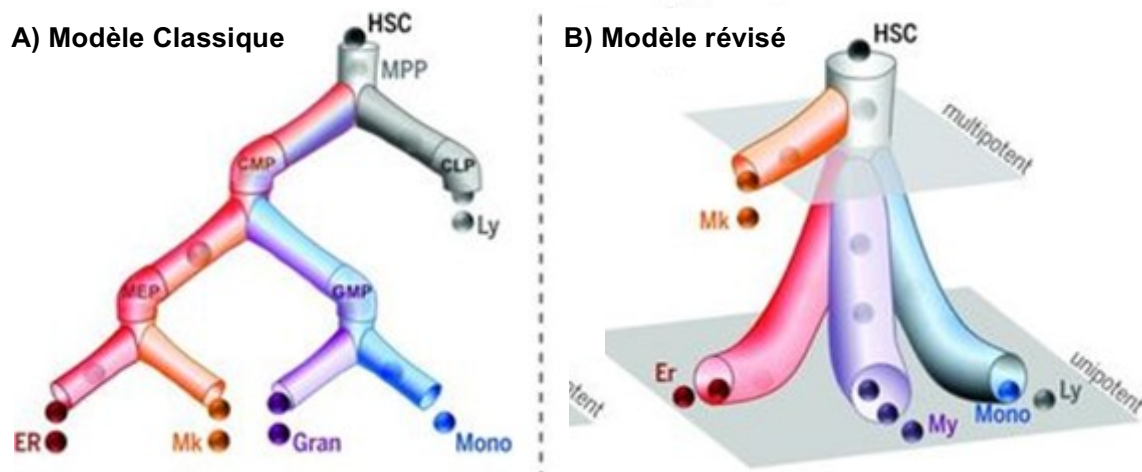


Figure 2 : Modèle classique et révisé de la différenciation des cellules souches hématopoïétiques

(Tiré de Notta et al; 2015).

A) Dans le modèle classique, les CSH sont représentées comme une population capable de se diviser en deux branches, myéloïdes et lymphoïdes, via des progéniteurs, commun myéloïde (CMP) et commun lymphoïde (CLP).

B) Le modèle actuel basé sur des analyses transcriptomiques à l'échelle d'une seule cellule et le traçage de lignée est moins rigide et apporte la notion nouvelle de continuum et de plasticité lors de la différenciation.

HSC : cellules souches hématopoïétiques, MPP : cellules progénitrices multipotentes, CMP : cellules progénitrices myéloïdes, CLP : cellules progénitrices lymphoïdes, MEP : cellules progénitrices érythro-mégacaryocytaires, GMP : cellules progénitrices monocytaires-granuleuses, ER : cellules érythroïdes, Mk : mégacaryocytes, Gran : granulocytes, Mono : monocytes, Ly : lymphocytes.

C'est en 1961, suite aux travaux révolutionnaires de Till et McCulloch que l'existence des cellules souches a été mise en évidence. Les expériences réalisées par les deux docteurs (un biophysicien et un hématologue) ont montré que l'injection de cellules provenant de la moelle osseuse à des souris irradiées augmentait leurs chances de survie. Leur étude a également montré l'apparition d'un nodule au niveau de la rate des souris et que le nombre de cellules présentes dans ce nodule était directement proportionnel à la quantité de cellules injectées. Ce nodule est nommé « Colony-forming units (CFU) » par Till et MacCulloch. Ils ont observé que cette colonie était dérivée de cellules individuelles et était capable de se différencier en cellules de la lignée hématopoïétiques : globules rouges, globules blancs et plaquettes [6].

ii. Généralité sur les cellules souches hématopoïétiques

Les recherches sur les CSH ont été initiées par des tests clonogéniques à partir de populations cellulaires isolées dans la moelle osseuse de souris. Les différents résultats obtenus ont ainsi permis de démontrer la multipotence des CSH. Cette capacité multipotente a été mise en évidence pour la première fois à partir d'expériences de

transplantation de moelle osseuse visant à protéger les souris d'une irradiation létale [7].

Ainsi, les cellules souches hématopoïétiques ont été définies sur la base de deux propriétés spécifiques :

- La capacité d'autorenouvellement : Ces cellules sont capables de donner naissance à de nouvelles cellules en conservant les mêmes propriétés. Cela permet de maintenir le pool de cellules souches tout au long de la vie.
- La capacité de différenciation : Ces cellules sont capables de donner naissance à différents types cellulaires matures en fonction des signaux qu'elles reçoivent de leur environnement [8].

Deux marqueurs de surface, Sca-1 et c-Kit, sont communément utilisés pour caractériser les cellules souches et progénitrices hématopoïétiques (CSPH) chez la souris. Ces cellules n'expriment pas les marqueurs de lignées, on dit qu'elles sont Lin⁻ [elles n'expriment pas TER119 (érythroïde), B220 (lymphocytes B), CD4 et CD8 (lymphocytes T), Gr-1 (granulocytes) et Mac-1 (monocytes)]. Les cellules hématopoïétiques de souris les plus immatures (CSPH) sont donc appelées cellules LSK [9], [10], [11]. Chez l'Homme, la population équivalente exprime le marqueur CD34 et les plus immatures n'expriment pas CD38. Cependant, des études ont montré que le compartiment des CSPH se compose d'au moins 2 sous-populations de CSH fonctionnellement distinctes. En effet, les événements initiaux de la différenciation des CSPH comprennent la transition des CSH à long terme (LT, CD34⁺) hautement auto-renouvelables en CSH à court terme (ST, CD34^{-/lo}) qui possèdent une activité d'auto-renouvellement limitée. Les CSH-LT ont démontré une capacité de radioprotection immédiate chez les souris exposées à une irradiation létale. En revanche, les CSH-ST étaient responsables de la reconstitution à court terme des lignées myéloïdes et lymphoïdes chez ces souris irradiées [12], [13]. Selon le modèle « classique », la différenciation des cellules souches ST aboutit aux progéniteurs multipotents : les MPP, l'avant-dernière étape avant la spécification de la lignée [14].

Le premier branchement se produit entre les progéniteurs myéloïdes communs (CMP, à potentiel myéloïde, érythroïde et mégacaryocytaire) [15] et les progéniteurs lymphoïdes communs (CLP, à potentiel lymphoïde uniquement) [16]. Il a été montré qu'il existait des cellules myéloïdes comme les cellules dendritiques qui peuvent être générées par des progéniteurs lymphoïdes [17], [18], qui sont des progéniteurs engagés dans la lignée dérivée des MPP. Les CMP, subissent une séparation pour générer des progéniteurs granulocytes-monocytes (GMP) et des progéniteurs mégacaryocytes-érythroïdes (MEP). Des analyses récentes étudient les divisions

cellulaires des CSH chez la souris ont montré que les cellules Mk peuvent être générées directement des CSHs sans passer par les étapes intermédiaires [19] .

B. Les monocytes : la monocytopenèse

Le système immunitaire, est constitué d'un réseau complexe d'organes, de cellules et de protéines qui ont pour rôle de protéger l'organisme de multiples agressions : pathogènes, éléments étrangers, cellules altérées ou transformées. Les globules blancs (polynucléaires neutrophiles, éosinophiles, neutrophiles et basophiles), lymphocytes (lymphocytes T et B) et monocytes, ont un rôle essentiel au sein de ce réseau. C'est pourquoi, il existe un grand intérêt à manipuler ces cellules pour des fins thérapeutiques.

Au cours de ma thèse, je me suis focalisée sur les monocytes, des cellules sanguines circulantes de grande taille (15 à 20 μm) dont la durée de vie dans la circulation est de maximum 2 jours et s'ils n'ont pas été programmés pour répondre à une infection, ils sont éliminés. Chez la souris et l'Homme, les monocytes représentent respectivement 4% et 10% des cellules nucléées du sang et sont également présents dans les poumons et la rate. Ils peuvent migrer dans les tissus où ils se différencient, en particulier en macrophages [20], [21]. Ils jouent un rôle dans la réponse immunitaire et dans la réparation des tissus. Les monocytes sont retrouvés chez différentes espèces [22], [23].

i. Historique et développement

C'est au XVI^{ème} siècle, suite à l'invention du microscope par Hans et Zacharias Janssen, que l'ensemble des composants du sang a été analysé. Les globules rouges ont été les premières cellules sanguines à être observées puis des cellules dites « incolores » ou « particules centrales » ont été visualisées par William Hewson en 1874 qui correspondaient aux globules blancs nommés aujourd'hui leucocytes. En se basant sur des études fonctionnelles et de mouvements des globules blancs, Schultze a découvert la présence de quatre différents types de leucocytes (Identifiés aujourd'hui comme étant des monocytes, lymphocytes, neutrophiles et éosinophiles) [24]. La mise au point de colorants a permis le développement de cette cytologie du sang à la fin du XIX^{ème} siècle, permettant à Paul Ehrlich de classer les globules blancs en leucocytes mononucléés et polynucléés [25]. La combinaison des travaux d'Ehrlich avec ceux de Metchnikoff où ils démontrent la présence de différents types cellulaires avec pour certaines la capacité de phagocyter des corps étrangers. Cette découverte leur a permis de partager un prix Nobel en 1908 [26].

Suite aux différentes découvertes, de nombreuses techniques ont été développées ayant pour but d'isoler les leucocytes. En 1926, Szilard [27] développe une technique permettant la séparation des leucocytes par hémolyse en utilisant des acides. D'autres investigateurs améliorèrent le rendement en leucocytes et réduisent la contamination avec les cellules érythrocytaires [28], [29], [30]. C'est en 1947 que Vallée, Hughes et Gibson [31] proposent une méthode basée sur la sédimentation par densité, consistant à déposer le sang sur une solution de densité nommée FICOLL. Après centrifugation, 3 phases distinctes sont obtenues : les globules rouges au fond du tube, le plasma en haut du tube et à l'interphase un anneau contenant les globules blancs. Ainsi, par cette méthode, d'autres chercheurs ont optimisé la technique afin d'isoler les différents constituants de l'anneau (lymphocytes, granulocytes et monocytes) [32], [33]. Ces différentes découvertes ont permis la séparation des monocytes des autres cellules aidant à une analyse en profondeur de ce type cellulaire.

Le terme « monocyte » a été cité dans la littérature pour la première fois par Arthur Pappenheim en 1910 [34]. Les monocytes circulants sont des cellules matures qui sont produites en continu dans la moelle osseuse et sont libérées dans le sang périphérique avant d'atteindre leur stade mature et de différencier pour donner naissance à des macrophages [34] ou des cellules dendritiques (DC), parfois à des ostéoclastes.

Monocytopoïèse

Nous avons vu précédemment, que le destin myéloïde et lymphoïde se sépare au niveau des MPP qui deviennent des CMP ou des CLP. Par profilage unicellulaire, il a été montré que les CMP peuvent se diviser en deux sous-populations selon l'expression du facteur de transcription GATA1. Les CMP exprimant GATA1 donnent naissance à des progéniteurs mixtes, mégacaryocytes/érythrocytes et à des progéniteurs de mastocytes/basophiles/éosinophiles (MEP) et l'absence de ce gène donne naissance aux monocytes et neutrophiles (GMP) [15], [35]. Dans la catégorie GMP, un autre progéniteur, le MDP (progéniteurs des monocytes et cellules dendritiques), a récemment été découvert chez l'Homme [36]. Ce MDP humain partage le phénotype des GMP, c'est-à-dire $Lin^{-}SCA1^{-}IL7R\alpha^{-}CD117^{-}CD34^{+}CD16^{+}$ et exprime CSF-1R (CD115) et CX3CR1 (récepteur de chimiokine). Le récepteur CX3CR1 est connu pour être assimilé à l'engagement des lignées monocytes/macrophages/DC [37], [38]. Un progéniteur limité à la lignée monocyttaire a également été identifié chez la souris sous le nom de cMoP, dérivé des MDP [39] qui nécessite l'expression du facteur régulateur de l'interféron 8 (IRF8) pour la différenciation des monocytes [40]. Au sein des MDP, la présence de cMOP humains a été identifiée à l'aide des marqueurs de surface CD64 (FcyRI) et CLEC12A (lectine de type C), exprimés spécifiquement sur les monocytes et

les macrophages [41]. Le cMoP humain a pour phénotype CD64⁺CLEC12A⁺ [42] et ne donne naissance qu'aux monocytes [38]. Une analyse approfondie des populations progénitrices a mis en évidence une diversité interne au sein de ces populations, remettant potentiellement en question le modèle classique de différenciation [43]. Des expériences de transfert adoptif ont montré que les CMP peuvent se différencier en GMP et MDP. Les MDP donneront naissance au cMoP [39], [44] tandis que les MP seront issus des GMP [44]. Pris ensemble, ces études ont établi un modèle d'ontogénie binaire des monocytes [45] (Figure 3).

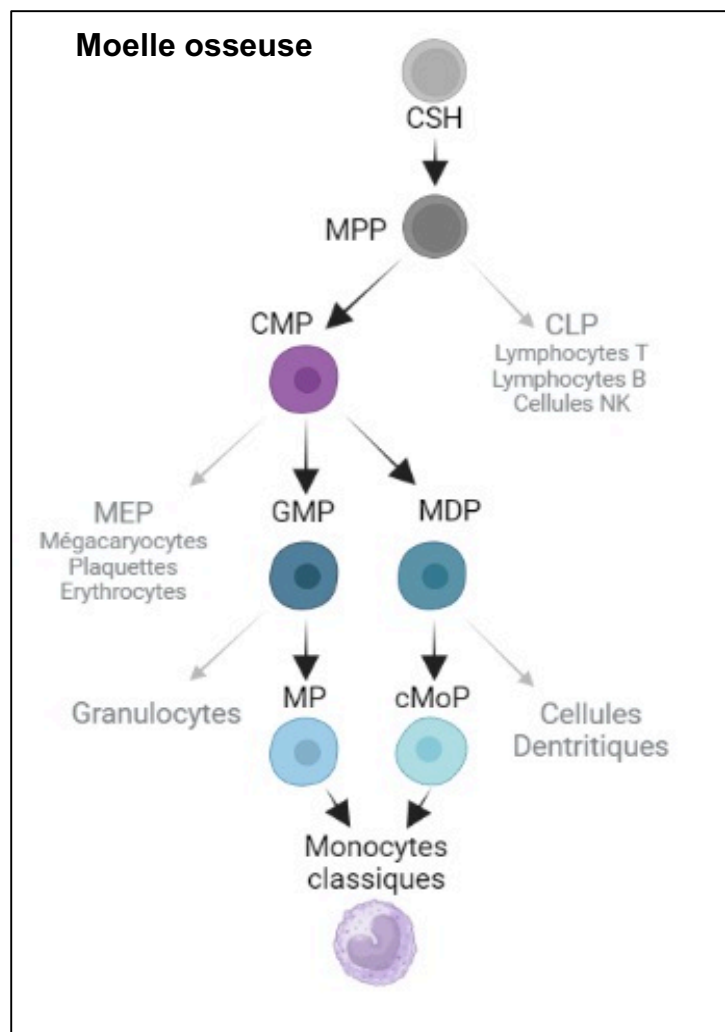


Figure 3 : Modèle de différenciation des monocytes

(Tiré de Williams H et al ; 2023)

Les monocytes apparaissent dans la moelle osseuse à partir de la différenciation de plusieurs niveaux de précurseurs. Ils sont ensuite libérés dans la circulation. CSH (Cellule souche hématopoïétique) ; MPP (progéniteurs multipotents) ; CMP (progéniteurs myéloïdes) ; CLP (progéniteurs lymphoïdes communs) ; MEP (progéniteurs érythro-mégacaryocytaires) ; GMP (progéniteurs granulo-macrophagiques) ; MDP (progéniteurs des monocytes et cellules dendritiques) ; MP (progéniteurs monocytes) ; cMoP (ancêtre commun des monocytes).

Les cytokines majeures de la monocytopenie

La régulation de la différenciation granulo-monocytaire est fortement influencée par des signaux extracellulaires, notamment les cytokines. Ces signaux jouent un rôle crucial dans la prolifération, la survie, la différenciation et la fonction des progéniteurs et des cellules matures. Le M-CSF et GM-CSF font partie des cytokines impliquées dans la régulation de la lignée granulo-monocytaire.

- Le GM-CSF (Granulocyte-Macrophage Colony Stimulating Factor) est une cytokine principalement sécrétée par des fibroblastes, les cellules endothéliales, les macrophages, les lymphocytes, les éosinophiles et les cellules tissulaires résidentes [46], [47]. Son expression est induite en réponse à des signaux inflammatoires, tels que IL-1 α/β , le TNF- α [48], [49]. Le GM-CSF signale par l'intermédiaire de son récepteur CSF-2R. En tant que cytokine pro-inflammatoire, le GM-CSF contribue à la réponse immunitaire en recrutant des cellules myéloïdes. Dans plusieurs modèles murins, le blocage de cette cytokine s'est traduit par une diminution notable du niveau de production des monocytes et des neutrophiles [50]. Des concentrations faibles de GM-CSF favorisent la différenciation des progéniteurs GMP en monocytes, tandis que des concentrations plus élevées favorisent la différenciation granulo-cytaire [51], [52].
- Le M-CSF (Macrophage-Colony stimulating factor) aussi connu sous le nom CSF1 (Colony Stimulating Factor 1) est une glycoprotéine homodimérique synthétisée de manière constitutive par une variété de cellules, y compris les cellules endothéliales, les fibroblastes, les ostéoblastes et les macrophages [53], [54]. Ses effets biologiques sont transmis par le CSF-1R (récepteur 1 du facteur de stimulation des colonies de macrophages), qui est un récepteur transmembranaire à tyrosine kinase de type III. Ce récepteur est également connu sous le nom de proto-oncogène c-fms [55]. Chez l'Homme, des altérations génétiques dans CSF-1R ont été liées à plusieurs conditions, notamment une leucoencéphalopathie diffuse héréditaire, le développement d'une leucémie myéloïde aiguë et à l'apparition des syndromes myélodysplasiques [56], [57]. Les premières expériences *in vitro* ont montré que les cellules précurseurs de la moelle osseuse cultivées dans le CSF1 donnent lieu à la formation de macrophages. Cela suggère une implication du CSF1 dans le processus de génération des monocytes et des macrophages. Cependant, chez les souris qui présentent une mutation dans le locus CSF1, le nombre de monocytes dans le sang reste dans les limites de la normale malgré cette mutation [58]. Ces souris développent une ostéopétrose du fait d'un défaut de

résorption osseuse. Cette observation suggère qu'il existe un mécanisme de compensation impliquant d'autres voies de signalisation pouvant être impliquées dans la régulation de la production des monocytes.

- MIF (macrophage migration inhibitory factor) est exprimé de manière constitutive par tous les tissus et types cellulaires et se comporte comme une cytokine pro-inflammatoire [59]–[61]. Les monocytes de nouveau-nés possèdent d'importantes réserves intracellulaires de MIF qu'ils sécrètent en cas d'infection [62]. Le MIF peut stimuler l'activation des monocytes et macrophages et il est nécessaire à l'expression de cytokines telles que TNF- α et IL-1 [63], [64].

Ces différentes observations montrent la complexité de la régulation de la différenciation qui permet au système hématopoïétique de s'adapter en fonction des signaux présents dans l'environnement. Cette adaptation est essentielle pour maintenir l'équilibre et la fonctionnalité du système immunitaire.

Les facteurs de transcription impliqués dans le développement des monocytes

En plus des signaux extracellulaires, la différenciation d'une lignée hématopoïétique nécessite l'implication de plusieurs facteurs de transcription qui jouent un rôle crucial dans la différenciation monocyttaire. Les principaux facteurs de transcription impliqués sont :

- PU.1 (SPI1) : Ce facteur de la famille des facteurs de transcription ETS (E-twenty-six) joue un rôle essentiel dans la différenciation des progéniteurs hématopoïétiques vers la lignée monocyttaire. C'est un régulateur clé du développement des monocytes. Les souris déficientes en Pu.1 n'ont pas de monocytes, de granulocytes et de cellules B [65]. Les souris dans lesquelles le gène Pu.1 est muté ont une diminution du nombre de monocytes dans la moelle osseuse, la circulation sanguine et les tissus. La production de cellules dendritiques a également été montrée réduite. Parallèlement à la diminution des monocytes et des DC, les souris mutantes pour Pu.1 (des mutations homozygotes et hétérozygotes de Pu.1) montrent souvent une augmentation du nombre de granulocytes dans la moelle osseuse et dans la circulation sanguine. Ces observations mettent en évidence le rôle crucial de Pu.1 dans la différenciation et le développement normal des monocytes et des cellules dendritiques, ainsi que dans le maintien de l'équilibre entre les différentes lignées cellulaires du système hématopoïétique [66]. Pu.1 est

exprimé à des niveaux relativement faibles dans les CMP et son expression augmente progressivement au cours de la différenciation granulomonocytaire [67]. PU.1 régule l'expression des gènes spécifiques des monocytes en se liant à des régions régulatrices (Analyse de motif de Pu.1 : (AAGAGGAAGT). La surexpression de PU.1 induit l'activation du facteur régulateur de l'interféron 8 (IRF8) [68], [69] et le facteur 4 de type Krüppel (KLF4) [70]. Dans le même temps, il peut inhiber l'activité de certaines protéines de liaison, notamment GATA1 et GATA2 [71], [72]. Ces interactions entre les facteurs de transcription génèrent un circuit de contre-régulation qui favorise la génération de monocytes plutôt que celle des neutrophiles.

- IRF8 (*Interferon Regulatory Factor 8*) est un facteur essentiel au développement des monocytes. Exprimé principalement dans les cellules de la lignée myéloïde, il joue un rôle essentiel dans la différenciation des monocytes. Les souris déficientes en *Irf8* révèlent des défauts dans la production et la maturation des monocytes. Les rares monocytes qui se développent chez ces souris ont une capacité réduite à se différencier en macrophages fonctionnels [73]. IRF8 régule l'expression de KLF4 [74].
- KLF4 (*Krüppel-like factor 4*) appartient à la famille des facteurs de transcription KLF. KLF4 est exprimé dans les cellules souches hématopoïétiques et les cellules myéloïdes. Dans le contexte des monocytes, KLF4 est impliqué dans la régulation de leur différenciation et de leur fonction. L'équipe de Civin a montré que le facteur de transcription KLF4 est essentiel pour le développement des monocytes classiques murins [75].
- CEBP α /CEBP β : sont deux facteurs de transcription de la famille des protéines de liaison CCAAT/enhancer (C/EBP). Ils jouent un rôle crucial dans la différenciation et la fonction des monocytes ainsi que d'autres cellules du système immunitaire [76]. CEBP α est impliqué dans l'engagement des progéniteurs hématopoïétiques vers la voie monocytaire, dans la maturation et dans l'acquisition de fonctions spécifiques des monocytes. Les souris *Cebpa*^{-/-} manquent d'éosinophiles et de neutrophiles sanguins mais pas de monocytes [77]. CEBP β , quant à lui, joue un rôle important dans la participation des monocytes à la réponse inflammatoire ou immunitaire. Les souris *Cebpb*^{-/-} ont une baisse des monocytes du sang périphérique sans atteinte des autres lignées [78].
- RUNX1 (Runt-related transcription factor 1) est un facteur de transcription essentiel de l'hématopoïèse définitive. Les souris n'exprimant pas *Runx1* présentent une létalité embryonnaire due à l'absence d'hématopoïèse normale [79]. Sans RUNX1, la différenciation des cellules souches

hématopoïétiques en cellules de la lignée myéloïde, dont font partie les monocytes, est gravement altérée. Des études ont montré que RUNX1 interagit avec la région promotrice du gène *Pu.1* et agit comme un activateur ou un répresseur de sa transcription en fonction du contexte cellulaire et des cofacteurs présents, plaçant RUNX1 en tant que régulateur précoce de l'hématopoïèse [80].

- EGR (Early Growth Response) : Cette famille de facteurs de transcription comprend : EGR-1, EGR-2, EGR-3, EGR-4 et WT-1. Ces facteurs partagent des caractéristiques structurales et fonctionnelles communes. Ils interviennent dans des contextes cellulaires et tissulaires différentes. EGR-1 est un facteur de transcription impliqué dans la régulation de nombreux processus biologiques. Il est exprimé dans divers types de cellules, y compris les monocytes dont il régule la différenciation et les fonctions. Il a été montré que l'induction d'EGR-1 favorise la différenciation des progéniteurs hématopoïétiques en monocytes [81]–[83] (**Figure 4**). Il se lie à un élément régulateur appelé FIRE dans le gène *CSF-1R* pour promouvoir son expression [84]. CSF-1R est un récepteur de surface cellulaire essentiel dans le développement et la fonction des monocytes et des macrophages. La fixation de CSF-1R à son ligand est essentielle pour la différenciation des progéniteurs hématopoïétiques en monocytes matures [85]. L'expression d'EGR-1 augmente au cours de la différenciation. En résumé, EGR-1 est impliqué dans la monocytopoïèse. Il joue un rôle important dans la régulation de la différenciation et de la maturation des monocytes à partir de progéniteurs hématopoïétiques.

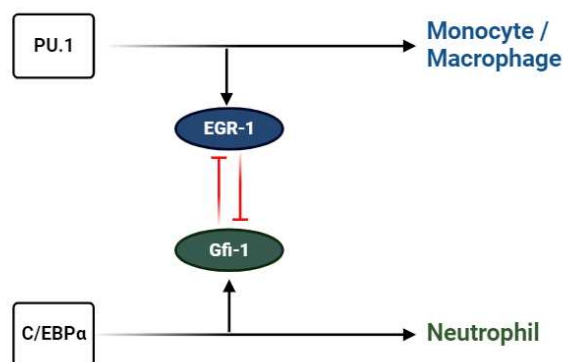


Figure 4 : Le destin des cellules myéloïdes

(Tiré de Laslo P et al, 2008)

Schéma représentant les gènes clés dans la génération des monocytes/macrophages et des neutrophiles. PU.1 et C/EBP α sont les facteurs de transcription importants dans le destin des cellules myéloïdes. Ensuite, les facteurs de transcription (EGR-1 et GFI-1) jouent un rôle clé, chacun neutralisant la voie de l'autre. EGR-1 avec PU.1 activent les gènes spécifiques aux monocytes/macrophages tandis que GFI-1 avec C/EBP α sont à l'origine de la génération des neutrophiles.

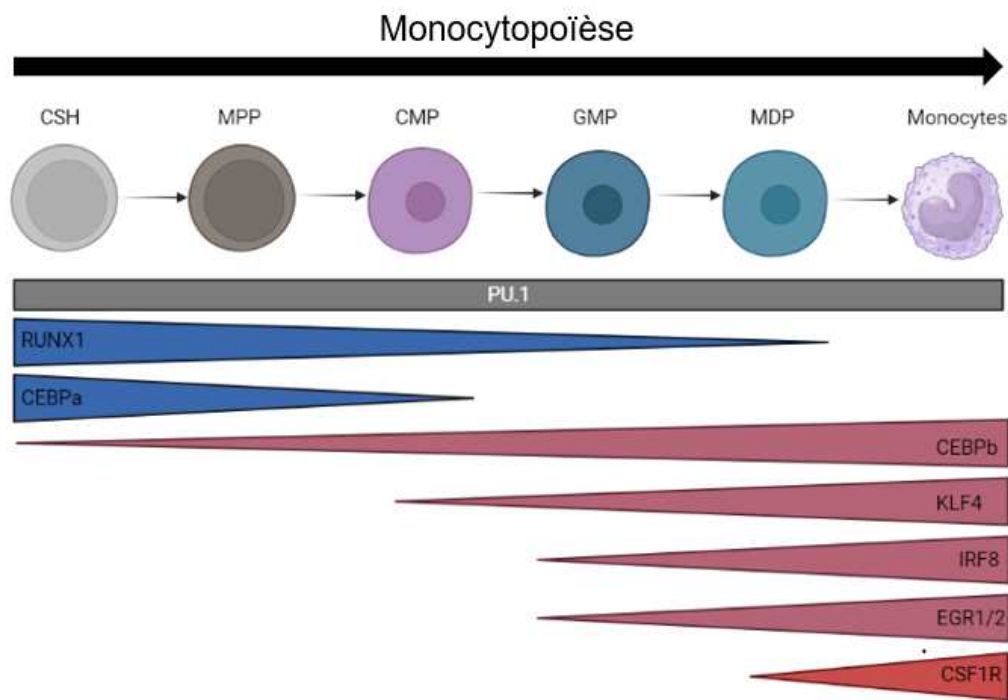


Figure 5 : l'influence des facteurs clés de transcription sur la monocytopoïèse

Illustration des facteurs de transcription les plus importants contribuant à l'expansion des cellules progénitrices vers la différenciation des monocytes à chaque étape de la maturation. En bleu on trouve les facteurs de transcriptions impliqués au début de la différenciation, en rose les facteurs de transcriptions impliqués au cours de la différenciation des progéniteurs vers le monocyte et en rouge le récepteur de CSF1 (CSF1R), un marqueur de surface clé lors des dernières étapes de la maturation des monocytes.

Il convient de noter que le développement des monocytes n'est pas un processus linéaire et strictement hiérarchique. Les facteurs de transcription interagissent de manière complexes et coordonnés pour réguler le développement des monocytes à partir des progéniteurs hématopoïétiques, en contrôlant l'expression des gènes impliqués dans la différenciation, la maturation et la fonction des monocytes (**Figure 5**).

La cytométrie en flux comme outil pour l'identification et la caractérisation des cellules monocytaires

Les efforts récents du consortium EuroFlow ont permis d'améliorer considérablement la caractérisation des cellules (pré)monocytaires de la moelle osseuse, y compris dans le contexte des hémopathies malignes. Ce consortium a validé des panels d'anticorps (reconnaissant une combinaison de plusieurs marqueurs de surface) qui permettent de déterminer l'immunophénotype des cellules par cytométrie en flux et leur stade de maturation.

La maturation monocyttaire normale dans la moelle osseuse commence avec les cellules progénitrices précoces qui expriment des marqueurs tels que le CD34 et CD117/KIT. L'engagement des précurseurs dans la lignée monocyttaire est associé à des niveaux plus élevés d'expression du marqueur CD64 et une expression plus faible pour le marqueur CD34 [86]. Ensuite, les monoblastes et les promonocytes sont distingués par l'expression du CD14 et CD35. Enfin, les monocytes matures sont caractérisés par l'expression de marqueurs tels que CD14⁺⁺, CD36⁺⁺ et le CD300E⁺⁺. Le CD36 et le CD14 sont largement utilisés pour identifier et définir les sous-ensembles de cellules monocytaires associés à la maturation [87]. Le CD300E (ou autrement appelé IREM-2) est un récepteur de type immunoglobuline. Son utilisation est moins courante que CD14 et CD36. [88]. Son expression est limitée aux cellules hématopoïétiques les plus matures des lignées de cellules dendritiques et monocytaires (**Figure 6**).

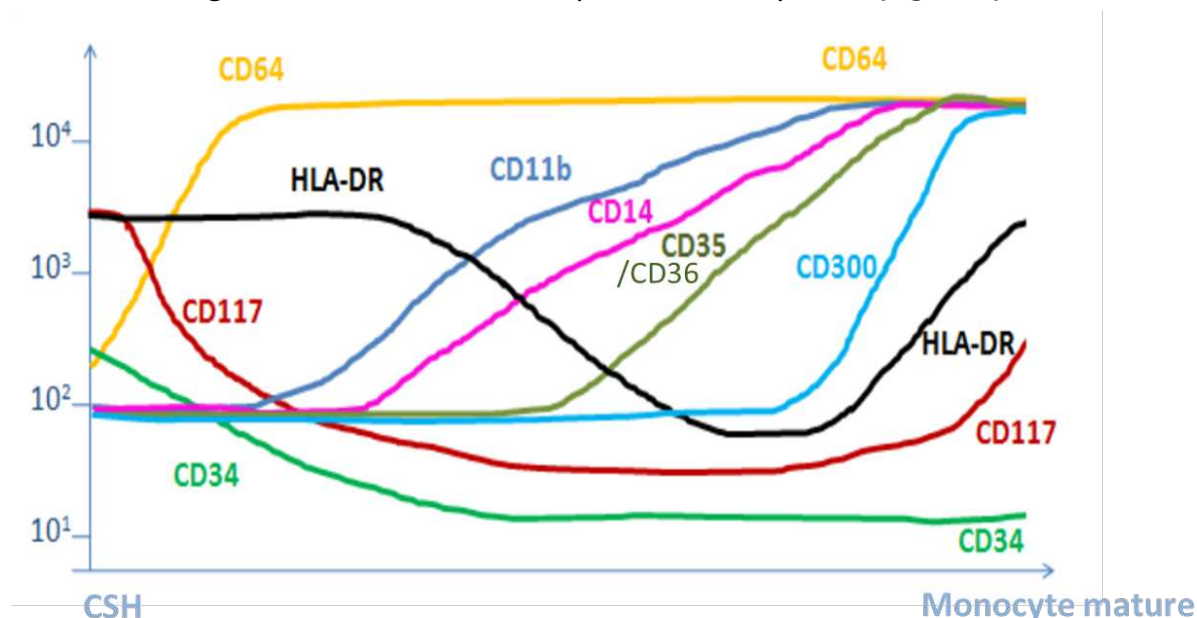


Figure 6 : Expression des marqueurs de surfaces au cours de la monocytopoïèse

Tiré de Wojciech Gorczyca

Au cours de la monocytopoïèse, CD64 est le premier marqueur à être exprimé puis nous avons respectivement le CD11b, le CD14 et le CD35 qui commencent à être exprimés au cours la maturation des monocytes. Le CD300E est le marqueur de surface à être exprimé au stade final de la maturation monocyttaire.

Cette identification des étapes du développement monocyttaire est une amélioration significative dans l'évaluation du degré de maturité des cellules monocytaires en situations pathologiques. Cependant, les changements sous-jacents dans l'expression des gènes qui régissent ces différentes étapes de maturation restent encore relativement peu étudiées et mal compris.

ii. Définition des sous-populations monocytaires : phénotypique et fonctionnelle

Les monocytes ont été décrits comme une population cellulaire unique sur la base de sa morphologie. Meuret et son équipe ont été les premiers à diviser les monocytes selon leur morphologie nucléaire en utilisant la radiographie par injection de 3H-tymidine (3H-TDR) : des monocytes immatures de type 'a' qui étaient « ovale rond », suivis du type 'b' qui étaient « légèrement pliés » et du type 'c' qui étaient « distinctement pliés » [89]. La mise au point de nouvelles technologies telles que la cytométrie en flux a eu un impact majeur sur l'identification de l'hétérogénéité des monocytes chez diverses espèces. C'est en 1989, que les monocytes ont été décrits sur la base de l'expression de deux marqueurs de surface chez l'homme CD14 et CD16 [90]. L'étude de ces deux marqueurs de surface a permis d'identifier la présence de trois sous-groupes de monocytes humains : les monocytes classiques qui sont CD14⁺⁺CD16⁻, les monocytes dites intermédiaires qui sont CD14⁺⁺CD16⁺ et enfin les monocytes non-classiques qui sont CD14^{Low}CD16⁺⁺ [91] (Figure 7).

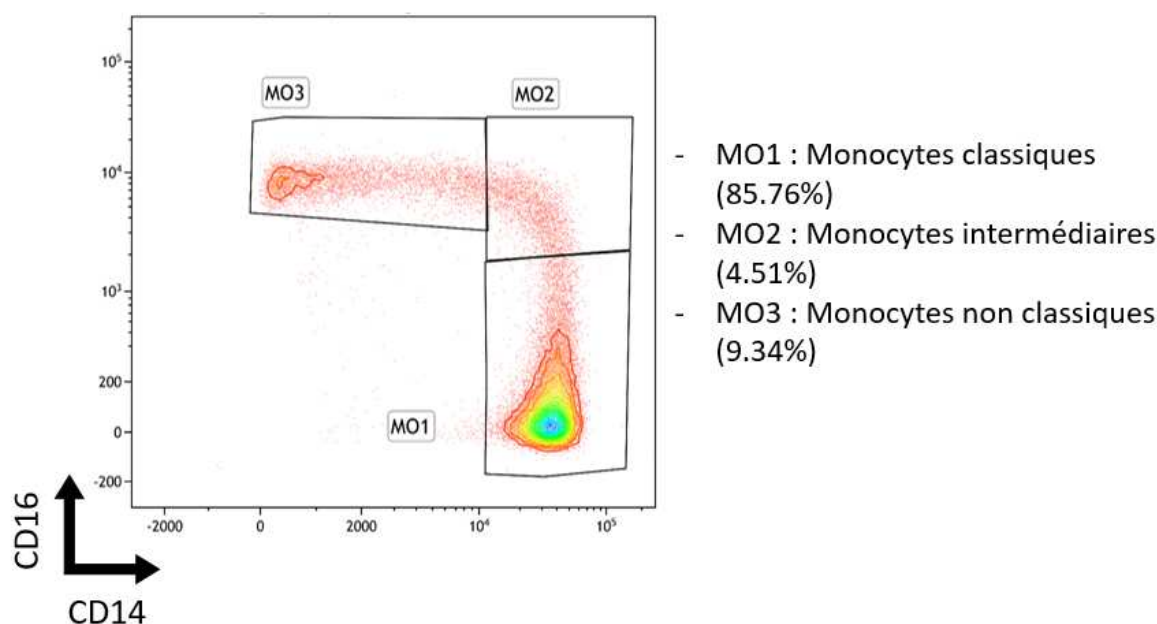


Figure 7 : Caractérisation des sous-populations monocytaires

Les sous-populations monocytaires peuvent être séparées en fonction de deux marqueurs de surface : le CD14 et le CD16. On différencie les monocytes classiques (MO1) qui sont CD14⁺⁺/CD16⁻ qui représentent environ 85% des monocytes totaux, les monocytes intermédiaires (MO2) qui sont CD14⁺⁺/CD16⁺ qui représentent environ 5% des monocytes totaux et les monocytes non classiques (MO3) qui sont CD14^{Low}/CD16⁺⁺ qui représentent 10% des monocytes totaux.

Les sous populations chez l'Homme

Au cours des dernières années, les chercheurs se sont beaucoup intéressés aux sous-populations monocytaires en utilisant des technologies récentes. Des études de microarray ont été employées pour évaluer les phénotypes, le transcriptome a été étudié par la technique de SuperSAGE [92] et enfin la cytométrie de masse ou la cytométrie spectrale ont été utilisées pour identifier les différents marqueurs de surface sur des cellules individuelles. Toutes ces différentes techniques, ont permis de montrer que les trois sous-populations de monocytes sont phénotypiquement et fonctionnellement différentes. Elles diffèrent par leur capacité de différenciation, de migration et de sécrétion de cytokines (**Tableau 1**).

| | Monocytes classiques : CD14 ⁺⁺ /CD16 ⁻ | Monocytes intermédiaires : CD14 ⁺⁺ /CD16 ⁺ | Monocytes non-classiques : CD14 ^{Low} /CD16 ⁺⁺ |
|------------------------------|--|--|---|
| Marqueurs de surfaces | CCR1, CCR2, CD1d, CD9, CD11b, CD33, CD36, CD62L, CD64, CD99, CLEC4D, CLEC5A, CXCR1-4 | CCR5, CD11b, CD32, CD40, CD47, CD54, CD64, CD80, CD86, CD163, GFRa2, HLA-ABC, HLQ-DR ,TNFR1 | CD45, CD97, CD116, CD123, CD294, CD11c, CX3CR1, P2RX1, Siglec10, SIRPα, SLAN, TNFR2 |
| Cytokines | IL13Rw1, G-CSF, CCL2, MCP-1 | IL-6, IL-8, IL-10, TNFα | TNFα, IL-1b, IL-6, IL-8 |
| Fonctions | Phagocytose, Adhérence, Migration, Inflammation, Réponse anti-bactérienne | Présentation d'antigène, Participation à la prolifération et à la réponse inflammatoire, Régulateur de l'apoptose, Forte production de ROS | Complément et phagocytose dépendant de FcR, Migration trans-endothéliale Adhérence, Réponses antivirales, Patrouiller l'endothélium |
| Formation | Moelle osseuse | Circulation sanguine ou tissus | Circulation sanguine ou tissus |
| Durée de vie | 1 jour | 3-4 jours | 4-7 jours |

Tableau 1 : Les sous-groupes de monocytes du sang périphérique humain avec les marqueurs de surface, les cytokines produites, les fonctions physiologiques activées, les localisations de formation et la durée de vie. (Tiré de Mongirdiene et al, 2022)

- Les monocytes classiques

Ces cellules expriment le CD14, HLA-DR, CCR2 et le CD63L à des niveaux élevés. Elles expriment en revanche très faiblement CD16 et CXCR1. Cette population a des fonctions pro-inflammatoires avec une forte capacité de phagocytose [92] [93]. Les monocytes classiques jouent également un rôle important dans la réparation des tissus

par leur capacité à migrer vers les tissus endommagés et expriment des récepteurs de chimiokines [94]. L'autre particularité des monocytes classiques est leur capacité à se différencier en macrophages, DC et ostéoclastes, ce que les monocytes intermédiaires et non-classiques semblent moins capables de faire [95].

- Les monocytes intermédiaires

Les monocytes intermédiaires représentent environ 5% des monocytes totaux. Ils partagent des propriétés phénotypiques et fonctionnelles avec les monocytes classiques et non-classiques. Ils expriment CD14, CD16 et CCR2. Cette population se distingue des deux autres par l'expression du CCR5, ce qui leur confère un rôle dans la sécrétion des cytokines. Ces cellules expriment des molécules du CMHII telles que CD74 et HLA-DR et ont une fonction de présentation d'antigène [92].

- Les monocytes non-classiques

Les monocytes non-classiques, représentent 10% de tous les monocytes et expriment fortement le CD16 et ont une expression relativement faible de CD14. Ils se distinguent des monocytes classiques par l'expression de CX3CR1. Leur stimulation par des LPS (lipopolysaccharides) provoque une production importante de cytokines inflammatoires, comme le TNF α , IL-1 β , ou l'IL-6, suggérant leur rôle dans l'inflammation [96], [97]. La principale fonction qui leur est attribuée est de rouler sur la paroi vasculaire pour en surveiller l'intégrité. D'autre part, les monocytes non-classiques montrent une expression importante de SLAN, qui est un marqueur utilisé pour différencier plus clairement les sous-ensembles intermédiaires et non-classiques [98].

Les monocytes classiques résident environ 24 heures dans la circulation. Tandis que les monocytes non-classiques ont une durée de vie plus longue, c'est à dire d'environ 2 jours chez la souris et 7 jours chez l'Homme [99], [100].

Les sous populations monocytaires chez la souris

Les sous-groupes de monocytes chez la souris ont été détectés plus d'une décennie après leur découverte chez l'Homme. En effet, une analyse par cytométrie en flux des cellules d'une souris transgénique portant un gène rapporteur fluorescente GFP dans le locus du récepteur de chimiokine CX3CR1 a révélé la présence de deux sous-ensembles de monocytes qui pouvaient être discriminés par différents marqueurs dont Ly6C, CCR2, CX3CR1 et CD62L [101]. Les monocytes classiques sont CX3CR1^{int}CCR2⁺CD62L⁺Ly6C⁺ et les non classiques sont CX3CR1⁺CCR2⁻CD62L⁻Ly6C⁻. Les

études fonctionnelles ont confirmé que les monocytes Ly6C⁺ et Ly6C⁻ correspondent respectivement aux sous-populations de monocytes humains classiques (CD14⁺⁺) et non-classiques (CD14^{low}) [102] [103] .

En revanche, leur répartition est différente. La population, Ly6C⁺ représente 50% des monocytes circulants. Elle est également dite « inflammatoire » car ces cellules sont généralement recrutées vers les sites d'inflammation ou d'infection aiguë. Elles ont une forte capacité accrue à produire des cytokines pro-inflammatoires et à phagocyter les agents pathogènes. Elles peuvent également se différencier en cellules dendritiques ou en macrophages une fois recrutées dans les tissus.

Les monocytes Ly6C⁻ sont souvent décrits comme une population anti-inflammatoire ou tolérogène. Ils sont principalement présents dans les tissus sains ou patrouillent dans les vaisseaux sanguins et les tissus, surveillant l'intégrité de leur environnement et contribuant à la régulation de l'homéostasie tissulaire. Ces monocytes semblent être impliqués dans la résolution de l'inflammation et la cicatrisation des tissus [104] [105].

C. Les monocytes en condition physiologique et pathologique

Les monocytes jouent un rôle clé dans le soutien de l'homéostasie tissulaire en ciblant directement les agents pathogènes. La répartition des sous-populations monocytaires est influencée par divers facteurs : l'infection, l'inflammation, les maladies auto-immunes ou les traitements médicamenteux. Ainsi, au cours de l'homéostasie et dans des conditions pathologiques, chaque sous-population joue un rôle différent. Dans certains cas, l'étude des sous-populations monocytaires, peut contribuer à faire le diagnostic d'une maladie ou apporte une information pronostique (**Figure 8**).

En condition physiologique, les monocytes matures patrouillent pendant quelques jours dans la circulation sanguine, dans un état inactif, à la recherche d'indications d'infection, d'inflammation ou de dommage cellulaire [106], [107]. Enfin, soit ils quittent la circulation soit ils meurent par apoptose [91]. Dans le cas où les monocytes sont face à un dommage, ils peuvent se différencier en macrophages et/ou en DC dotés de fonctions spécialisées par la production de cytokines/chimiokines/enzymes, la phagocytose et la présentation des antigènes [107]–[109].

i. Monocytes et mode de vie

Même à l'état physiologique, le pourcentage de monocytes varie selon les critères simples tels que le sexe et l'âge. La proportion de monocytes non-classiques est plus importante chez les hommes (6,1%) par rapport aux femmes (3,5%) [110]. Une

augmentation du pourcentage de monocytes intermédiaires et non-classiques a été observée chez les personnes âgées [111]. Il a aussi été montré que les habitudes alimentaires peuvent affecter le nombre et la répartition des trois sous-populations monocytaires. Par exemple, l'obésité induit une augmentation du nombre de monocytes intermédiaires et non-classiques. Il a également été observé, une sécrétion accrue de cytokines pro-inflammatoires, telles que IL-1 β et TNF α , dans les populations intermédiaires et non-classiques [112].

ii. Monocytes en pathologie

Les monocytes et macrophages sont des acteurs centraux de la réponse immunitaire, de son initiation à sa résolution et au rétablissement de l'homéostasie [26], [113]. En conséquence, le dysfonctionnement des monocytes génère des infections chroniques et participe à l'expression de maladies inflammatoires ou auto-immunes (par exemple de maladies inflammatoires chroniques de l'intestin (MICI), de polyarthrite rhumatoïde ou du psoriasis) [114]–[117].

Lorsqu'une infection ou une lésion se produit, cela implique une séquence d'événements cellulaires et moléculaires. L'étape la plus critique est la reconnaissance de l'agent pathogène ou du dommage cellulaire qui passe par la libération de multiples signaux reconnus notamment par des récepteurs de la surface des monocytes. Il s'agit par exemple de récepteurs de reconnaissance des pathogènes (PRR) pour la détection des micro-organismes en identifiant des structures/molécules conservées à la surface des pathogènes (virus, bactéries, champignons et parasites) telles que les PAMP et les DAMP [118]. Dans la famille de PRR, on trouve les récepteurs de type TLR (Toll Like Receptor), les récepteurs de lectine de type c et des protéines cytoplasmiques. Les TLR sont caractérisés par la présence de séquences répétées N-terminales riches en leucine (LRR). Chez l'Homme, 10 TLR ont été identifiés. TLR4 est présent à la surface des monocytes et macrophages et reconnaît les bactéries Gram-négatives tandis que TLR2 sont capables de reconnaître les bactéries Gram-positives [119].

Il est connu que les monocytes exprimant le marqueur de surface CD16 sont essentiels dans un contexte infectieux. Par exemple, dans les cas de septicémie sévère, la sous-population des monocytes intermédiaires augmente [120], dans le cas de la tuberculose, les populations intermédiaires et non-classiques sont les plus répandues [121]. Il en est de même lors d'une hépatite B [122], d'une hépatite C chronique [123] et d'une infection par le VIH-1 [124]. La fraction des monocytes CD16⁺ est indicative de

la sévérité des maladies inflammatoires [73], [125], [126] et corrèle au risque d'événements cardiovasculaires [127].

Une réponse inflammatoire excessive ou chronique peut entraîner des dommages tissulaires et des maladies inflammatoires telles que l'arthrite rhumatoïde, la maladie inflammatoire de l'intestin, l'athérosclérose, etc. Par conséquent, la régulation équilibrée de l'activité des monocytes est essentielle pour permettre une réponse inflammatoire appropriée et éviter les conséquences néfastes d'une réponse excessive.

iii. Monocytes et Cancer

Les monocytes peuvent jouer un rôle anti-tumoral dans le contexte de certains cancers. Certains monocytes, notamment ceux qui expriment CD16, peuvent avoir des propriétés cytotoxiques et participer à la destruction des cellules tumorales. Leur longue durée de vie et leur activité de patrouilleur les rendent bien adaptés pour détruire la tumeur et générer des cytokines qui favorisent la réponse immunitaire antitumorale [128].

Les monocytes peuvent tuer directement les cellules malignes en produisant des protéines inductrices de la mort cellulaire telles que TRAIL [129]. Cependant, il est important de noter que de nombreuses cellules cancéreuses sont résistantes à l'apoptose dépendante de TRAIL [130].

La phagocytose induite par les monocytes joue également un rôle important dans l'élimination des cellules cancéreuses. Cependant, les cellules cancéreuses peuvent développer une résistance contre la phagocytose en exprimant CD47, un paramètre de mauvais pronostic en clinique dans un certain nombre de cancers comme les cancers de l'ovaire et du poumon [131].

Le microenvironnement tumoral peut influencer la polarisation des monocytes et leurs fonctions, orientant ces cellules vers un phénotype pro-tumoral ou anti-tumoral. Leur impact sur la progression tumorale dépend de divers facteurs, comme le type de cancer, le stade de la maladie et l'interaction avec d'autres cellules du système immunitaire. Dans certains cas, les monocytes se différencient en macrophages tumoraux. Ces monocytes et macrophages peuvent présenter un phénotype pro-tumoral et favoriser la croissance, l'invasion tumorales et la formation de métastases. Ils peuvent également favoriser l'angiogenèse, c'est-à-dire la formation de nouveaux

vaisseaux sanguins qui nourrissent la tumeur. Dans le cas des tumeurs de glioblastomes, les macrophages produisent des métalloprotéinases matricielles (MPP) qui libèrent le facteur VEGF et stimulent l'angiogenèse. [132]. Chez l'Homme, un sous-ensemble de monocytes CD16 exprimant Tie-2, le récepteur de l'angiopoïétine, est capable de promouvoir l'angiogenèse par la sécrétion de facteurs [133], [134].

Des modifications de la répartition des populations monocytaires peuvent être associées aux cancers. Par exemple, on observe une augmentation des sous-populations intermédiaires et non classiques dans les leucémies aiguës lorsque leur génération n'est pas inhibée par l'expansion du clone leucémique [135]. Ils peuvent être utilisés comme un marqueur pronostique :

- Le rapport lymphocytes/monocytes dans le sang périphérique est devenu un facteur pronostique pour plusieurs types de cancers [136]–[138].
- Dans les cancers colorectaux (CCR), les monocytes ont une signature génique (composée de 23 gènes) distincte par rapport à des monocytes sains, faisant du monocyte un candidat dans le diagnostic du CCR [139].

Il est important de souligner que le rôle des monocytes dans le cancer est complexe et varie selon le contexte. Des recherches supplémentaires sont nécessaires pour mieux comprendre les mécanismes spécifiques régissant l'interaction entre les monocytes et les cellules tumorales afin de développer des approches thérapeutiques ciblées plus efficaces et d'améliorer l'efficacité des traitements anticancéreux préexistants.

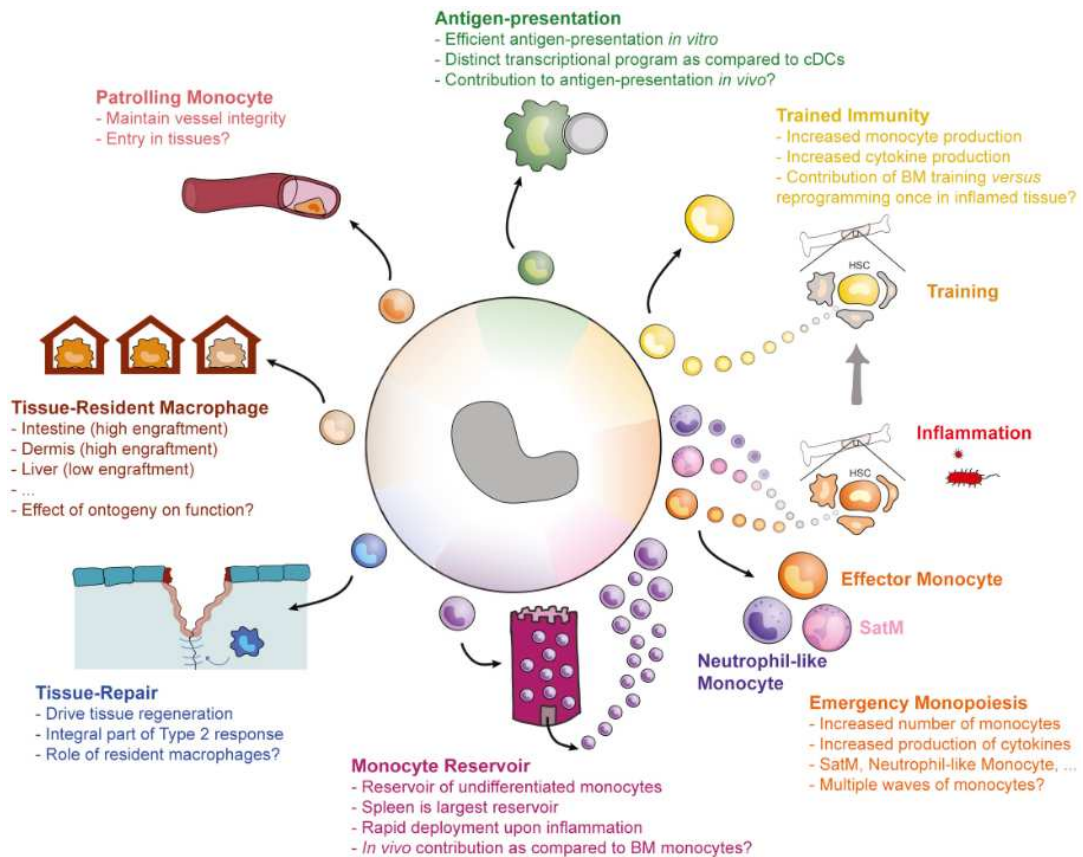


Figure 8 : Fonctions des monocytes en condition physiologique et pathologique

(Tiré de Guiliams M et al, Immunity ; 2018)

Les monocytes peuvent se greffer dans des réservoirs de monocytes périphériques ou peuvent se différencier en monocytes non classiques ou en macrophages résidant dans les tissus à l'état d'équilibre. Les monocytes peuvent également acquérir des capacités inflammatoires, régénératives ou de présentation antigénique lorsqu'ils pénètrent dans les tissus enflammés. Une inflammation sévère peut conduire à une monocytopoïèse d'urgence avec la production de nouveaux sous-ensembles de monocytes aux caractéristiques fonctionnelles altérées. L'inflammation peut avoir des effets à long terme sur l'hématopoïèse et entraîner une immunité entraînée associée à une production accrue de monocytes produisant davantage de cytokines inflammatoires.

Conclusion générale de la partie 1 :

Les monocytes jouent un rôle essentiel :

- Dans la réponse immunitaire en participant à la reconnaissance et à l'élimination des agents pathogènes ou des cellules altérées.
- Dans la réponse inflammatoire, leur dysfonctionnement ou leur activation excessive pouvant contribuer au développement et à la progression des maladies inflammatoires, auto-immunes et des cancers.
- Leur plasticité cellulaire leur permet de différencier en macrophages, en cellules dendritiques et en ostéoclastes.
- En tant que marqueurs diagnostiques et pronostiques : des altérations dans les populations de monocytes ou des changements dans l'expression de certains marqueurs peuvent être associés à des maladies spécifiques, fournissant ainsi des informations utiles pour le diagnostic et la prise en charge des patients.

Ainsi, l'étude des monocytes permet de mieux comprendre les mécanismes de l'immunité, de mieux caractériser les maladies inflammatoires, d'explorer la plasticité cellulaire et d'identifier de nouveaux marqueurs diagnostiques et pronostiques. Ces avancées contribuent à l'amélioration des connaissances scientifiques et ouvrent des perspectives prometteuses pour le développement de nouvelles approches thérapeutiques.

2. La Leucémie MyéloMonocytaire Chronique

A. Généralité sur la LMMC

La thématique de recherche de l'équipe porte sur la Leucémie MyéloMonocytaire chronique (LMMC). C'est une maladie clonale de la cellule souche hématopoïétique faisant partie, au sein de la classification OMS, de la catégorie des syndromes myélodysplasiques (SMD) et des néoplasmes myéloprolifératifs (SMP).

i. Historique

Un premier cas de LMMC a été observé en 1972, chez des personnes de plus de 50 ans présentant une augmentation des monocytes [140]. La maladie a d'abord été décrite par le groupe de travail franco-américain-britannique (FAB) comme un SMD [141]. De nombreux scores pronostiques ont ensuite tenté de stratifier les patients atteints de LMMC. Des formes prolifératives ont été distinguées des formes dysplasiques [142]. Par la suite, le score proposé par le MD Anderson (MDAPS) à partir d'une cohorte provenant de Düsseldorf (Allemagne), a classé les patients en trois catégories de risque [143]. Ensuite, des chercheurs ont analysé l'impact pronostique indépendant des anomalies cytogénétiques chez 414 patients d'un registre espagnol, montrant que les patients avec un caryotype anormal avaient une survie plus faible et un risque plus élevé d'évoluer en une Leucémie myéloïde aiguë (LAM) [144]. L'Organisation Mondiale de la Santé (OMS) a ensuite classé la LMMC comme un syndrome SMD/SMP dont les critères ont évolué par étapes jusqu'en 2022 [145]. La survie médiane des patients LMMC se situe entre 12 et 31 mois et le risque de transformation en LAM se situe entre 15 et 30% [143], [146]. La transformation est une des causes de mortalité chez les patients atteints de LMMC [147]. Lorsque la maladie ne se transforme pas, elle entraîne une fatigue croissante, une détérioration progressive de l'état général, une anémie ou une thrombopénie, ces symptômes pouvant être liés à des comorbidités liées à l'âge. L'origine de la maladie n'est pas identifiée, mais dans la plupart des cas, la LMMC survient en raison du vieillissement des cellules souches hématopoïétiques (CSH). La signature mutationnelle est caractéristique du vieillissement. La LMMC peut provenir dans certains cas d'un SMD antérieur [148] ou apparaître après le traitement d'un autre cancer (« therapy-related ») [149].

ii. Diagnostic d'une LMMC

Les critères selon la classification de l'OMS

En 2016, l'OMS définit la LMMC comme une maladie clonale de la cellule souche hématopoïétique caractérisée par une accumulation de monocytes supérieure ou égale à $1 \times 10^9/L$ pendant plus de 3 mois dans le sang périphérique représentant plus de 10% des leucocytes, ainsi que des caractéristiques dysplasiques dans la moelle osseuse [150]. Le seuil de la monocytose a été révisé à $0,5 \times 10^9/L$ à condition que les monocytes représentent plus de 10% de l'ensemble des GB par l'OMS en 2022 [151]. La LMMC est une hémopathie rare avec une incidence estimée à 4 cas pour 100 000 personnes par an [152], [153]. Au moment du diagnostic, l'âge médian est d'environ 73 à 75 ans avec une prédominance masculine [154], [155], sans qu'on en connaisse la cause. La LMMC est une maladie très hétérogène comme l'indique sa classification.

Compte tenu de la présence d'une monocytose, le premier objectif est alors d'exclure les monocytoses transitoires. Il faut savoir que la monocytose peut faire référence à plusieurs autres maladies telles que la tuberculose, les infections fongiques et virales, la monocytose réactionnelle... Par la suite, des analyses moléculaires sont envisagées pour évaluer le chromosome de Philadelphie et la fusion BCR-ABL1 afin d'exclure une leucémie myéloïde chronique (LMC) [156]. L'absence d'une hyperéosinophilie et de réarrangement génique affectant les récepteurs A ou B du PDGF (PDGFRA/B) permet d'écarter un autre néoplasme myéloïde sensible à l'imatinib [157], [158]. L'existence d'une dysplasie dans une ou plusieurs lignées cellulaires de la moelle osseuse contribue au diagnostic [150].

La LMMC est dite proliférative si le taux de leucocytes est supérieur ou égale à $13 G/L$, cette forme est de mauvais pronostic [159] par rapport aux formes dites dysplasiques où le taux de leucocytes est inférieur à $13 G/L$. En plus de ces caractéristiques, la LMMC était classée jusqu'en 2022 en 3 variants selon le pourcentage de blastes dans le sang périphérique et la moelle osseuse : on distingue les LMMC-0, LMMC-1 et LMMC-2 (tableau 2). Les LMMC-0 et LMMC-1 ont été fusionnées en un seul sous-groupe dans la version 2022 de la classification OMS. Le pourcentage de blastes permet d'exclure les LAM (>20% de blastes dans le sang périphérique et la moelle osseuse) (**Tableau 2**).

| | LMMC-1 | LMMC-2 | LAM |
|-------------------|--------|--------|-------|
| Blaste dans le SP | 2-4% | 5-19% | > 20% |
| Blaste dans la MO | 5-9% | 10-19% | > 20% |

Tableau 2 : Les sous-types de LMMC

Décompte des blastes présents dans le sang périphérique (SP) et moelle osseuse (MO).

Les anomalies cytogénétiques

Le caryotype des cellules médullaires et sanguines est normal chez 70% des patients. Les anomalies cytogénétiques clonales sont observées chez 30% d'entre eux. Parmi les anomalies cytogénétiques les plus répandues on retrouve la trisomie 8, la trisomie 21, la perte du chromosome Y, l'anomalie du chromosome 7 ou la délétion del(7q) [160].

Les anomalies moléculaires

Des anomalies génétiques sont détectées chez plus de 90% des patients atteints de LMMC. En moyenne, les patients atteints de LMMC présentent environ 10 à 15 mutations par kilobase de régions d'ADN [161] [162]. Les mutations génétiques impliquent les modificateurs épigénétiques tels que *TET2* (60%), *ASXL1* (40%), [163], [164], des composants du spliceosome tels que *SRSF2* (50%), *U2AF1* (10%) et *SF3B1* (10%) [155], [165] [166], des facteurs de transcription tels que *RUNX1* (15%) et *SETBP1*, des gènes impliqués dans les voies de signalisation tels que *RAS* (30%), *JAK2* (10%) et *CBL* (15%) [167] et des facteurs suppresseurs de tumeurs tels que *TP53* (très rare, <2%) et *PHF6* [168] [169]. Aucune de ces altérations n'est spécifique de la LMMC mais la combinaison de mutations bialléliques de *TET2* et d'une mutation hétérozygote de la proline 95 de *SRSF2* est très évocatrice de LMMC. Les mutations d'*ASXL1*, de *RUNX1*, de *NRAS* et *SETBP1* sont des marqueurs de mauvais pronostic [169].

Le clone leucémique

La LMMC présente des similitudes génétiques avec les SMD et les SMP. Aucune mutation génétique n'est exclusivement spécifique à la LMMC, ce qui rend leur diagnostic plus complexe. L'hétérogénéité phénotypique observée malgré des altérations génétiques identiques peut être expliquée par divers facteurs tels que la répartition des mutations dans le clone, la cellule d'origine des altérations génétiques et l'ordre d'apparition des mutations. Ainsi, pour mieux comprendre ces aspects, des

études sur l'évolution clonale et l'architecture du clone leucémique en utilisant des techniques de séquençage de l'ADN ont été réalisées.

L'analyse des monocytes CD14⁺ (par séquençage complet du génome (WGS)) de patients atteints de LMMC a permis de mettre en évidence que les mutations somatiques observées dans cette maladie résultent d'un processus de mutagenèse liée à l'âge [170] [161]. Dans la LMMC, la majorité (75%) des cellules souches les moins différenciées (CD34⁺CD38⁻) portent des mutations géniques. Cette constatation indique que le clone leucémique domine sur les cellules normales dès le compartiment de cellules souches. L'analyse à l'échelle unicellulaire, menée par Itzykson et ses collègues, a mis en évidence, dans le compartiment de cellules souches, la présence de sous-clones qui ont émergé suite à l'accumulation graduelle de mutations génétiques [171]. Les mutations additionnelles sont impliquées dans la progression de la maladie vers une forme plus sévère. L'ordre d'apparition des mutations dans ce contexte semble aléatoire. Cependant, la première mutation se produit le plus souvent dans un gène qui code pour un régulateur épigénétique, tel que *TET2* ou *ASXL1*. La seconde mutation survient généralement dans un gène impliqué dans l'épissage des ARN tel que *SRSF2* ou dans la régulation de la signalisation tel que *RAS* aboutissant à une maladie clinique et/ou biologique. Certaines mutations génétiques semblent se présenter de manière concomitante. C'est le cas de la combinaison des mutations *TET2* et *SRSF2* qui sont généralement présentes chez un même patient. Cette association entre les deux mutations a évoqué une relation d'épistatique [168]. Cependant, les mutations du gène *SRSF2* peuvent conférer un désavantage compétitif aux CSH lorsqu'elles surviennent seules [172]. Dans un contexte où il y a déjà une mutation du gène *TET2*, la présence de la mutation *SRSF2* semble conférer un avantage supplémentaire aux CSH. Cet équilibre entre le désavantage compétitif et l'avantage contextuel pour la mutation *SRSF2* dans le cadre d'une mutation *TET2* pourrait contribuer à expliquer l'ordre d'apparition observé pour ces mutations dans la progression de la LMMC [171].

Les études moléculaires et fonctionnelles ont révélé que l'expansion granulomonocytaire qui est la caractéristique centrale du phénotype de la LMMC, peut-être le résultat d'au moins deux mécanismes distincts [173] **(Figure 9)**:

- L'hypersensibilité des progéniteurs myéloïdes à la signalisation GM-CSF entraîne une activité phospho-STAT5 augmentée [174]. Cette activité de STAT5 agit comme un transducteur jouant un rôle important dans la régulation de la prolifération et de la différenciation des cellules myéloïdes [175]. Des études récentes ont mis en évidence que cette hypersensibilité n'est pas une caractéristique présente dans tous les cas de LMMC (35 à 50% des cas de LMMC). Ces cas correspondent en partie aux formes prolifératives

de la LMMC (MP-LMMC) et présentant des mutations dans les voies de signalisation (*RAS*) [174], [176].

- Les données provenant d'études menées sur des modèles murins et des cellules primaires humaines suggèrent que la mutation de *TET2* confère un avantage sélectif aux CSH mutées, leur permettant de se multiplier plus efficacement par rapport aux CSH non mutées. Cependant, cet avantage concurrentiel peut conduire à des changements dans le développement des progéniteurs cellulaires et à des altérations de la différenciation des cellules [171].

Ce modèle peut ne pas être applicable à tous les cas de LMMC, car la maladie peut être hétérogène sur le plan génétique et présenter des mécanismes différents entre différents patients.

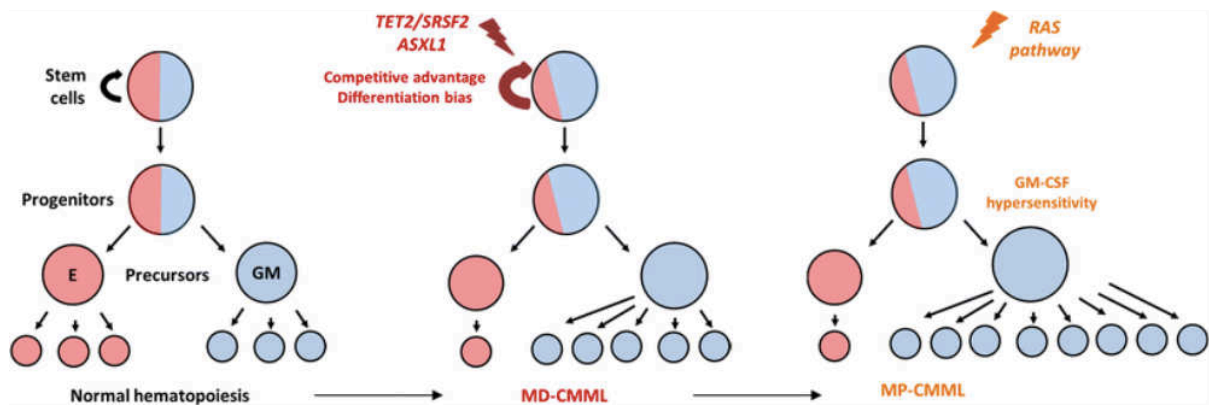


Figure 9 : La LMMC comme modèle

Acquisition en deux étapes du biais granulomonocytaire (GM) chez les cellules souches mutées. Tout d'abord, ces CSH mutées acquièrent un avantage sélectif, conduisant à l'expansion granulomonocytaire. Ensuite, ces progéniteurs mutés développent une hypersensibilité au GM-CSF en raison de mutations dans la voie de signalisation RAS. MD-CMML (myelodysplastique CMML), MP-CMML (myeloproliférative CMML)

Au final, comprendre les caractéristiques du clone leucémique dans la LMMC est essentiel pour le diagnostic et le développement de traitements ciblés.

Répartition des sous-population monocytaires

L'étude des sous-populations monocytaires dans le sang des patients atteints de LMMC par cytométrie en flux a clairement fait avancer le diagnostic de la LMMC. Elle montre typiquement une accumulation des monocytes classiques (MO1), exprimant le CD14 mais pas le CD16, supérieure à 94% des monocytes totaux, avec une diminution des

monocytes intermédiaires et non-classiques. Ce phénotype est commun aux deux groupes de patients : proliférative ou dysplasique, et est indépendant du statut mutationnel. L'analyse des sous-populations monocytaires distingue facilement une LMMC d'une monocytose réactionnelle, caractérisée par une augmentation de la population de monocytes intermédiaires et/ou non-classiques [177] (**Figure 10**).

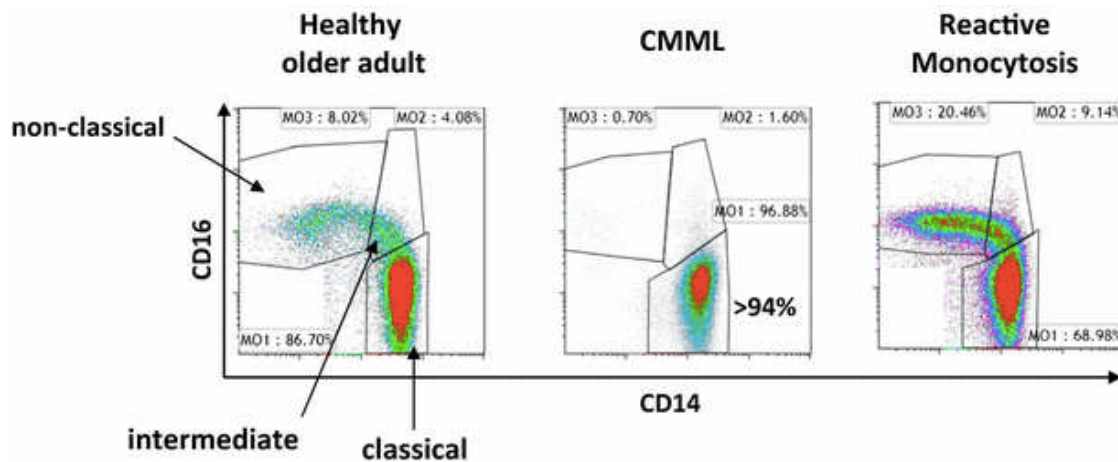


Figure 10 : Répartition des sous-populations monocytaires

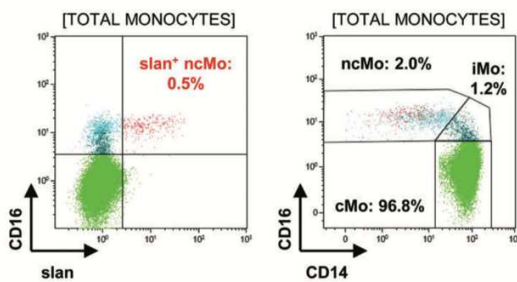
(Tiré de Selimoglu-Buet Dorothée, 2015)

Représentation de la répartition des sous-ensembles de monocytes dans les PBMC collectées d'échantillons contrôles, de patients atteints de LMMC et de patients atteints d'une monocytose réactionnelle. Le pourcentage de chaque sous-ensemble de monocytes est indiqué.

L'équipe a montré qu'une diminution de l'expression du gène MIR150, par méthylation d'un promoteur spécifique des monocytes, est à l'origine de l'accumulation de monocytes classiques dans le sang périphérique des patients atteints de LMMC. Cette diminution est liée à une forte expression de TET3 dans les monocytes classiques qui empêche la conversion des monocytes classiques en monocytes intermédiaires et non-classiques [178].

Malgré tout, il est possible de rencontrer des exceptions lors de l'utilisation de cette technique. Les patients LMMC présentant une maladie auto-immune ou inflammatoire sont associés à une augmentation du nombre de monocytes intermédiaires, ce qui masque l'accumulation des monocytes classiques. Dans ce cas, il est recommandé d'associer le marqueur SLAN, exprimé uniquement dans les monocytes non-classiques, et qu'il soit inférieur à 1,7% [179], [180] (**Figure 11**). L'utilisation d'autres marqueurs de monocytes tels que CCR2 et CD36, ainsi que le développement de la cytométrie de masse (CyTOF) sont des approches prometteuses [162].

A) LMMC



B) LMMC inflammatoire

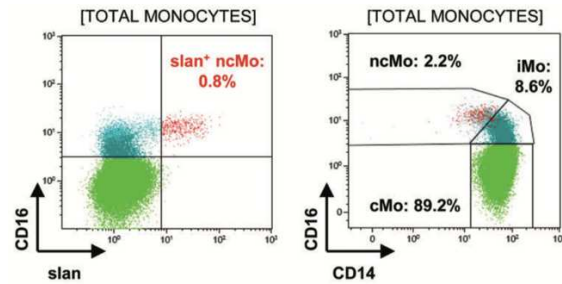


Figure 11 : Impact de l'inflammation associée à la LMMC sur les sous-populations monocytaires et le pourcentage de SLAN

(Tiré de Tarfi S et al ; 2020)

Représentation de la répartition des sous-populations monocytaires d'un patient atteint de LMMC avec un profil typique (monocytes classiques cMo > 94%) **(A)** et d'un patient atteint de LMMC avec un état inflammatoire (monocytes classiques cMo < 94% et accumulation de monocytes intermédiaires iMo) **(B)**. Dans une telle situation, la diminution de la fraction de monocytes non classiques SLAN+ en dessous de 1,7% soutient toujours le diagnostic de LMMC.

B. Traitement

La prise en charge des patients atteints de LMMC reste difficile en raison de l'âge, des comorbidités, du faible nombre d'essais cliniques dédiés spécifiquement à la maladie et de son hétérogénéité. Deux groupes de patients existent :

- Les patients à faible risque c'est-à-dire ceux qui sont asymptomatiques, ont une faible évolutivité et notamment un faible risque de transformation en une LAM. Ces patients sont sous surveillance jusqu'à l'apparition de signes cliniques de la maladie et ne reçoivent pas de traitement.
- En revanche, pour les patients à haut risque, des traitements doivent être programmés pour éviter l'évolution de la maladie ou le décès. Le traitement repose principalement sur la thérapie cytoréductrice, les agents hypométhylants et la greffe allogénique de cellules souches hématopoïétiques. Cette dernière thérapie est recommandée chez les patients jeunes et en bonne santé [181], [182].

i. Thérapie cytoréductrice

La thérapie cytoréductrice est utilisée pour traiter la leucocytose et la splénomégalie associée à la LMMC. Parmi les agents cytoréducteurs, on utilise la cytarabine, l'étoposide et l'hydroxyurée. A ce jour, l'hydroxyurée est le médicament le plus utilisé, suite à un meilleur taux de réponse et de survie par rapport à l'étoposide chez les

patients atteints de LMMC [183]. En plus de son bénéfice clinique, l'hydroxyurée est un médicament oral facile d'utilisation.

Pour améliorer l'anémie induite par l'hydroxyurée, les traitements par un agent stimulant l'érythropoïétine (ASE) ou la transfusion de plaquettes peuvent être utilisés comme dans les SMD. La répétition des transfusions de globules rouges nécessite une chélation du fer [181].

ii. Les agents hypométhylants (HMA)

Les patients LMMC ayant une forte prolifération ou une dysplasie sévère peuvent être traités par des HMA tels que l'Azacitidine (AZA) et la Décitabine (DAC). Le rôle principal des HMA est l'induction d'une hypométhylation en inhibant l'ADN méthyltransférase. Les HMA sont des traitements de fond pour les SMD et la LMMC. Seule l'azacitidine est autorisée en Europe. Les données concernant l'efficacité des HMA dans la LMMC proviennent d'étude en phase II, avec un taux de réponse de 40-50% [161], [162]. Le séquençage de l'exome entier (WES) chez les patients LMMC montre que la HMA n'ont aucun effet sur la charge allélique mutationnelle, même chez les patients répondeurs, suggérant que le clone leucémique n'est pas éradiqué [161], [184]. Chez les patients répondeurs, on observe une restauration de la balance hématopoïétique et aussi une amélioration de la répartition des sous-populations monocytaires, notamment des monocytes classiques [177] [161].

Il est donc nécessaire d'améliorer les traitements par les HMA. À cette fin, plusieurs études sont en cours afin de voir s'il faut associer les HMA avec d'autres molécules. Plusieurs associations ont été faites :

- HMA + HDAC : cela n'a pas donné de résultat concluant dans le cas de la LMMC [185].
- Azacytidine + lénelidomide : Pour cette étude, 160 patients ont été recrutés : les résultats étaient satisfaisants mais il n'y a pas eu d'amélioration des taux de réponse et de survie par rapport à l'azacytidine seule [186].
- Azacytidine + Vénétoclax (inhibiteur de Bcl-2) : l'étude en phase I a été réalisée, 23 patients ont été recrutés, l'âge médian était de 68 ans et parmi eux 78% étaient de sexe masculin. Les résultats sont encourageants, le taux de réponse global était de 87%. Dans l'ensemble, il y'a eu 3 décès dus à la septicémie, qui ont été déterminés comme n'étant pas liés au protocole. Le fait que les décès ne soient pas liés au protocole de traitement est rassurant dans le contexte de la tolérance de la thérapie. Les essais actuels évaluent plus en détail les avantages et les éventuels inconvénients de cette approche [187].

iii. Greffe de cellules souches hématopoïétiques

La transplantation de cellules souches hématopoïétiques allogéniques reste la seule option curative pour les patients atteints de LMMC [162]. Néanmoins, en raison de l'âge avancé et des comorbidités, cette option est très peu utilisée et seul environ 10% des patients atteints de LMMC sont éligibles à cette thérapie. Concernant les critères d'éligibilités pour cette transplantation : avoir une LMMC sévère et être âgé de moins de 70 ans. La survie à 5 ans est de l'ordre de 20 à 40%. La rechute est importante après la greffe (17-52%) [188], [189].

C. Modèles d'étude pour la LMMC

Le manque de modèles appropriés constitue une limitation majeure dans la recherche sur la physiopathologie de la LMMC.

Modèles murins

La présence de mutations somatiques récurrentes dans les SMD/SMP a permis la génération de nombreux modèles murins ayant des caractéristiques proches des SMD/SMP.

Les mutations de *TET2* sont considérées comme l'événement déclencheur de la LMMC. Des souris avec perte de fonction de *TET2*, développent une hémopathie myéloïde évoquant une LMMC. Ce phénotype se développe à l'âge de 2 à 4 mois. Ces souris présentent une splénomégalie, une monocytose, une thrombopénie et une anémie, évocateur d'une LMMC de type « proliférative » [190], [191].

Les études sur les souris mutées dans la voie *Ras* telles que *KRas*, *NRas*, *HRas* [192] [193], *Flt3* [194], *Shp2* [195], *Cbl* [196] et *Nf1* [197], ont fourni des informations démontrant leur association avec des caractéristiques similaires aux LMMC de type prolifératif (excès de monocytes dans le sang périphérique, une hypersensibilité à la cytokine GM-CSF et une splénomégalie)

D'autres études ont montré que la surexpression du gène *Kdm6b* suivie d'une stimulation avec des agents pro-inflammatoires (LPS/TLR) entraîne des anomalies hématopoïétiques et des caractéristiques évocatrices de la LMMC [198].

L'étude menée par Mei-Yi Wu et ses collègues en 2008 a rapporté un phénotype intéressant de type LMMC chez des souris déficientes pour *Arid4A*. ARID4A est une protéine impliquée dans la régulation de la transcription dépendante d'E2F en

interagissant avec la protéine RB (répresseur E2F) du rétinoblastome. Dans les souris knock-out *Arid4a*, les chercheurs ont observé une perturbation importante du schéma de méthylation des histones. Une des manifestations observées était la monocytose et les souris présentaient une splénomégalie. Ainsi, ces souris ont présenté des symptômes similaires à ceux observés chez les patients atteints de CMML [199].

La LMMC étant une maladie complexe et hétérogène, impliquant souvent des altérations génétiques multiples, les modèles murins monogéniques peuvent ne pas reproduire cette complexité génétique et moléculaire observée chez les patients, avec en moyenne 14 mutations (138).

Modèle de xénogreffe

Les modèles de xénogreffe de cellules de patients dans des souris immunodéficientes ont joué un rôle crucial dans l'étude des leucémies. Ces modèles permettent d'analyser l'ensemble des mutations et des altérations épigénétiques présentes dans les cellules des patients, et de mieux comprendre l'hétérogénéité clonale observée dans ces maladies. Grâce à ces modèles de xénogreffes, les chercheurs ont pu identifier et étudier les cellules souches leucémiques (CSL).

Les études réalisées par Yoshimi et ses collègues en 2017, consistaient à greffer des cellules CD34⁺ provenant de patients atteints de LMMC dans des souris NOD/SCID-II2rgnull exprimant des cytokines humaines comme le GM-CSF, IL-3, SCF. Les cellules greffées étaient capables de reproduire les caractéristiques génétiques, immunophénotypiques et morphologiques observées chez les patients d'origine, ce qui a permis de récapituler l'hétérogénéité clonale et sous-clonale présente chez ces patients. Malgré l'efficacité des transplantations primaires, les greffes secondaires n'ont été possibles que dans un nombre limité de cas. Il est important de noter que malgré ces limitations, les modèles de xénogreffes de cellules de patients restent des outils précieux pour étudier les caractéristiques de la LMMC ou d'autres leucémies [200].

Dans notre équipe, Beke *et al* en 2020 ont utilisé la technologie des cellules souches pluripotentes induites (iPSC) pour générer un modèle *in vitro* de la LMMC. Pour cela, ils ont utilisé des cellules CD34⁺ issues de patients pour générer des iPSC. Ce modèle *in vitro* a permis de récapituler une partie de l'hétérogénéité moléculaire et phénotypique observée dans la LMMC. Les CSPH générées à partir des patients ont été différenciées en cellules de la lignée myéloïde, notamment des cellules de la lignée granulocytaire et des cellules de la lignée monocytaire. Ces cellules présentaient des

caractéristiques moléculaires et phénotypiques similaires à celles observées chez les patients atteints de LMMC [201].

Conclusion générale de la partie 2 :

La LMMC est une maladie chronique rare de la personne âgée. Le diagnostic de la maladie est plutôt bien caractérisé. Elle s'accompagne de symptômes généraux (asthénie, amaigrissement, fièvres parfois...) et parfois des signes d'autoimmunité. Les traitements actuels sont utilisés pour corriger les symptômes mais, à l'exception de la greffe de CSH, ne permettent pas d'éradiquer la maladie. Il est donc crucial d'identifier des approches thérapeutiques permettant de prolonger l'expérience de vie tout en préservant la qualité de vie des patients. Il est donc important que les modèles d'études pour traiter la LMMC évoluent afin que de nouvelles thérapies soient développées.

3. TET2 et ses mutations

La méthylation de l'ADN est générée par les ADN méthyltransférases (DNMT), qui transfèrent un groupe méthyle à la position 5 des bases de cytosine [202], [203]. La déméthylation est un processus important dans le développement et la différenciation des cellules, car elle permet de réguler l'activation ou la désactivation des gènes.

Les protéines TET (TET1, TET2 et TET3), abréviation de *ten-eleven translocation*, sont des dioxygénases dépendantes du fer {Fe(II)} et de l' α -cétoglutarate (α -KG) qui permettent la déméthylation active de l'ADN par la conversion de la 5-méthylcytosine (5-mC) en 5-hydroxyméthylcytosine (5-hmC) puis en 5-formylcytosine (5fC) et 5-carboxylcytosine (5caC) [204], [205] (**Figure 12**). Ces protéines jouent un rôle important dans la régulation de l'expression génique en déméthylant l'ADN.

Le développement de nouvelles techniques de séquençage a permis l'identification de plusieurs gènes mutés dans les cancers myéloïdes dont *TET2* [206]–[208], qui est l'un des gènes les plus mutés dans ces maladies et le plus muté dans la LMMC.

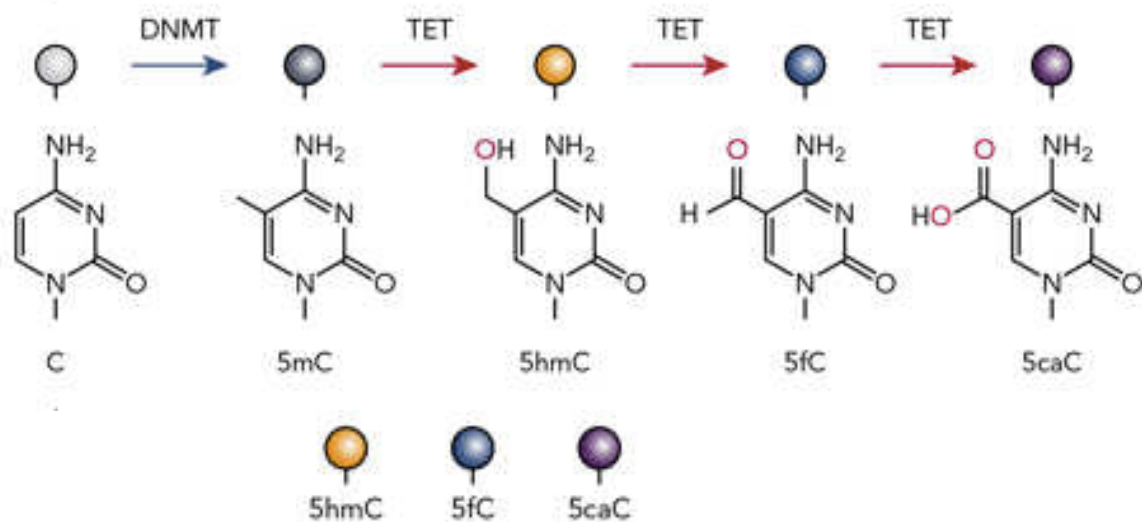


Figure 12 : Enzymes TET dans le processus de déméthylation de l'ADN

(Tiré de Lio CJ, Yuita H, Rao A, Blood, 2019)

Les cytosines non modifiées dans l'ADN sont méthylées par les DNMT pour donner 5 mC. Les protéines TET oxydent successivement 5mC en 5hmC, 5fC et 5caC.

A. TET2 : Ten-eleven translocation 2

TET2 est un gène suppresseur de tumeur qui est localisé sur le chromosome 4q24.1. Il contient 11 exons sur une séquence de 96kb et la protéine est constituée de 2002 acides aminés et peut être synthétisée sous 3 formes différentes. La protéine TET2 est composée d'une extrémité C-terminale où on trouve le domaine catalytique, qui est composé d'un domaine riche en cystéine et d'un domaine en hélice beta double brin (**Figure 13**).

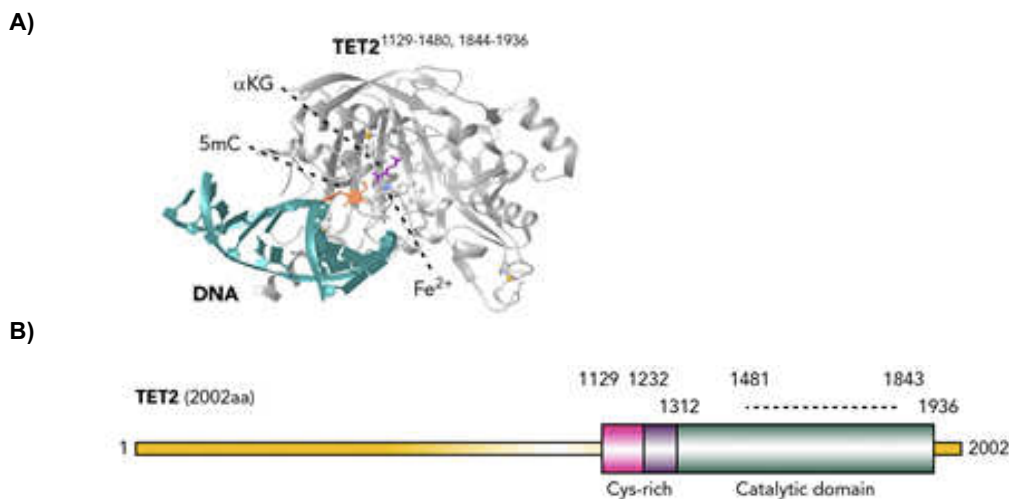


Figure 13 : Enzyme TET2

(Tiré de Lio CJ, Yuita H, Rao A, Blood, 2019)

A) Interaction TET2-ADN. Le diagramme montre une partie du domaine catalytique TET2 (gris ; acides aminés [aa]1129-1480, 1844-1936) interagissant avec 5mC (orange) sur l'ADN double brin (turquoise). **B)** Représentation schématique de la structure du domaine de TET2, y compris le domaine riche en cystéine (rose et violet : riche en cys ; aa1129-1312) et le domaine catalytique (vert ; aa1313-1936).

B. Rôle de TET2 dans l'hématopoïèse

Les 3 protéines TET sont exprimées dans le tissu hématopoïétique murin. TET1 est la moins abondante en termes d'expression [209]. TET2 et TET3 sont exprimées de manière ubiquitaire dans le compartiment hématopoïétique. Cependant, il a été observé que TET3 est moins exprimé dans les cellules différenciées par rapport aux CSH [191], [210]. Des études où le gène *Tet2* a été invalidé, ont suggéré que TET2 est responsable d'environ 60% de l'activité de l'ADN dioxygénase dans les cellules souches progénitrices et hématopoïétiques (CSPH) mettant en évidence l'importance cruciale de Tet2 dans la régulation du processus hématopoïétique [211].

TET2 joue un rôle important dans l'hématopoïèse en favorisant l'auto-renouvellement des cellules souches [212]. Plusieurs études ont montré l'impact de la perte de TET2 dans l'hématopoïèse [213]. Des expériences réalisées au sein du laboratoire ont montré que la suppression de TET2 dans les cellules CD34⁺ de sang de cordon favorise la différenciation vers la lignée monocyttaire, des résultats similaires ont été observés chez la souris [214] (**Figure 14**). Les travaux sur des cellules primaires issues de patient SMP/SMD et de souris ont montré l'impact de la perte de *TET2/Tet2* dans la progression des tumeurs malignes myéloïdes et lymphoïdes [191], [215], 90% des souris *Tet2*^{-/-} ont développé des néoplasmes prolifératifs myéloïdes de types LMMC [190]. Ces observations identifient TET2 comme suppresseur de tumeur dans le tissu hématopoïétique.

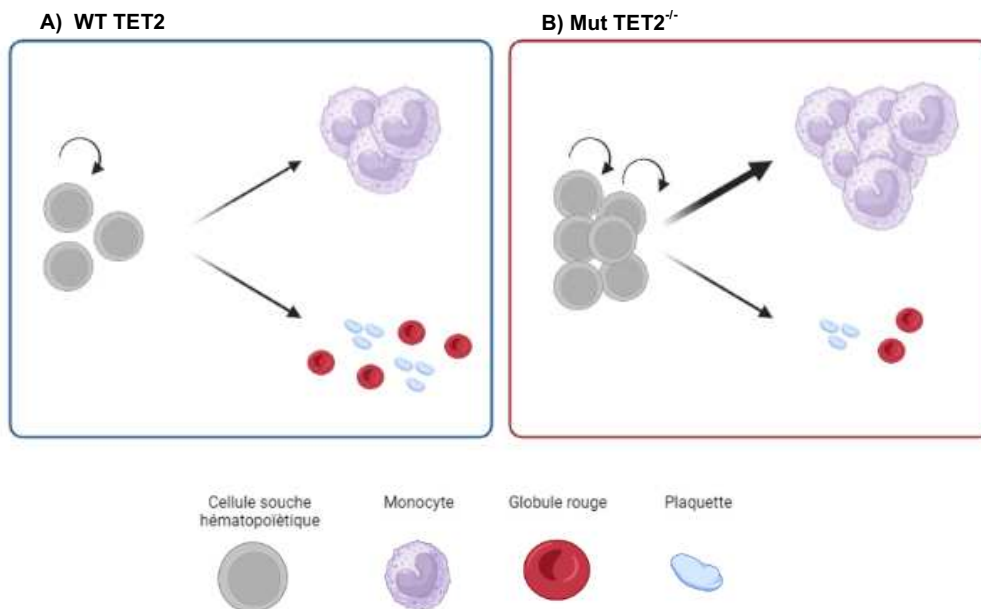


Figure 14 : La perte de TET2 entraîne un auto-renouvellement aberrant des CSH et une différenciation hématopoïétique altérée

A) Les cellules souches hématopoïétiques normales (CSH) maintiennent un auto-renouvellement coordonné et une différenciation hématopoïétique équilibrée, contribuant à l'homéostasie sanguine.
B) Les CSH déficientes en *Tet2* montrent un auto-renouvellement amélioré et une différenciation hématopoïétique altérée, y compris une distorsion myéloïde et une érythropoïèse perturbée.

L'expression de TET2 peut être régulée par différents composants tels que des micro-ARN (miR-125, miR101, miR22), des petites molécules (Vitamine C, Fe(II)) et des facteurs de transcription (EBF1, WT1) [216]. Dans plusieurs études il a été montré que les microARN régulent négativement l'expression du gène *TET2*, environ 30 miARN ont été identifiés comme étant capable d'inhiber l'expression de *TET2* [217]. Par exemple,

dans la leucémie aiguë myéloïde certains microARN comme mir-22 et miR-29 régulent négativement le messager *TET2* [218].

Les mutations de *TET2* ont été identifiées dans diverses pathologies hématologiques, notamment dans les SMD (avec 20-35%) [219], la LMMC (60%) [163] et dans la LAM (10-20%) [220]. Les mutations de *TET2* sont très fréquentes dans la LMMC et sont considérées comme des événements initiateurs dans le développement de la maladie. De manière intéressante, des mutations inactivatrices de *TET2* ont également été retrouvées dans l'hématopoïèse clonale à potentiel indéterminé (CHIP), impliquant une expansion clonale aberrante des cellules souches et progénitrices [221]. Ces données suggèrent une mutation clonale précoce dans le compartiment souche. Tet2 seul est capable de former une prédisposition leucémogène. Cependant, des mutations supplémentaires sont requises pour une progression maligne complète [191]. Dans le cas où Tet2 est le clone dominant, les mutations additionnelles sont définies par la présence de *SRSF2*, *ASXL1*, *DNMT3A* et *EZH2* [222] (**Figure 15**). Ce sont ces mutations en cascade qui vont contribuer à l'évolution de la maladie.

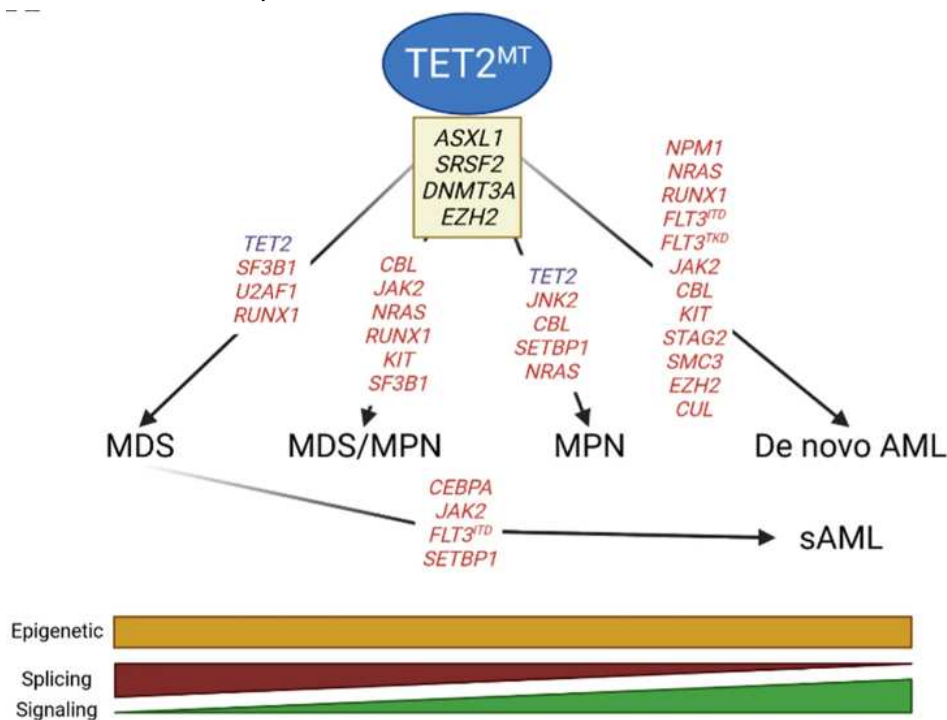


Figure 15 : Mutations génétiques concomitantes des tumeurs malignes myéloïdes humaines TET2 muté

(Tiré de Joshi, K., Zhang, L., Breslin SJ, P. et al, *J Exp Clin Cancer Res*, 2022)

Les mutations de *ASXL1*, *SRSF2*, *DNMT3A* et *EZH2* concourent dans tous les types de tumeurs malignes myéloïdes avec les mutations de *TET2*. Les mutations du second allèle de *TET2* sont couramment détectées dans les SMD et les MPN, mais pas dans la LAM *de novo*. De plus, des mutations dans des facteurs d'épissage tels que *SF3B1* et *U2AF1* sont couramment détectées dans les SMD, tandis que des mutations de molécules de signalisation sont couramment détectées chez les patients atteints de MPN et de LAM.

Plusieurs types de mutations *TET2* ont été décrites telles que les mutations faux sens, non-sens, de décalage du cadre de lecture et des délétions/insertions. Dans la plupart des cas, les mutations de *TET2* induisent une protéine tronquée dans le domaine catalytique [215], [223].

Dans certaines pathologies, les mutations de *TET2* ont été identifiées comme des marqueurs pronostiques. Dans la leucémie aiguë myéloïde (LAM), les mutations de *TET2* sont associées à un mauvais pronostic [224]. Dans une étude sur une cohorte de patients atteints de SMD, il a été observé que ceux qui présentaient des mutations de *TET2* avaient tendance à montrer une meilleure réponse au traitement par agents hypométhylants par rapport aux patients sans mutations de *TET2* [225].

Des mutations dans le gène *TET2* ont été identifiées chez un pourcentage significatif de patients atteints de LMMC. Une étude publiée en 2020 a montré un impact pronostique favorable de la perte de fonction de *TET2* dans la LMMC mais pas dans les LAM, MDS, PV, et DLBCL [226]. Les patients présentant des mutations de *TET2* ont été associés à des caractéristiques spécifiques, telles qu'un âge plus avancé, une sous-catégorisation plus élevée de CMML-1, des numérations leucocytaires et plaquettaires plus basses, une augmentation des blastes dans le sang périphérique et la moelle osseuse, des taux d'hémoglobine plus élevés, une charge mutationnelle tumorale plus élevée et une fréquence accrue de mutations d'épissage, ainsi que des anomalies caryotypiques [227]. Dans la LMMC, les patients porteurs de mutations *TET2* ont démontré un avantage en terme de survie (confirmé par deux études cliniques) [163], [167]. Pris ensemble, ces données soulignent l'importance de l'hétérogénéité biologique de *TET2* et suggèrent l'existence de mécanismes supplémentaires impliqués dans la leucémogénèse.

4. Le facteur de transcription p53

TP53 (ou *Tumor Protein 53*) est un gène qui code pour une protéine appelée p53. Cette protéine a été décrite par David Lane comme le « gardien du génome » en raison de son rôle crucial dans la régulation de la division cellulaire et la prévention de la formation de tumeurs. La protéine p53 agit comme un suppresseur de tumeur en surveillant l'intégrité de l'ADN dans les cellules. En cas de dommages à l'ADN, tels que des mutations ou des lésions, p53 est activée et déclenche une série de réponses cellulaires afin de réparer, si possible, ces dommages et si la réparation n'est pas possible, induire la mort cellulaire des cellules. Le suppresseur de tumeur *TP53* est le gène le plus fréquemment muté dans les tumeurs solides humaines.

A. L'histoire de p53

Un virus nommé SV40 a été découvert pour la première fois en 1960. Ce virus a suscité un intérêt particulier en raison de sa capacité à transformer les cellules normales en cellules tumorales. Il a été montré que dans les cellules transformées par le virus SV40, l'antigène T de ce virus est capable de fixer et de stabiliser la p53 *in vivo*. L'infection des cellules de souris par le SV40 a été associée à deux nouvelles espèces d'antigènes : i) le premier une protéine d'une taille de 130 kDa qui a été détectée que dans certaines cellules, ii) le deuxième une protéine d'une taille de 56 kDa qui a été observée dans toutes les cellules infectées par le virus [228]. En 1979, la protéine de 56 kDa a été nommée *p53* en raison du poids moléculaire de la protéine correspondante, obtenue après analyse par électrophorèse sur gel de polyacrylamide [229], [230]. Cette estimation était fautive en raison de la présence d'une région riche en proline dans la protéine p53 qui altère sa migration dans le gel SDS. Le poids moléculaire réel de p53 est d'environ 43,7 kDa. Après sa découverte, p53 est devenue un sujet d'intérêt dans la recherche scientifique de nombreux secteurs. Cependant, en raison du manque d'un nom officiel et de différentes appellations utilisées par différentes institutions, une confusion a eu lieu dans la littérature scientifique. Pour remédier à cela, en 1983, lors du 1^{er} atelier international sur p53 qui s'est tenu à Oxted, au Royaume-Uni, des scientifiques de divers groupes de recherche et pays ont collaboré pour établir une nomenclature commune pour cette protéine nouvellement découverte. Lors de cette réunion, il a été décidé d'utiliser le nom « p53 » pour désigner cette protéine [231]. L'analyse de l'ADNc de *TP53* dans les premières études ont mis en évidence une mutation de *TP53* [232] et c'est ainsi que *TP53* est devenu un gène suppresseur de tumeur au lieu d'un oncogène. Confirmant cette activité suppresseur de tumeur, la ré-expression de p53 sauvage dans des cellules d'ostéosarcome déficientes pour son

expression a permis de changer le phénotype de ces cellules qui ont perdu certaines caractéristiques cancéreuses [233].

B. Caractéristiques transcriptomiques et biochimiques de *TP53*

i. Structure du gène

Le gène *TP53* est situé sur le bras court du chromosome 17 humain (17p13.1) et s'étend sur environ 20kb. Ce gène est composé de 11 exons. Alors que l'exon 1 est un exon non codant, mesurant entre 8 et 10kb, les exons 2 à 11 contiennent les informations génétiques nécessaires à la synthèse de la protéine p53 fonctionnelle. L'exon non codant peut jouer un rôle dans la régulation de l'expression du gène *TP53* et peut contenir des éléments de contrôle importants pour son activation et sa fonction. En plus du promoteur classique situé en amont de l'exon 1, il existe également un promoteur interne situé dans l'intron 4 du gène. Cependant, le promoteur classique (P1/P1') est le site d'initiation de la transcription le plus couramment utilisé. Le promoteur interne de l'intron 4 (P2) est moins couramment utilisé mais peut aussi servir de site d'initiation de la transcription dans certaines conditions. La présence de ce promoteur interne ajoute un niveau supplémentaire de complexité à la régulation de l'expression du gène *TP53*. Le gène *TP53* peut aussi avoir des épissages alternatifs avec l'utilisation de promoteurs alternatifs, ce qui permet la production d'au moins 8 ARNm différents. Ces différents transcrits résultent de l'inclusion ou de l'exclusion de certains exons ou de l'utilisation de sites d'épissage alternatif [234] (**Figure 16**). Parmi les membres de la famille p53, on retrouve les protéines p63 et p73. Ces protéines partagent une homologie de séquence avec la protéine p53, en revanche elles ont des fonctions distinctes [234], [235].

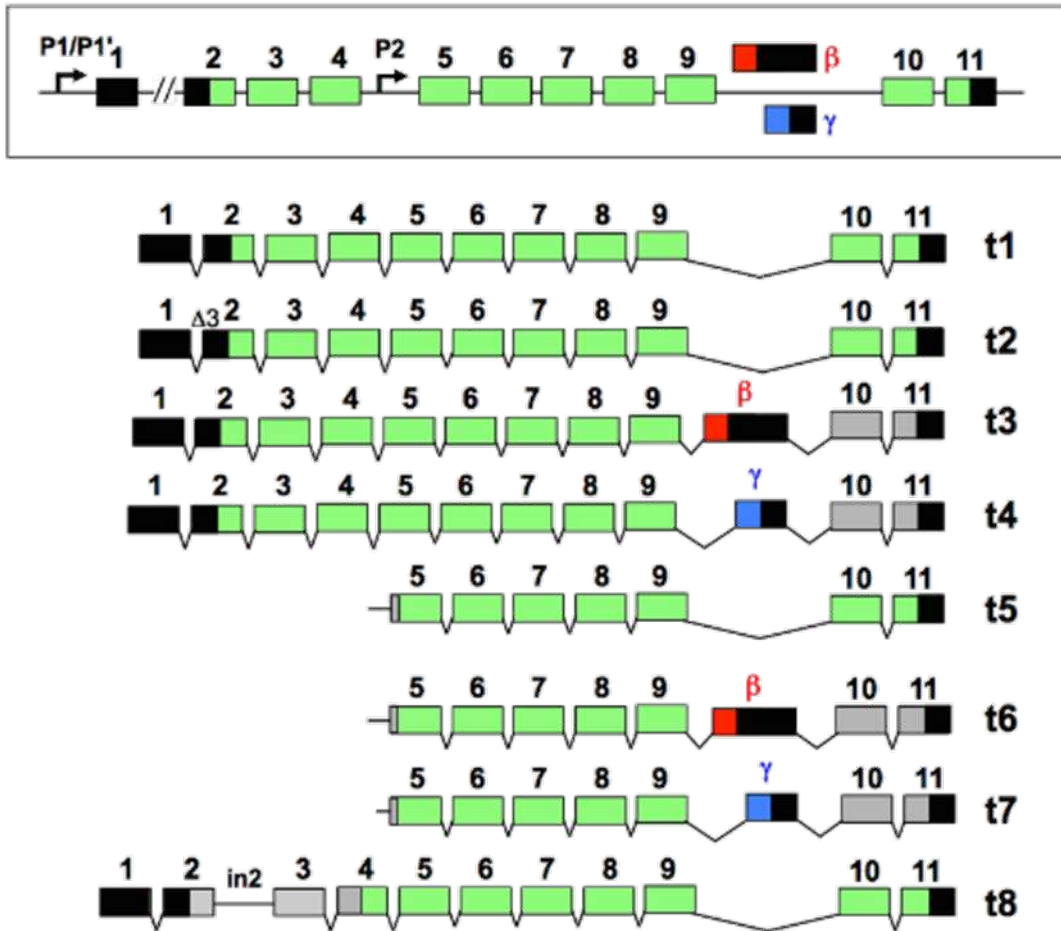


Figure 16 : TP53, un gène et au moins huit transcrits

(Tiré de Soussi Thierry et al, Human Mutation, 2014)

Le gène TP53 est transcrit en huit ARNm différents. Les transcrits t1 à t4 proviennent des promoteurs P1 et P1' localisés en amont du gène. Les transcrits t5 à t8 proviennent du promoteur P2 localisé dans l'intron 4. En vert, on trouve les exons qui peuvent être traduits. Les deux nouveaux exons β et γ sont représentés respectivement en rouge et bleu. Les exons non traduits sont représentés en noir. Pour les transcrits t3, t4, t6 et t7, qui incluent l'exon β ou γ , les exons 10 et 11 sont non codants (cases grises). Le transcrit t8 ne code que p8 (DeltaTP53 α) et les exons 1 à 3 sont non codants (cases grises).

ii. Structure de la protéine

La protéine p53 contient trois domaines structuraux (**Figure 17**) :

- Le domaine N-terminal (N-Terminus) est composé **i)** d'un domaine de transactivation (TAD), important pour activer des facteurs de transcription et **ii)** d'un domaine riche en proline (Pro), important dans l'activité apoptotique [236].
- Le domaine de liaison à l'ADN (DBD) : domaine clé permettant à la protéine p53 de se lier spécifiquement à l'ADN [237]. Il reconnaît des séquences

spécifiques appelées éléments de réponse p53 (RE) présentes dans les promoteurs des gènes cibles.

- Le domaine C-terminal (C-Terminus) est composé i) d'un domaine de tétramérisation (TD), jouant un rôle dans la régulation de la stabilité de la protéine p53, sa localisation cellulaire et ses interactions avec d'autres partenaires protéiques et ii) d'un domaine avec une région basique (RD) dans les 30 derniers acides aminés [238], [239].

Des altérations génétiques dans ces différents domaines peuvent affecter la fonction de p53, ce qui peut conduire à des dysfonctionnements cellulaires et à la prédisposition au développement de cancers.

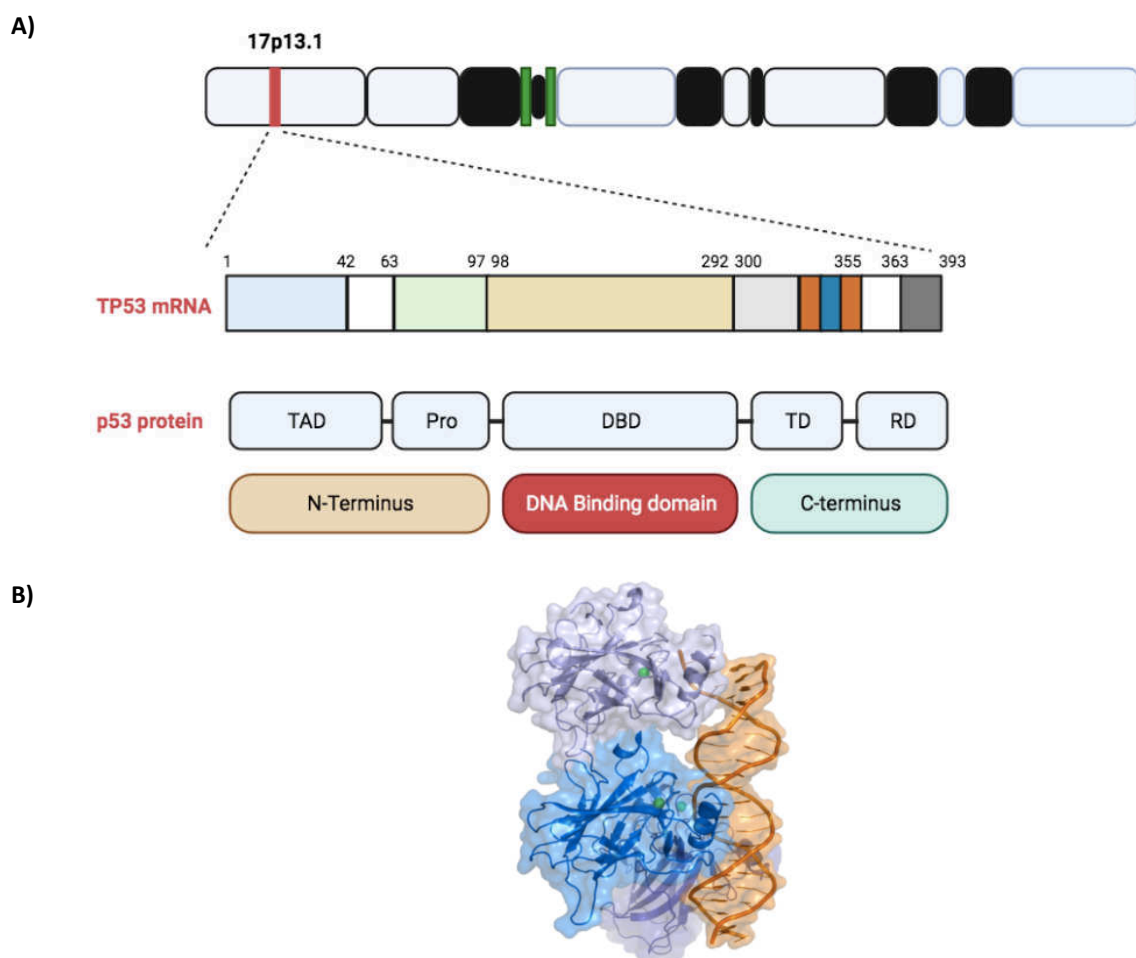


Figure 17 : Structure du gène et de la protéine humaine p53

(Tiré de Babamohamadi M et al, *Front. Mol. Biosci*, 2022)

A) Le gène *TP53* est localisé sur le chromosome humain 17p13.1. p53 est composée de 393 résidus d'acides aminés et possède un domaine de transactivation (TAD), un domaine riche en proline (Pro), un domaine de liaison à l'ADN (DBD), un domaine de tétramérisation (TD) et un domaine basique (RD).

B) Structure RMN (résonance magnétique nucléaire) de la protéine p53 en 3 dimensions. Tiré de *Protein data bank in Europe*

iii. Régulation de p53

De nombreux événements intrinsèques ou extrinsèques peuvent engendrer des modifications post-traductionnelles de p53, avec des phosphorylations, des méthylations, des acétylations, des ubiquitinations ou encore des sumoylations de certains acides aminés. Voici quelques exemples de ces modifications post-traductionnelles :

- Phosphorylation : les sites de phosphorylation de p53 sont généralement retrouvés au niveau des domaines C- et N-terminaux. Suite à un stimulus, p53 est phosphorylée au niveau de certaines sérine (Ser15 et Ser20) ou de la thréonine (Thr18). Cette phosphorylation est faite par la kinase ATM et entraîne un changement conformationnel qui permet sa stabilisation en inhibant l'affinité de p53 pour MDM2 (régulateur négatif de p53) [240]. La phosphorylation d'autres résidus par d'autres kinases permet la liaison de p53 à l'ADN.
- Méthylation : au niveau de l'extrémité carboxy-terminale, lors d'un stress, SET9 méthyle la lysine en position 372, entraînant la stabilisation de p53 [241]. Les fibroblastes de souris déficients pour Set9 n'expriment pas les gènes cibles de p53, *p21* et *Puma* [242].
- Acétylation : l'acétylation de p53 se déroule selon un processus réversible par des enzymes coactivatrices appelées histones acétyltransférases (HAT), telles que p300/CBP (Lys373, Lys382) et PCAF (Lys320). P300/CBF et PCAF se lient et acétylent p53 ce qui entraîne la trans-activation de p53 lors d'un stress [243], [244].
- Ubiquitination : l'ubiquitinylation de la lysine 48 de p53 par E4F1 permet l'arrêt du cycle cellulaire. En revanche, l'ubiquitinylation de p53 par le dimère MDM2/MDM4 conduit à sa dégradation par le protéasome [245], [246].
- Sumoylation : l'ajout du peptide SUMO sur la lysine 386 de p53, module positivement ou négativement la fonction transcriptionnelle de p53 [247].

iv. Fonctions de p53

La protéine p53 peut être présente dans le noyau et/ou le cytoplasme des cellules où elle joue un rôle important dans de nombreux processus cellulaires [248], [249] (**Figure 18**). Dans des conditions normales, l'expression de p53 dans la cellule est très faible en raison d'une dégradation par ubiquitinylation rapide par ses régulateurs négatifs tels que MDM2 et MDM4. Lors d'un stress, la protéine p53 est activée, stabilisée et

s'accumule dans la cellule. Cette accumulation est accompagnée par une liaison de p53 à l'ADN où elle régule la transcription de ses gènes cibles [250].

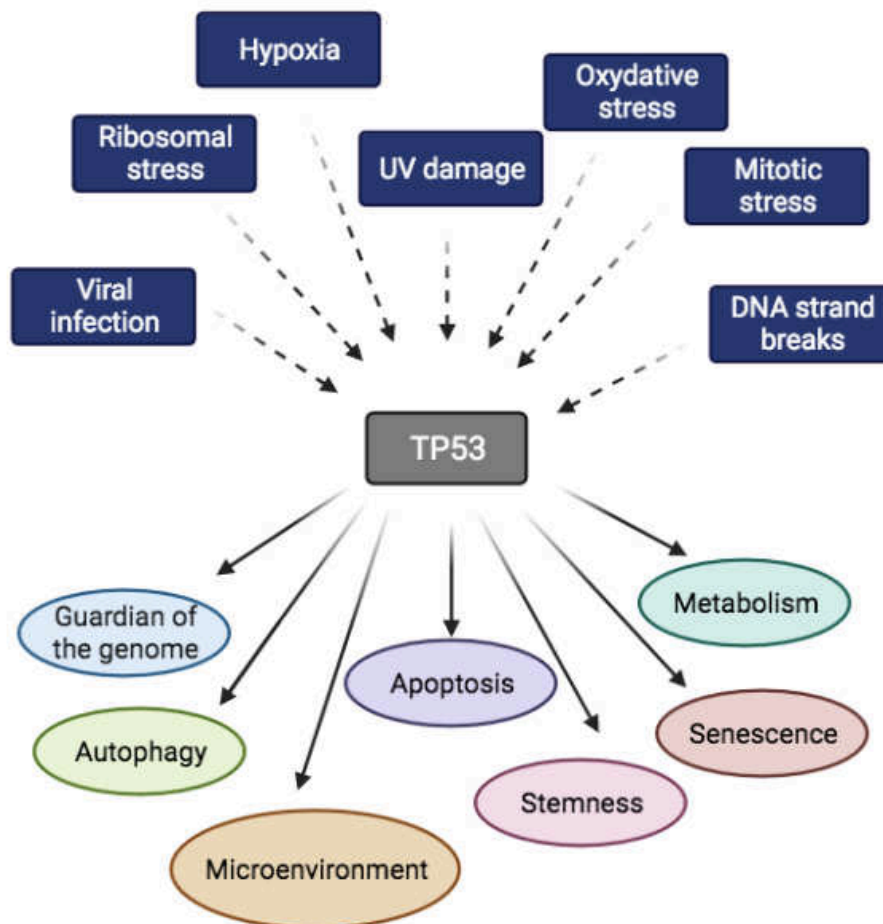


Figure 18 : p53 est une protéine centrale des réponses au stress

La protéine p53 est un pivot central qui régule l'expression de milliers de gènes, suite à un stress (infection virale, stress ribosomal, hypoxie, dommages aux UV, stress oxydatif, stress mitotique, cassures de l'ADN), qui contrôlent sa surveillance du génome, l'autophagie, l'apoptose, le microenvironnement, les cellules souches, la sénescence et le métabolisme.

P53 le « gardien du génome »

P53 est souvent surnommé « le gardien du génome » en raison de son rôle essentiel dans la surveillance et la préservation de l'intégrité de l'ADN. En effet, lorsque l'ADN subit des dommages, tels que des lésions ou des mutations, p53 est activée. Suite à cette activation, p53 induit l'arrêt du cycle cellulaire, répare l'ADN ou déclenche une mort cellulaire programmée par apoptose. L'arrêt du cycle passe par la fixation de p53 activée sur le promoteur du gène *CDKN1A* (p21) et permet son expression. p21 est un inhibiteur de kinases dépendant des cyclines (CDKs). Les CDKs sont des enzymes

impliquées dans la régulation du cycle cellulaire suite à diverses étapes de phosphorylation. Cette protéine se lie aux complexes CDK-cycline et inhibe leur activité kinase, ce qui entraîne l'arrêt du cycle cellulaire en phase G1/S et G2/M (**Figure 19**). Ce mécanisme permet la réparation de l'ADN endommagé avant la réplication et ainsi permet de réduire la propagation de mutations souvent oncogéniques [251]. Une fois l'ADN réparé, p53 induit l'expression de MDM2, qui à son tour dégrade p53 et permet aux cellules de passer le point de contrôle, en absence de p21, et d'entrer à la phase suivante. Des études sur des souris déficientes pour p21 ont montré qu'elles développent moins de tumeurs, ce qui suggère que p53 agit sur d'autres cibles (tels que p27, p16), qui sont impliquées dans la régulation du cycle cellulaire [252], [253].

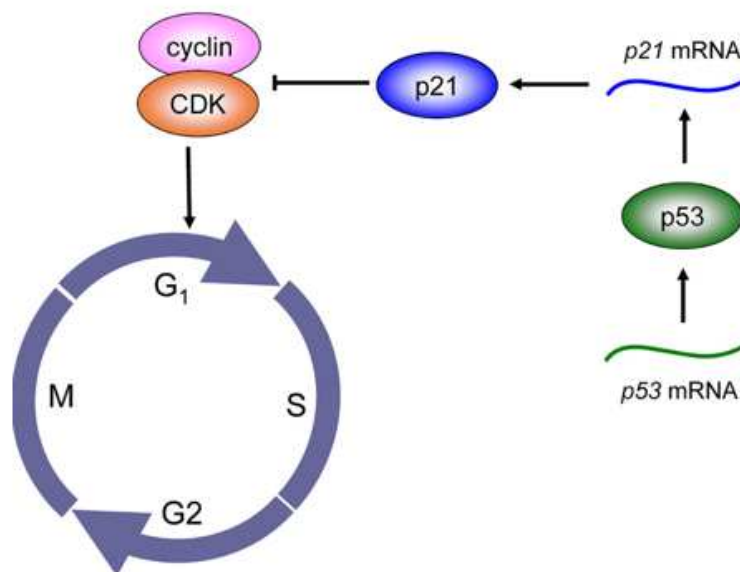


Figure 19 : Régulation du cycle cellulaire par la voie de signalisation p53-p21

(Tiré de Li-Na Zhang, Yong-Bin Yan, BBA, 2015)

Le suppresseur de tumeur p53 induit l'arrêt du cycle cellulaire en se fixant sur le promoteur du gène *CDKN1A* (p21) et induit son expression protéique. La surexpression de la protéine p21 conduit à l'inactivation de CDK et à l'arrêt du cycle cellulaire.

P53 et apoptose

L'apoptose est un processus de mort cellulaire programmée qui joue un rôle dans le développement et l'homéostasie des tissus. C'est un mécanisme complexe régulé par plusieurs voies de signalisation. L'apoptose se fait en plusieurs étapes : d'abord, la cellule se rétracte, ce qui entraîne une condensation du noyau et une fragmentation de l'ADN. Cela crée des corps apoptotiques qui sont ensuite éliminés par des cellules phagocytaires comme les macrophages. La fragmentation de l'ADN se fait via la sécrétion de protéases à cystéine-aspartique (caspases) [254], [255].

La capacité de p53 à réguler la mort cellulaire programmée contribue également à son rôle de suppresseur de tumeur. Lorsque des cellules subissent des dommages de l'ADN, p53 s'active et régule les voies apoptotiques. En effet, p53 a pour gènes cibles des gènes pro-apoptotiques tels que *BAX*, *PUMA*, *NOXA*, *BID* ou *FAS*. p53 est également capable d'inhiber l'expression des gènes codant pour les protéines anti-apoptotiques comme *BCL-2* ou permettant l'expression de microARNs [256] qui sont impliqués dans la répression des protéines pro-apoptotiques [257], [258]. p53 peut également interagir directement avec les membres pro-apoptotiques et anti-apoptotique dans le cytoplasme et la membrane des mitochondries [248], [259].

La sénescence par p53

La sénescence consiste à un arrêt du cycle cellulaire où les cellules cessent de se diviser en entrant dans un état de vieillissement. La sénescence est corrélée avec la diminution des télomères. Lorsque les télomères atteignent une longueur critique, ils déclenchent une réponse de signalisation qui active p53. Cette activation conduit à l'arrêt du cycle et à l'induction de la sénescence cellulaire [260]. D'autres types de stress (UV, agents chimiques...) ou des dommages à l'ADN, activent la voie p53, qui à son tour active p21 ce qui entraîne l'arrêt du cycle et la sénescence [261]. Il a également été montré que p53 peut induire directement la sénescence en stabilisant l'inhibiteur de l'activateur du plasminogène-1, qui est un marqueur de la sénescence répllicative [262].

En induisant la sénescence cellulaire, p53 joue un rôle clé dans la suppression de la prolifération de cellules endommagées et potentiellement cancéreuses. Il aide à prévenir la propagation de cellules qui pourraient avoir accumulées des mutations génétiques nuisibles, pouvant conduire au développement de tumeurs.

P53 dans la différenciation

Bien que moins bien étudié, p53 peut jouer aussi un rôle important dans la régulation de la différenciation cellulaire. Voici quelques exemples :

- Il a été montré que bloquer l'activité de p53 par l'introduction d'une protéine p53 dominante négative entrave la différenciation des neurones (sur des cellules d'oligodendrocytes, des neurones de rat et de lignée cellulaire PC12), et protège les neurones de la mort par apoptose. Dans les cellules PC12, l'activation de p53 entraîne l'arrêt de la croissance et la différenciation cellulaire des neurones via des cibles de p53 telles que *Wnt7b* et *Grhl3* [263], [264]. D'autres travaux ont montré que la suppression de p53 et PTEN (cible de p53) dans le système nerveux central de souris a abouti au développement de gliomes. Cette double inactivation a favorisé un auto-renouvellement accru des cellules souches neuronales et les a maintenues dans un état non différencié [265]. Ces observations impliquent le rôle direct de p53 dans le développement neuronal.
- Il a été montré que p53 agit en tant que régulateur négatif de la différenciation des ostéoblastes, du développement squelettique et du remodelage osseux. Des études menées par Wang *et al* [266] ainsi que par Legner *et al.* [267] ont révélé que les ostéoblastes de souris dépourvues de p53 présentent une différenciation accélérée, entraînant une augmentation du taux de formation osseuse et de densité osseuse.
- Il a été montré qu'au cours de la différenciation musculaire, la protéine p53 est stabilisée et coopère avec la protéine MyoD pour induire la transcription du gène *MCK* (créatine kinase musculaire). Le gène *MCK*, dont la transcription est stimulée pendant la différenciation musculaire, possède des sites de liaison pour la protéine p53. La diminution de l'expression de p53 dans ces cellules musculaires a entraîné une diminution de l'expression du gène *MCK* [268].

Ces études suggèrent que p53 peut agir de manière différente en fonction du type de cellule.

C. Mutations TP53 dans les tumeurs solides

p53 est l'une des protéines suppresseurs de tumeur les plus étudiées. L'analyse du génome de différentes cellules cancéreuses humaines a montré que dans environ 42% des cas, des mutations de *TP53* sont observées [269]. Le type de mutation le plus

courant dans le gène *TP53* est une mutation faux sens qui correspond à un changement d'un acide aminé qui peut entraîner une altération de la fonction de la protéine [270], [271]. Les mutations *TP53* sont largement présentes dans les tumeurs. Cependant, chaque tissu ou organe présente une prédisposition différente aux mutations de *TP53*. Les mutations de *TP53* sont fréquentes dans l'ovaire (47%), le côlon et le rectum (45%), le poumon (40%) et le sein (27%) (<https://cancer.sanger.ac.uk/cosmique>). Il existe également des différences dans les mutations de *TP53* selon la provenance des populations. Par exemple, la fréquence des mutations de *TP53* dans le cancer du sein est plus importante chez les Asiatiques (43%) que chez les Caucasiens (30%) [272].

Parmi les mutations observées, 80% sont cartographiées sur le domaine de liaison à l'ADN [273], [274] et environ 30% de ces mutations surviennent dans six points chauds (hotspot) : R175, G245, R248, R249, R273 et R282 [273], [275]. Ces mutations sont incapables d'activer les gènes cibles de p53 et d'inhiber la tumorigenèse. En plus de perdre leur fonction normale, certaines formes mutantes de p53 acquièrent de nouvelles propriétés oncogéniques. Ces propriétés oncogéniques acquises sont appelées propriétés de « gain de fonction ». Cela signifie que ces mutations confèrent à la protéine p53 des propriétés qui favorisent la formation de tumeurs. Ce terme a été employé suite à une transfection, chez les souris n'exprimant pas *Trp53*, d'un p53 mutant qui a provoqué le développement de tumeurs [276], [277]. Depuis lors, de nombreuses recherches ont mis en évidence les propriétés oncogéniques de certains variants de la protéine p53. Par exemple, des altérations génétiques dans *TP53* ont été corrélées à une résistance accrue aux médicaments [278], [279], et cela pourrait découler de l'activation de MDR-1 [280], [281]. Un autre mécanisme clé, conférant une nouvelle fonction, est la capacité des mutants de p53 à se lier et à désactiver les autres membres de la famille p53 comme p63 et p73 [282]. Des expérimentations menées sur des modèles murins ont clairement démontré que p63 et p73 peuvent partiellement compenser la perte de p53 [266]. En conséquence, l'inhibition de leur fonction est considérée comme un mécanisme par lequel les mutants de p53 acquièrent une fonction d'oncogène [283].

Les mutants « non-hotspot » et qui se retrouvent un peu partout représentent 70% de toutes les mutations faux-sens dans *TP53*, formant la majorité des altérations constatées chez les patients. L'effet majoritaire de ces mutations dans *TP53* est la perte des fonctions caractéristiques de p53 sauvage. Cela favorise le développement tumoral en inhibant les fonctions antitumorales, telles que l'arrêt du cycle cellulaire et l'apoptose [270]. Certaines de ces mutations localisées dans le domaine de liaison à l'ADN empêchent l'apoptose via la perturbation de la formation des complexes BCL-XL/p53 [284].

L'accumulation des mutants de p53 dans le cytoplasme peut également inhiber l'autophagie et favoriser la survie cellulaire [285]. Par contre, certaines mutations de p53, comme la mutation K382R, inhibent la capacité de p53 à supprimer l'autophagie [286].

Les mutations *TP53* créent un environnement propice à la formation de tumeurs. En effet, en absence d'une protéine p53 fonctionnelle, les cellules cancéreuses échappent aux mécanismes de surveillance et de réparation de l'ADN et elles présentent une résistance accrue à la mort cellulaire programmée.

D. P53 dans l'hématopoïèse

P53 dans la génération des cellules hématopoïétiques matures

Des travaux ont également montré que p53 pouvait être impliquée dans la différenciation des cellules souches hématopoïétiques. L'introduction de p53 de type sauvage dans la lignée cellulaire pré-B, déficiente pour l'expression de p53, a conduit à l'activation de l'expression de la chaîne lourde des immunoglobulines et du marqueur de surface B220, ce qui a induit la maturation des cellules pré-B précoces en cellules B matures [287].

Un rôle de p53 dans la différenciation érythroïdique a également été démontré dans les cellules K562 et dans les cellules d'érythroleucémie de Friend. Rotter et al, ont montré le rôle de p53 dans les étapes finales de la différenciation des érythroblastes (normoblastes). En effet, l'apoptose dépendante de p53 entraîne une condensation nucléaire et une expulsion du noyau sans mort cellulaire, poussant les cellules vers des stades terminaux de différenciation [288]. L'équipe de Fontenay et ses collègues a montré que le moment de l'extinction de la biogenèse des ribosomes et de l'activation de p53 est crucial pour le développement des érythrocytes. L'activation de p53 entraîne l'expression de gènes cibles impliqués dans la régulation de la différenciation terminale érythroïde [289].

L'expression de p53 a été associée à la régulation positive de la différenciation myéloïde. Les équipes de Sodu [290] et Banerjee [291] ont montré que l'introduction de p53 de type sauvage dans les cellules HL-60, qui sont déficientes en p53, peut induire leur différenciation vers les voies granulocytaires ou monocytaires. Une analyse plus approfondie des cellules HL-60 a montré que des niveaux élevés de p53 de type

sauvage induisent l'apoptose tandis que la différenciation de ces cellules est induite par de faibles niveaux de p53 [292].

p53 dans l'hématopoïèse normale

Des études récentes ont lié p53 au processus d'auto-renouvellement ainsi qu'au maintien de la quiescence des CSH [293]. L'analyse de souris déficientes pour *Trp53* a montré une augmentation de 2 à 3 fois de leur population de CSH [294]. Cette prolifération non-contrôlée des CSH en absence de p53 pourrait entraîner une accumulation de mutations et provoquer l'apparition de tumeurs hématologiques. Une analyse à long terme des CSH chez les souris p53^{-/-} n'est pas envisageable, du fait d'une tumorigénèse précoce et la mort des souris [295]. Il a également été montré que la perte de p53 en coopération avec les mutations *Kras* entraîne un auto-renouvellement des CSH et des cellules progénitrices myéloïdes murines avec une proportion à se transformer en cellules initiateurs de leucémie [296].

p53 sauvage, en réduisant le niveau d'expression des ROS, protège les CSH des dommages et des mutations de l'ADN [297] [298]. Cette fonction de p53, pourrait expliquer le développement de tumeurs hématopoïétiques chez les souris p53^{+/-} et/ou p53^{-/-}.

Des études sur les cellules souches mésenchymateuses (MSC), composants de la niche impliqués dans la régulation de l'hématopoïèse, ont montré que les MSC provenant de souris déficientes pour *Trp53* étaient défectueuses et donc avaient un impact dans le soutien de la croissance des progéniteurs hématopoïétiques [299].

Différentes études ont montré qu'une diminution de l'expression de Mdm2 et Mdm4 avait un impact sur le système hématopoïétique. L'équipe d'Evan a utilisé des souris n'exprimant pas *Mdm2* et a observé une suppression de la moelle osseuse, du thymus et de la rate, entraînant la létalité. Ils ont également analysé le sang périphérique où ils ont observé une réduction de toutes les lignées hématopoïétiques [300], indiquant que l'expression de p53 est importante pour la génération des différentes cellules hématopoïétiques. En effet, des niveaux faibles mais détectables de la protéine p53 ont été observés dans les populations matures non proliférantes telles que les cellules lymphoïdes, granulocytaires et monocytaires matures [301].

p53 dans l'hématopoïèse clonale

Le vieillissement est un processus continu entraînant des dommages persistants de l'ADN et donc l'apparition de mutations suite à cette instabilité. Au cours du vieillissement, les CSH acquièrent des mutations, ce qui peut conduire à une

hématopoïèse clonale à potentiel indéterminé (CHIP) encore appelée hématopoïèse clonale liée à l'âge (ARCH) [302]. Les CHIP sont associées à un risque accru d'hémopathies malignes, telles que les SMD et LAM mais aussi des maladies liées à l'âge telles que les maladies cardiovasculaires, goutte, maladies hépatiques.

Concernant les mutations identifiées dans les CHIP, on en retrouve 5 qui sont majoritaires : DNMT3A, TET2, ASXL1, JAK2 et TP53 [303], [304]. Les mutations de *TP53* augmentent avec l'âge et sont impliquées dans l'apparition des hémopathies malignes [305]. On retrouve également des mutations TP53 dans les CHIP liées aux traitements [306]. La majorité des mutations TP53 dans les CHIP sont des mutations faux-sens [221], [303]. Il a été observé que certaines mutations de p53 permettaient l'interaction avec EZH2, protéine clé dans la régulation de l'épigénétique. Cette association augmente les niveaux de H3K27me3 qui induisent des changements dans la structure de la chromatine et affectent ainsi la régulation des gènes cibles. Cette dynamique moléculaire entre p53, EZH2 et H3K27me3 déclenche l'auto-renouvellement et la différenciation des CSH mutées pour *TP53* [307].

P53 dans les hémopathies malignes

Les hémopathies malignes présentent une altération génétique du gène *TP53* dans 10-20% des cas [308]. Le gène *TP53* est également associé à plusieurs syndromes héréditaires de prédisposition au cancer, tels que le syndrome de Li-Fraumeni, dans lequel les individus héritent d'un gène *TP53* muté de leurs parents et présentent un risque significativement accru de développer plusieurs types de cancer au cours de leur vie [309], [310]. Les mutations de *TP53* sont plus fréquentes dans les formes les plus agressives et résistantes au traitement. La fréquence des mutations *TP53* dans la LAM est d'environ 10% [311], tandis que dans les LAM à caryotype complexe, la fréquence des mutations et/ou délétions de *TP53* est d'environ 70% [312]. On retrouve des mutations et délétions de ce gène dans la leucémie lymphoïde chronique (LLC), dans les lymphomes diffus à grandes cellules B... [313]. Dans certains cas, la perte de *TP53* peut être un marqueur pronostique [314]. Les mutations de *TP53* dans les hémopathies malignes ont été établies comme ayant un impact négatif sur la survie [312], [315], [316]. D'autres événements impliqués dans la diminution de l'expression p53, tels que les surexpressions de Mdm2/MDM2 et Mdm4/MDM4 peuvent être à l'origine des tumeurs d'origine hématologiques chez la souris [317], [318] et l'Homme. Chez l'Homme, la surexpression de MDM2 a été observée dans différentes maladies hématopoïétiques telles que la LLC, les lymphomes folliculaires, le lymphome diffus à grandes cellules B...[313].

Une étude visant à analyser les altérations de *TP53* chez les patients atteints de LMMC a été récemment publiée. Parmi les 1315 patients atteints de LMMC, seulement 1,9% présentaient des mutations de *TP53* et 0,6% présentaient des délétions ou réarrangement structurels de ce gène. Ainsi, les patients présentant une altération de *TP53* ont un taux plus élevé de blastes médullaires, ce qui suggère une proportion plus importante de patients atteints du sous-type CMML-2 qui est associé à une forme plus agressive de la maladie. Il a également été montré que les cas avec des mutations multiples (10 mois) ou l'isochromosome 17q (8 mois) avaient une survie inférieure par rapport aux cas avec des mutations *TP53* uniques (19 mois) [319], suggérant une relation entre le nombre de mutations et la gravité.

Conclusion générale de la partie 3 :

Le gène *TP53*, appelé « gardien du génome », est un gène suppresseur de tumeur très étudié qui joue un rôle essentiel dans la prévention du développement et de la progression des cancers. La protéine p53 est impliquée dans la régulation du cycle cellulaire, la réparation de l'ADN, l'apoptose et le maintien de la stabilité génomique. Ce gène contribue au maintien de l'intégrité du génome et empêche la propagation de cellules endommagées ou anormales. Il est couramment muté dans différents types de cancers solides et ces mutations sont associées à des formes plus agressives de cancer et à de mauvais résultats thérapeutiques. Les mutations de *TP53* sont très rares dans la LMMC (<2%). Malgré cette rareté, les altérations de *TP53* dans la LMMC sont de mauvais pronostic.

IV. Les objectifs du travail de thèse

Les cancers sont souvent caractérisés par des anomalies dans un ou plusieurs gènes. Ces anomalies sont appelées « mutations génétiques ». Ainsi les cellules avec plusieurs mutations arrêtent de fonctionner, se multiplient de façon incontrôlée et deviennent tumorales. Des mutations peuvent survenir à la naissance en héritant un gène muté de nos parents. Les facteurs environnementaux peuvent causer des dommages à l'ADN et générer ces anomalies ou au cours du vieillissement avec l'augmentation de l'inflammation les gènes acquièrent des anomalies.

Concernant les gènes qui sont souvent mutés dans les cancers, on retrouve les gènes suppresseurs de tumeur. Les rôles principaux de ces gènes sont de protéger les cellules en réparant les dommages dans l'ADN, en ralentissant la croissance et la division des cellules et en induisant l'apoptose des cellules présentant des taux anormaux d'erreurs dans leur ADN. Ainsi, une mutation dans ces gènes peut provoquer leur inhibition et induire une croissance aberrante des cellules avec des anomalies au niveau de l'ADN.

Mon projet de thèse a porté sur l'étude de deux suppresseurs de tumeur, TET2 et TP53, dans un contexte sain et dans la LMMC, une maladie présentant le pourcentage de mutations pour *TET2* le plus élevé et le pourcentage de mutation pour *TP53* le plus rare.

- Dans une première partie, j'ai voulu savoir s'il existait une signature cytokinique spécifique des mutations de *TET2*. J'ai identifié la surexpression de la cytokine MIF dans des modèles de souris et de lignées cellulaires invalidées pour l'expression de *Tet2/TET2* ainsi que dans des monocytes *TET2* mutés de patients atteints de LMMC. J'ai déterminé le mécanisme de sur-expression de MIF ainsi que son effet sur les CSH (Partie 1 : Article publié).
- Dans la seconde partie, je me suis intéressée à la diminution du gène *TP53* dans les monocytes de patients atteints de LMMC. Cette approche m'a permis de caractériser le phénotype de ce sous-groupe de patients n'exprimant pas ce gène ainsi que le rôle de p53 au cours de la monocytopenie (Partie 2 : First draft).

V. Partie 1 : Article publié

Macrophage migration inhibitory factor is overproduced through EGR1 in $TET2^{low}$ resting monocytes

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Résumé de l'article

Plusieurs études ont montré que les facteurs épigénétiques jouent un rôle essentiel dans le développement et la progression des hémopathies myéloïdes. Plusieurs études ont montré que le gène *TET2* est fréquemment muté dans les hémopathies malignes, notamment la LAM, LMC, les MDS et la LMMC. *TET2* code pour une enzyme qui est impliquée dans le processus de la déméthylation de l'ADN, un mécanisme épigénétique important pour la régulation de l'expression des gènes.

En utilisant le modèle de transduction lentivirale des cellules CD34+, une analyse par criblage des cytokines présentes dans les surnageants récupérés à partir de cellules CD34⁺ infectées par un shRNA dirigé contre *TET2* ou par un shRNA contrôle *SCR* a montré que seul MIF (Macrophage migration inhibitory factor) était significativement accumulé dans le surnageant des cellules CD34+ sous-exprimant *TET2*.

Dans les modèles murins déficients quant à l'expression de *Tet2*, développés par l'équipe d'Olivier Bernard, l'inactivation de *Tet2* est associée à une augmentation de la concentration plasmatique de cette cytokine en fonction du génotype des souris analysées avant toute apparition de la maladie.

En utilisant les lignées Kasumi, UT7, MO7E et TF-1a, nous avons montré que MIF, est sur-exprimée lors de l'inhibition de l'expression de *TET2*. *TET2* est fréquemment muté dans la LMMC. Dans des monocytes de patients atteints de LMMC mutés pour *TET2*, on observe une augmentation des messagers *MIF* comparé à des monocytes contrôles. Les monocytes humains de patients atteints de LMMC présentant des variants tronquants du gène *TET2* expriment plus de MIF que les patients LMMC avec des mutations ponctuelles.

Des expériences de ChIP-PCR ont montré que *TET2* se fixait directement au niveau du site d'initiation de la transcription de *MIF*. En utilisant les données de ChIP-seq de *Tet2* précédemment publiées dans des souris de type sauvage et *Tet2* knock-out, nous avons vérifié que *Tet2* était également recruté au niveau du promoteur de *Mif*.

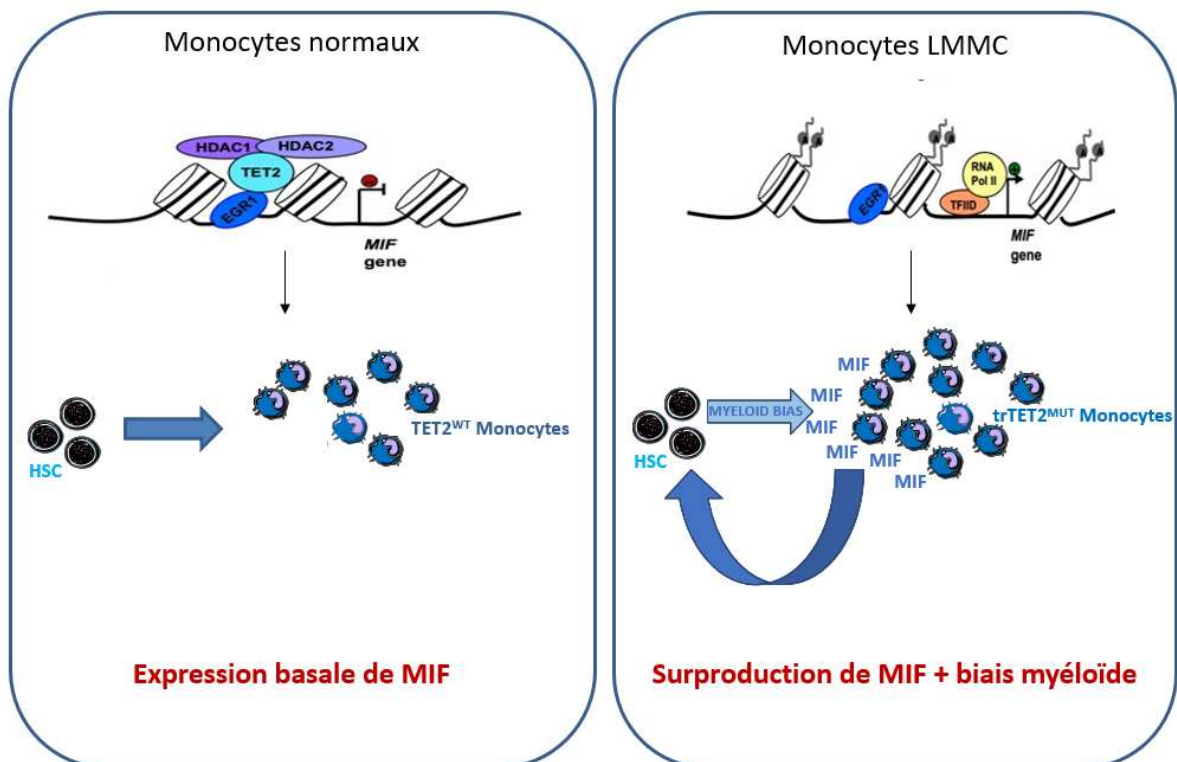
L'enrichissement de Tet2 a été observé chez des souris de type sauvage, mais pas chez des souris knock-out à la position de locus de *Mif* positions 75322311-75323742 sur le chromosome 10 en utilisant mm9.

Dans des monocytes TET2 p.W564X homozygote, nous avons identifié une diminution du recrutement de HDAC1 et 2 au niveau du promoteur de *MIF*. Ce changement de la structure chromatinienne favorise la transcription de *MIF* par le facteur de transcription EGR1 au niveau du TSS de *MIF*.

Nous avons étudié aussi l'expression de deux facteurs de transcription, PU.1 et GFI1, impliqué respectivement dans la différenciation monocytaire et dans la différenciation granulocytaire, dans les CSH provenant de donneur sain ou de patients LMMC avec ou sans mutation de TET2. Nos données montrent que l'expression de *PU.1* est augmentée tandis que l'expression de *GFI1* est diminuée dans les CSH des patients LMMC *TET2*^{MUT}. Par ailleurs, la différenciation granulomonocytaire des CSH de sang de cordon, avec ou sans MIF, a montré que MIF est suffisant en soi pour induire un biais vers la production de monocytes.

Nous avons ainsi mis en évidence une boucle de rétrocontrôle des monocytes mutés pour *TET2* sur la production de monocytes par les CSH via MIF. Cette étude met aussi en évidence l'importance des cellules matures du clone leucémique sur les cellules souches et sur le développement de la leucémie.

Schéma général de l'article













<https://doi.org/10.1038/s42003-022-03057-w>

OPEN

Macrophage migration inhibitory factor is overproduced through EGR1 in *TET2*^{low} resting monocytes

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Somatic mutation in *TET2* gene is one of the most common clonal genetic events detected in age-related clonal hematopoiesis as well as in chronic myelomonocytic leukemia (CMML). In addition to being a pre-malignant state, *TET2* mutated clones are associated with an increased risk of death from cardiovascular disease, which could involve cytokine/chemokine overproduction by monocytic cells. Here, we show in mice and in human cells that, in the absence of any inflammatory challenge, *TET2* downregulation promotes the production of MIF (macrophage migration inhibitory factor), a pivotal mediator of atherosclerotic lesion formation. In healthy monocytes, *TET2* is recruited to *MIF* promoter and interacts with the transcription factor EGR1 and histone deacetylases. Disruption of these interactions as a consequence of *TET2*-decreased expression favors EGR1-driven transcription of *MIF* gene and its secretion. MIF favors monocytic differentiation of myeloid progenitors. These results designate MIF as a chronically overproduced chemokine and a potential therapeutic target in patients with clonal *TET2* downregulation in myeloid cells.

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Ten-eleven-translocation (TET) proteins are iron [Fe(II)]- and α -ketoglutarate (α -KG)-dependent dioxygenases that promote active DNA demethylation through iterative oxidation of 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC), and 5-carboxylcytosine (5caC), eventually leading to the replacement of 5mC by native C¹. hmC also prevents DNA methylation by decreasing cytosine accessibility to DNA methyltransferases². This activity of TET enzymes is regulated via substrate and cofactor availability, post-transcriptional regulation, and post-translational modifications³.

TET family member interacting proteins may tether them to DNA. For example, TET2 could interact with NANOG in mouse embryonic stem cells⁴, SPI1/PU.1 in differentiating B-cells⁵ and monocytes⁶, early growth response 2 (EGR2) in IL4/GM-CSF-driven human monocyte differentiation⁷ and Wilms' tumor suppressor gene1 (WT1) in acute myeloid leukemia cells^{8,9}. The binding of TET proteins to 5mC-free promoters with diverse partners suggested that they could function independently of their catalytic activity³. In brain epigenome programming during postnatal development, EGR1 recruits TET1 to demethylate EGR1 binding sites¹⁰. Tet1 regulates gene transcription in mouse embryonic stem cells through associating with the Sin3A co-repressor complex¹¹ and MOF histone acetyltransferase¹², while a catalytically dead Tet2 mutant represses *Interleukin-6 (Il6)* gene transcription in mouse macrophages by recruiting Hdac1 and Hdac2 histone deacetylases¹³. Some of the catalytic-activity independent effects of TET proteins are mediated by the recruitment of OGT [O-linked N-acetylglucosamine (O-GlcNAc) transferase] to gene promoters^{14–16}.

Mono- or bi-allelic somatic mutations along the entire coding *TET2* region are recurrent events in human hematopoietic malignancies^{17,18}, especially in chronic myelomonocytic leukemia (CMML) in which mono- or bi-allelic mutations in *TET2* gene are detected in 57% of patients¹⁹. Mouse models with *Tet2* gene deletion in hematopoietic stem cells develop a myeloid or a lymphoid malignancy^{20–23}. The long latency and low penetrance of these diseases suggest that cooperation with another genetic event and/or a permissive environment may be needed for malignancy emergence^{24–26}.

Somatic mutation in *TET2* gene is also one of the most common clonal genetic events detected in the peripheral blood of ageing healthy individuals, named Clonal Hematopoiesis of Indeterminate Potential (CHIP)²⁷. These *TET2*-mutated clones can be a first step towards a malignancy such as CMML²⁸. A *TET2*-mutated CHIP also increases the risk of death from cardiovascular disease^{29,30}. A deregulated production of inflammatory cytokines by mutated myeloid cells may explain the cardiovascular risk associated with *TET2* CHIP as these cytokines may promote leukocyte recruitment to atherosclerotic plaques^{31,32}. Accordingly, *Tet2*-deletion in murine macrophages induces a constitutive expression of lipopolysaccharide (LPS)-induced genes³³ and cardiovascular risk is reduced on a genetically decreased IL-6 signaling background³⁴ or prevented by a selective NLR family pyrin domain-containing 3 (NLRP3) inflammasome inhibitor decreasing IL-1 β secretion by innate immune cells³⁵.

Here, we show that, in the absence of any inflammatory challenge, *TET2* gene downregulation induces an overproduction and secretion of MIF (macrophage migration inhibitory factor). Initially identified as a lymphocyte-derived soluble product^{36,37}, MIF is released by a variety of cells³⁸ and behaves as a pro-inflammatory cytokine with pathogenic roles in inflammatory and autoimmune disorders^{39–44}. Genetic deletion of *Mif* impairs the production of inflammatory mediators by monocytes/macrophages^{45,46} and prevents the inflammasome activation⁴⁷. We show that in CMML human monocytes harboring truncating

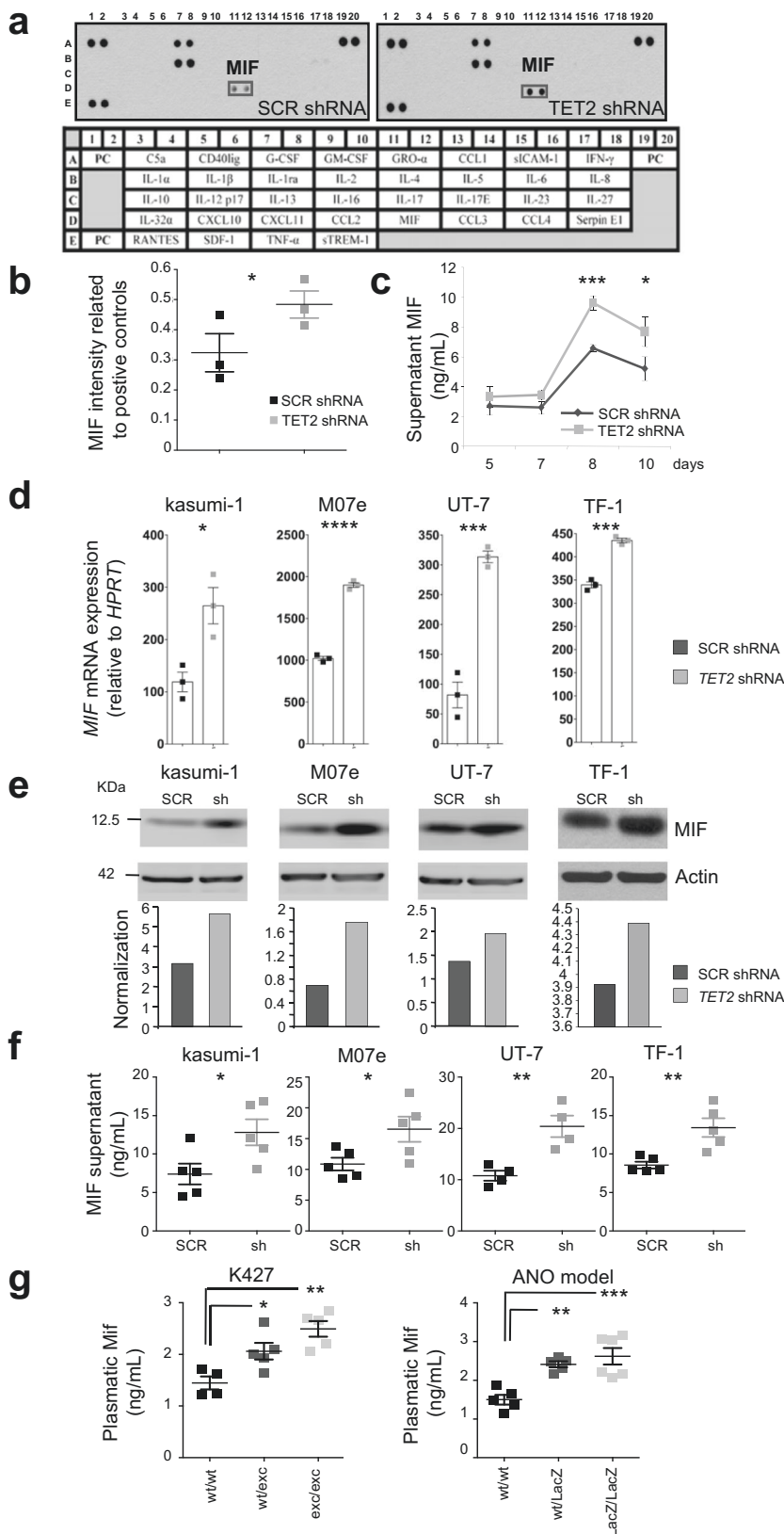
variants of *TET2* gene, MIF secretion is increased through EGR-1 transcription factor recruitment to its promoter. These results identify MIF as a potential therapeutic target to prevent atherosclerosis and progression of *TET2* mutated CHIP towards a chronic myeloid malignancy.

Results

***TET2* downregulation induces MIF overproduction.** We investigated whether *TET2* gene downregulation could alter cytokine secretion by myeloid cells. Human cord blood CD34⁺ cells, transduced with *TET2* or scrambled (SCR) shRNA lentiviruses, were sorted and cultured with SCF, IL-3, TPO, and GM-CSF to promote granulocytic/monocytic differentiation⁴⁸. Using cytokine-arrays to analyze day-10 cell culture supernatant, three cytokines were readily detected: MIF, G-CSF, and IL-1RA. MIF was repeatedly increased, while G-CSF and IL-1RA were not affected when *TET2* expression was decreased (Fig. 1a, b). ELISA measurements confirmed MIF overproduction upon *TET2* silencing at days 8–10 of culture (Fig. 1c). Consistent with these observations, *MIF* mRNA (Fig. 1d) and secreted MIF (Fig. 1e, f) were increased in four human leukemic cell lines (kasumi-1, M07e, UT-7, and TF-1) in which *TET2* gene expression was decreased by using lentiviral shRNA (Supplementary Fig. 1a), as previously described⁴⁸. Finally, MIF concentrations were increased in blood (Fig. 1g) and supernatant of bone marrow aspirations (Supplementary Fig. 1b) of two *Tet2*-deficient mouse models²⁰. In these models, *Mif* plasma levels increased in mice 1–3 aged months, i.e., before changes in white blood cell count (Supplementary Fig. 1c, d), thus monocyte counts in the peripheral blood as a confounding factor. These results indicated that, in vitro and in vivo, in mouse and human cells, *TET2* downregulation increased MIF secretion.

***MIF* is overexpressed in *TET2*-mutated CMML monocytes.** CMML is a clonal disorder, mostly observed in the elderly with a median age at diagnosis of 73 years. *TET2* mutations are early somatic events⁴⁹ and the most frequent genetic alteration of this disease⁵⁰. To explore the link between *TET2* mutations and *MIF* expression in human primary samples, we sorted peripheral blood monocytes from 60 CMML patients (17 *TET2*-wild-type [*TET2*^{WT}] and 43 *TET2*-mutated [*TET2*^{MUT}] cases) (Supplementary Table 1) and from 10 age-matched healthy donors and performed bulk RNA sequencing. Focusing on cytokine genes, *IL6* mRNA was not expressed in control and CMML resting monocytes while a significant increase in *MIF* mRNA was observed in *TET2*^{MUT} compared to healthy donors and *TET2*^{WT} CMML (Fig. 2a; Supplementary Data 1). We also observed an inverse correlation between *TET2* and *MIF* mRNA expression levels in CMML patient monocytes (Fig. 2b). *MIF*-increased mRNA detected in *TET2*^{MUT} monocytes was validated by RT-qPCR analysis in an independent cohort of 146 CMML patients (56 *TET2*^{WT} and 90 *TET2*^{MUT} cases including 68 truncating variants) compared to 19 young and 8 age-matched healthy donors (Supplementary Table 2 and Fig. 2c). Importantly, we did not detect any significant recurrent change in the expression of other cytokine-encoding genes in *TET2*-mutated patient monocytes, i.e., *MIF* gene expression was frequently increased in cells expressing truncating *TET2* variant whereas the increased expression of other cytokine-encoding genes was heterogeneous and independent of *TET2* status (Fig. 2d).

In a multi-institutional study ($n = 1084$ CMML patients), we recently reported an overall survival advantage associated with *TET2* mutations in CMML patients. This was especially significant in the context of multiple or truncating *TET2* mutants (*trTET2*^{MUT}), i.e., nonsense and frameshift variants, compared to



non-truncated variants (*non-trTET2^{MUT}*), i.e., in frame insertion/deletion, missense, and splice site variants¹⁹. From our RNA sequencing data, the decreased expression of *TET2* gene expression observed in *trTET2^{MUT}* cells correlated with an increased expression of *MIF* mRNA, which was not detected in

non-trTET2^{MUT} cases (Fig. 2e). Among CMML samples tested by RT-qPCR (Fig. 2c), we observed an increased expression of *MIF* gene in *trTET2^{MUT}* samples only (Fig. 2f). The previously described better survival of *TET2^{MUT}* CMML patients was validated in the present cohort (Supplementary Fig. 2a) with no

Fig. 1 TET2 downregulation promotes MIF production. **a, b** Cytokine profile arrays of supernatant collected from cord blood CD34⁺ cells infected with SCR- and TET2-shRNA-GFP lentiviruses, sorted on GFP expression, and induced to differentiate with stem cell factor (SCF), interleukin-3 (IL-3), Fms-related tyrosine kinase 3 ligand (FLT3L) and granulocyte-colony stimulating factor (G-CSF). **a** Representative cytokine array with supernatants collected at day 10 of differentiation. The rectangle points to MIF detection. **b** Quantification of MIF signals, normalized to positive controls. Data are mean \pm SEM of three independent experiments. Paired t test: * $P < 0.05$. **c** MIF concentrations determined by ELISA in the supernatant of cells induced to differentiate for indicated time. Data are mean \pm SEM of indicated independent experiments (day 5: $n = 6$; day 7: $n = 5$; day 8: $n = 3$; day 10: $n = 7$). Paired t test: * $P < 0.05$; *** $P < 0.001$. **d** RT-qPCR analysis of MIF mRNA expression in four TET2-depleted (TET2 shRNA, gray bars) and control (SCR shRNA, black bars) human leukemic cell lines. Data are mean \pm SEM of three biological replicates. Unpaired t test: * $P < 0.05$; *** $P < 0.001$; **** $P < 0.0001$. **e** Immunoblot of SCR or TET2 shRNA infected leukemic cell lines sorted on GFP expression. Lower panels, quantification after actin normalization using Image J software. **f** MIF concentrations determined by ELISA in the supernatants of kasumi-1 ($n = 5$), MO7e ($n = 5$), UT-7 ($n = 4$) and TF-1 ($n = 5$) cells transduced 24 h before with SCR (black squares) or TET2 (gray squares) shRNA. Data are mean \pm SEM of indicated biological replicates. Unpaired-t test: * $P < 0.05$; ** $P < 0.01$. **g** MIF concentrations determined by ELISA in the plasma of two Tet2-deficient models (1-3 months): K427 knock-out model (wt/wt or Tet2^{+/+} $n = 4$, wt/exc or Tet2^{+/-} $n = 5$ and exc/exc or Tet2^{-/-} $n = 5$); ANO knock-down model (wt/wt or Tet2^{+/+} $n = 5$, wt/LacZ or Tet2^{+/-} $n = 5$ and LacZ/LacZ or Tet2^{-/-} $n = 6$). Data are mean \pm SEM of indicated biological replicates. Dunnett's multiple comparison tests using wt/wt as control: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

significant difference between *trTET2^{MUT}* and *non-trTET2^{MUT}* CMML (Supplementary Fig. 2b). No difference in white blood cell, monocyte, neutrophil, lymphocyte count, and hemoglobin level was observed between the three groups whereas, interestingly, the platelet count was significantly lower in *trTET2^{MUT}* patient subgroup (Supplementary Fig. 2c).

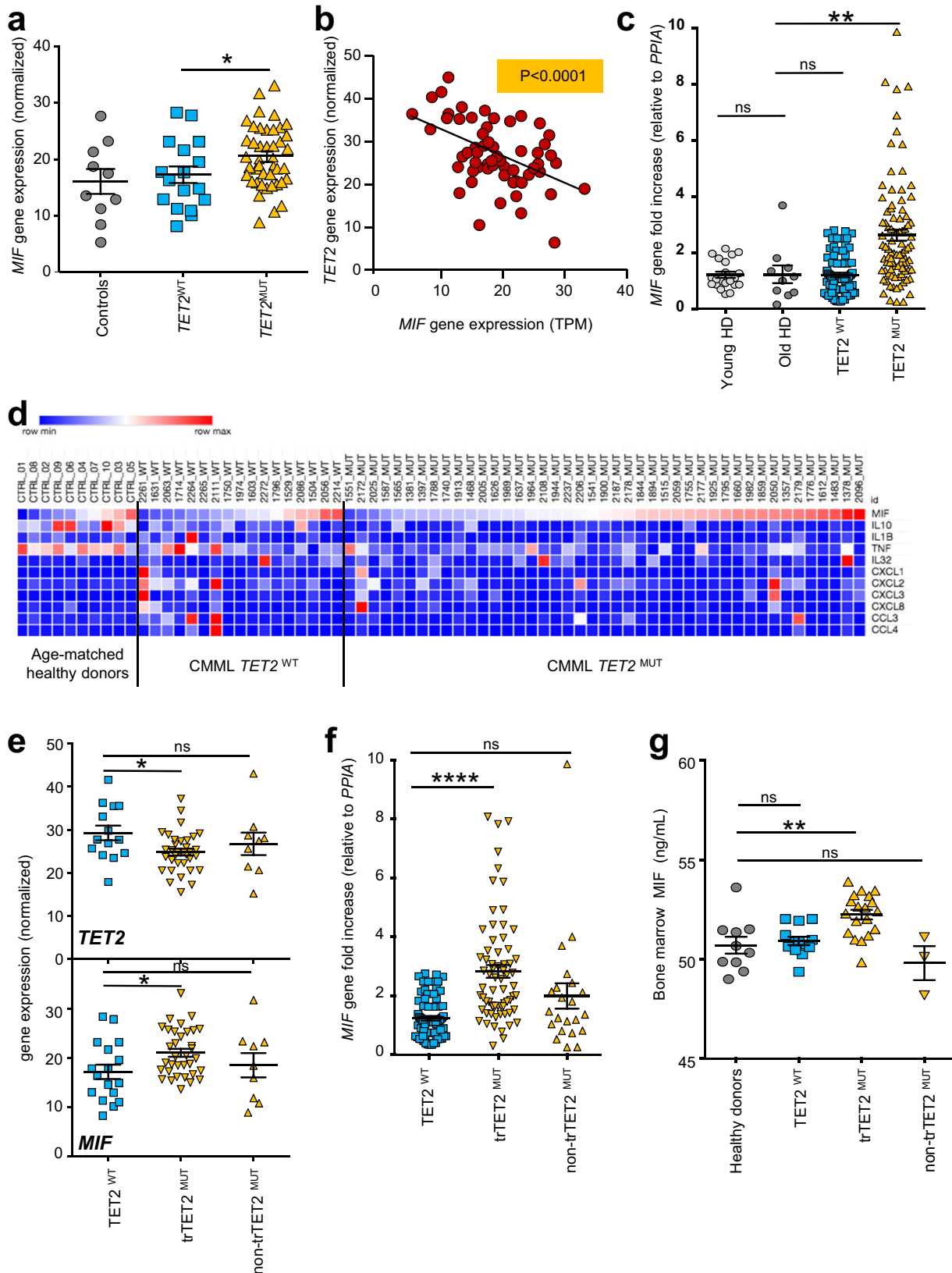
Immunoblot analysis of monocytes from 3 controls and 5 CMML patients (1 TET2^{WT}, 4 *trTET2^{MUT}*) further showed an increased expression of MIF protein in *trTET2^{MUT}* patients (Supplementary Fig. 3a). MIF level was higher in the supernatant (18 h in serum-free medium) of *trTET2^{MUT}* CMML-monocytes from 2 patients compared to healthy donors (Supplementary Fig. 3b, c) and in the serum of 3 *trTET2^{MUT}* CMML patients at diagnosis compared to 3 healthy donor serum samples (Supplementary Fig. 3b, d). Finally, MIF concentrations in the supernatant of bone marrow aspirates from 10 healthy controls and 35 CMML patients (13 TET2^{WT}, 19 *trTET2^{MUT}*, and 3 *non-trTET2^{MUT}*) (Supplementary Table 3) revealed a significant increase of MIF in *trTET2^{MUT}* CMML samples (Fig. 2g). Together, these results identified a correlation between TET2 mutation, especially truncated mutants, and increased production of MIF.

TET2 protein binds MIF promoter region. Tet2 chromatin immunoprecipitation-sequencing (ChIP-seq) performed with mouse wild-type bone-marrow cells¹⁶ indicated the recruitment of Tet2 at the *Mif* promoter (chromosome 10, locus position 75,322,231–75,323,742 using the mouse genome NCBI Build 37/UCSC mm9). Using two distinct antibodies against TET2 (Ab1:C2 and Ab2: sc-136926), we performed ChIP-qPCR to map TET2 binding in 3 regions (R2-R4) spanning the MIF promoter (Fig. 3a) in control (shSCR) and TET2-depleted (shTET2) kasumi-1 cells. In control cells, TET2 was enriched around the transcription start site (TSS) (Fig. 3b). In both humans and mice, downregulation of TET2 precluded chromatin immunoprecipitation, supporting the specificity of TET2 binding. We then analyzed H3K4me3 and H3K27me3 histone marks and recruitment of active polymerase II (phosphorylated on serine 5, pS5 Pol II) to the MIF promoter. H3K4me3 signal increased from the distal region of MIF promoter to the TSS region in shSCR cells, while it was decreased in shTET2 cells (Fig. 3c) as previously shown¹⁶. H3K27me3 was not detected at the MIF promoter (Fig. 3c). Finally, active polymerase II was recruited in the TSS area of the MIF gene at much higher levels in shTET2 than shSCR cells (Fig. 3d), in accordance with an increased transcription of MIF gene under TET2 depletion.

To determine the minimal DNA sequences regulating MIF promoter activity, we used fragments of the human MIF

promoter, ranging from position -1083 to +129, into a luciferase reporter vector that was transiently expressed into SCR- and TET2-shRNA kasumi-1 cells. The -25/+129 and +44/+129 vectors drove only background luciferase activity while the other vectors induced a stronger luciferase activity in TET2-depleted compared to control cells (Fig. 4a). Since the -81/+129 sequence was sufficient to drive optimal MIF promoter activity, we explored the role of cis-acting regulatory elements in that region. Deletion of c-MYB, CRE^d, SP1^d, AML1a, AP4, and HIF1 sites⁵¹ did not affect the luciferase activity measured in SCR- and TET2-shRNA kasumi-1 cells. Deletion of the SP1^P and CRE^P sites decreased luciferase activity in both control and TET2-depleted cells, indicating that these sites are critical for MIF gene expression (Fig. 4b). Using the Transcription Element Search System (TESS, <http://www.cbil.upenn.edu/tess/>), we identified a potential EGR binding site overlapping the SP1^P site (Fig. 4c). We noticed a higher recruitment of EGR1 to the R4 region of the MIF promoter in TET2-depleted cells, whereas EGR2 was only slightly more recruited and SP1 recruitment remained unchanged (Supplementary Fig. 4). Together, these results identify EGR1 recruitment to the minimal promoter sequence required for MIF gene expression.

TET2 interacts with EGR1. In silico analysis using MethylPrimer Express (Applied Biosystems) identified a CpG island (CG >65% of 710 bp) within MIF gene promoter. Sequencing of bisulfite-treated DNA failed to identify any differential methylation of the minimal promoter sequence (-81/+129) in a cohort of 21 CMML monocytes, including 11 TET2^{WT} and 10 TET2^{MUT} samples, compared to 17 healthy donors, including 10 young and 7 age-matched donors, respectively (Supplementary Fig. 5). We next performed ChIP-qPCR in controls, TET2^{WT}, and TET2^{MUT} CMML patient monocytes, focusing on the R3 region, close to the TSS (Fig. 3a). EGR1 was strongly enriched in *trTET2^{MUT}* cells compared to controls and TET2^{WT} cells (Fig. 5a). To demonstrate EGR1-dependent MIF expression in *trTET2^{MUT}* cells, we transfected EGR1-siRNA in *trTET2^{MUT}* monocytes collected from 2 CMML patients. EGR1 mRNA downregulation, validated by RT-qPCR (Fig. 5b and Supplementary Fig. 6a), prevented MIF upregulation in these cells. HDAC1 and HDAC2 were enriched on MIF promoter in controls and TET2^{WT} cells, but their recruitment decreased in *trTET2^{MUT}* cells (Fig. 5c). To determine if EGR1 and TET2 could interact, we transiently co-transfected 293T cells with empty vectors or pcDNA3-EGR1 and pcDNA3-TET2-HA. Having checked EGR1 and TET2-HA protein expression in 293T cells (Fig. 5d), co-immunoprecipitation experiments validated the ability of TET2 to interact with EGR1. Interestingly, HDAC1 was only immunoprecipitated with



TET2-HA antibody, suggesting a stronger interaction with TET2 in the complex (Fig. 5d). Co-immunoprecipitation experiments in human blood monocytes confirmed TET2 interaction with EGR1 (Supplementary Fig. 6b, c) and validated the ability of TET2 to interact with HDAC1 and HDAC2 (Supplementary Fig. 6d).

TET2 downregulation disrupts TET2/EGR1 interaction. To explore if TET2 downregulation globally affects EGR1 recruitment to DNA in monocytes, we performed ChIP-seq in monocytes from two healthy donors and three TET2^{MUT} CMML patients selected for having trTET2^{MUT} with diverse variant allele frequencies (VAF). CMML1818 combined TET2 A1241fsX11

Fig. 2 Increased expression of MIF gene in TET2-mutant CMML monocytes. a–c Sequencing of total polyA-RNA was performed in sorted peripheral blood monocytes collected from age-matched healthy donors ($n = 10$, gray circles) and CMML patients ($n = 60$) including $TET2^{WT}$ ($n = 17$, blue squares) and $TET2^{MUT}$ ($n = 43$, orange triangles) cases. **a** *MIF* gene expression after count normalization. Data are mean \pm SEM of indicated biological samples. Mann–Whitney test using $TET2^{WT}$ as control: $*P < 0.05$. **b** Inverse correlation between *TET2* and *MIF* gene expression in all samples (10 age-matched healthy donors and 60 CMML patients); $R^2 = 0.2325$; $P < 0.0001$. **c** RT-qPCR analysis (normalized to *PPIA* gene) of *MIF* mRNA expression in sorted peripheral blood monocytes of 19 young healthy donors (age < 65), 8 age-matched healthy donors (age > 64) and 146 CMML patients ($TET2^{WT}$ 56; $TET2^{MUT}$ 90). Data are mean \pm SEM of indicated biological samples. Dunnett’s multiple comparison test using Old HD as control, $***P < 0.01$, ns, non-significant. **d** Heatmap of the expression of a selection of cytokine and chemokine genes in the three cohorts. **e** *TET2* and *MIF* gene expression after count normalization was compared in CMML monocytes of patients with truncating *tr-TET2^{MUT}* ($n = 34$) and non-truncating ($n = 9$) *non-tr-TET2^{MUT}*. Data are mean \pm SEM of indicated biological samples. Dunnett’s multiple comparison test using $TET2^{WT}$ ($n = 17$) as control, $*P < 0.05$, ns, non-significant. **f** *MIF* gene expression normalized to *PPIA* gene was compared in CMML monocytes of patients with truncating *tr-TET2^{MUT}* ($n = 68$) and non-truncating ($n = 22$) *non-tr-TET2^{MUT}*. Data are mean \pm SEM of indicated biological samples. Dunnett’s multiple comparison test using $TET2^{WT}$ ($n = 56$) as control, $****P < 0.0001$, ns, non-significant; **g** *MIF* concentrations in bone marrow fluid from healthy controls ($N = 10$), $TET2^{WT}$ ($n = 12$), *tr-TET2^{MUT}* ($n = 19$) and *non-tr-TET2^{MUT}* ($n = 3$) CMML patients. Data are mean \pm SEM of indicated biological samples. Dunnett’s multiple comparison test using Old HD as control, $**P < 0.01$, ns non-significant.

(VAF = 40%) and E1513GfsX9 (VAF = 49%), CMML1900 exhibited a *TET2* W564X mutant (VAF = 100%) and CMML1268 showed a *TET2* H800fs variant (VAF = 16%). EGR1 recruitment was increased in CMML1818 and CMML1900 monocytes, both exhibiting high *tr-TET2^{MUT}* allele frequencies, compared to CMML1268 and control monocytes (Fig. 6a). Altogether, Cis-Regulatory Annotation System (CEAS) indicated that EGR1 was preferentially recruited to intergenic regions in control and CMML1268 monocytes, whereas it was highly recruited at TSS in the two CMML samples with high *tr-TET2^{MUT}* allele frequencies (Fig. 6b). Ranking heatmaps centered on TSS confirmed EGR1 distribution around the TSS in the two CMML samples with high *tr-TET2^{MUT}* VAF (Fig. 6c). Examples of EGR1 distribution around TSS are shown for *RAD50* (Fig. 6d), *RPL30*, *RPL37A*, *RPS13*, *RPS23*, *RPS3A*, *POLR1B*, *POLR1D*, and *POLR1E* (Supplementary Fig. 7a). In control samples and CMML1268, EGR1 peaks were very similar and mainly intergenic, as illustrated for *CEBPD* (Fig. 6d), *ALDH3B2* and *BCL2* regions as well as intergenic areas on chromosome 2, 7, 9, 13, 16, and 22 (Supplementary Fig. 7b).

MACS2 algorithm identified 11,393 and 19,763 peaks in control samples, of which 2,069 (13%) were common to the two samples (Fig. 6e; Supplementary Data 2). The algorithm identified 17,324 and 32,651 peaks in CMML1900 and CMML1818 monocytes respectively, with 4,424 (18%) common peaks (Fig. 6f; Supplementary Data 3). Comparison of peak localization in monocytes from controls and CMML with *tr-TET2^{MUT}* at high VAF identified only 29 common peaks (Fig. 6g; Supplementary Data 4), whereas comparison of peak localization in healthy donors and CMML1268 monocytes identified 1,510 common peaks (Fig. 6h; Supplementary Data 5), further arguing for CMML1268 being closer than other CMMLs to *TET2* wild-type monocytes regarding EGR1 peak localization (Fig. 6e). Gene Ontology (GO) analysis of EGR1-interacting genes, using peaks that are common to healthy donor and CMML monocytes, showed a dramatic change in CMML1900 and CMML1818, with a global enrichment in genes involved in RNA processing, ribosome biogenesis, and translation (Supplementary Fig. 8). Together, these experiments indicate that *tr-TET2^{MUT}* mutants with decreased expression of *TET2* gene induce chromatin remodeling that, at the level of *MIF* gene TSS, promotes the recruitment of EGR1 that may account for its overproduction.

MIF favors monocyte differentiation of myeloid progenitors.

Tet2 deletion was associated with an increased monocyte count in ageing mice²⁰. We analyzed transcriptomic data generated from CD34⁺ cells sorted from healthy donor and CMML patients with and without *TET2* mutation (Supplementary Table 4 and

Supplementary Data 6). Interestingly, SPI1/PU.1 transcription factor expression, promoting monocytic differentiation, was increased, while the expression of GFI1, involved in granulocytic differentiation, was decreased in $TET2^{MUT}$ compared to $TET2^{WT}$ CD34⁺ cells (Fig. 7a). An inverse correlation was observed for the expression of these two genes in CD34⁺ samples (Fig. 7b). These observations could account for the increase monocytes to granulocytes ratio measured in the peripheral blood of $TET2^{MUT}$ CMML patients (Fig. 7c). Since mutations decreasing *TET2* gene expression increased MIF production, and MIF could increase SPI1/PU.1 dependent transcriptional activity⁵², we explored whether MIF affects myeloid cell differentiation. Cord blood CD34⁺ cells were cultured with SCF, FLT3L, IL-3, and G-CSF in the presence or absence of 20 ng/mL recombinant MIF for 48 h before bulk RNA-seq analysis: 33 genes were differentially expressed in the presence of MIF (Supplementary Fig. 9a). GO Molecular Function analysis of these differentially deregulated genes (DEGs) identified kinase activity (p value = $3.44e^{-6}$; FDR q value = $2.94e^{-3}$) (Supplementary Fig. 9b) and signaling receptor binding (p value = $3.34e^{-5}$; FDR q value = $1.43e^{-2}$) (Supplementary Fig. 9c) signatures. GO Biological Process analysis of these DEGs identified cell morphogenesis involved in differentiation (p value = $3.33e^{-6}$; FDR q value = $6.23e^{-3}$) (Supplementary Fig. 9d) and anatomical structure formation involved in morphogenesis (p value = $4.43e^{-5}$; FDR q value = $2.76e^{-2}$) (Supplementary Fig. 9e) signatures. Finally, CIBERSORT analysis identified more monocytes and macrophages in the culture when MIF was added (Supplementary Fig. 9f), further suggesting that MIF may promote monocytic differentiation of stem and progenitor cells. Cord blood CD34⁺ cells were cultured in medium with SCF, FLT3L, IL-3, and G-CSF in the presence or absence of recombinant MIF for 6 to 9 days before flow cytometry analysis. The addition of recombinant MIF significantly increased the fraction of CD14⁺ monocyte cells, at the expense of CD15⁺ granulocytes (Fig. 7d). Single cell analysis of cells collected at day 7 showed 7 clusters defined by the expression of characteristic genes (Supplementary Fig. 10a) and further validated by the ten most expressed genes (Supplementary Fig. 10b). MIF induced an increase in the number of cells in cluster 6 (monocytes) while this number was decreased in cluster 4 (granulocytes; Fig. 7e). Accordingly, MIF increased the expression in *CSF1R* gene, a SPI1/PU.1 transcription factor target, in cluster 6 while decreasing the expression of *GFI1* gene in cluster 4 (Fig. 7f). These results further argue for the ability of MIF to promote the expansion of monocytes at the expense of granulocytes (Fig. 7g). Altogether, these data suggested that MIF overproduced by *TET2*-mutant cells could promote monocyte differentiation, generating a feedback loop that may promote disease progression.

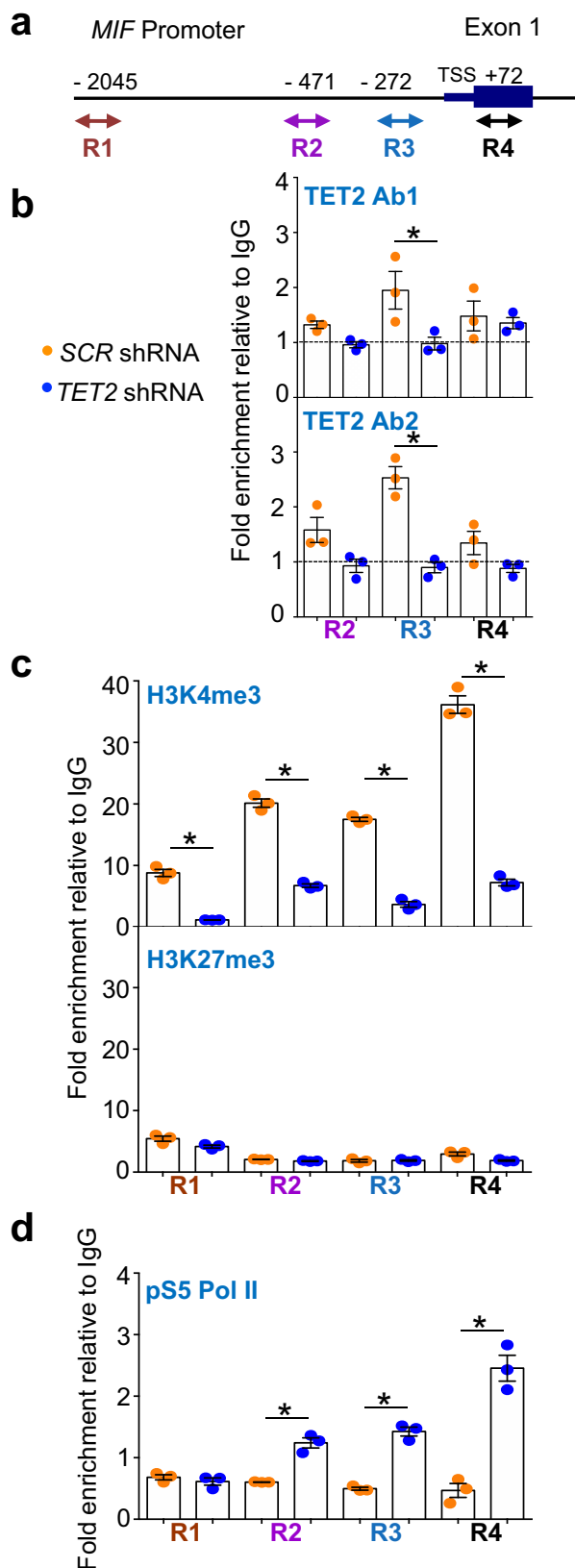


Fig. 3 TET2 binds to the proximal promoter of *MIF*. **a** Schematic representation of *MIF* gene promoter. Quantitative ChIP-PCR analyses were performed in kasumi-1 cells infected by SCR (yellow) or *TET2* (blue) shRNAs by using a set of primers targeting regions located in distal (R1), intermediate (R2), proximal (R3) promoter, and in exon 1, closed to the TSS (R4). **b** TET2-associated DNA was immunoprecipitated with either C2 (TET2 Ab1 upper panel) or sc-136926 (TET2 Ab2, lower panel) antibodies. **c** H3K4me3 (upper panel) or H3K27me3 (lower panel) marks associated with DNA immunoprecipitation. **d** RNA polymerase II phosphorylated on CTD serine 5 (pS5 Pol II) associated to DNA immunoprecipitation. **b-d** Results are expressed as fold enrichment relative to DNA immunoprecipitated with control immunoglobulin G (IgG). Data are mean \pm SEM of each sample run in triplicate. Unpaired t Test, * $P < 0.05$.

cells into monocytes. Given the role of monocytes and MIF in atherosclerosis pathogenesis⁴¹, MIF overproduction might also account for the cardiovascular risk identified in patients with *TET2* mutated clonal hematopoiesis²⁹.

TET2 diversely modulates the expression of cytokine genes, according to the cell type and their environment. In immune cells such as dendritic cells and macrophages^{13,53} as well as in microglia cells⁵⁴, Toll-like receptor (TLR) agonists upregulate *TET2* expression. Conversely, *TET2* binding to target genes, such as *IL6*, downregulates their expression in a catalytic activity-independent manner. *TET2* favors inflammation resolution acting on immune cells and downregulating IL-6 production through HDAC1 and HDAC2 recruitment to *IL6* gene promoter^{13,53}. *TET2* also stimulates the inflammatory response of microglial cells⁵⁴. In a hypoxic tumor microenvironment, upregulated *TET2* promotes *IL6* gene expression in tumor cells, an effect that may involve the catalytic activity of dioxygenase since it is associated with the demethylation of *IL6* promoter⁵⁵. Here, we show that, in the absence of inflammatory cue, *TET2* downregulation in myeloid cells promotes the displacement HDAC1/HDAC2 from *MIF* promoter, leading to *MIF* gene expression. Strengthening this observation, HDAC inhibitors strongly decreased MIF expression in a variety of malignant cell lines and primary cells⁵⁶. Importantly, *TET2* regulates *MIF* gene expression in resting monocytes while *TET2*-mediated *IL6* regulation was detected at the recovery phase of macrophage activation¹³. MIF is the main cytokine overproduced by resting monocytes in which *TET2* expression is decreased, suggesting that overproduction of other cytokines by *TET2*-mutated cells may involve less direct effects of *TET2* mutation.

Unlike *TET1* and *TET3*, *TET2* does not have a canonical CXXC domain binding unmethylated CpG, and *IDAX/CXXCA* gene, leading to *TET2* interaction with CpG islands⁵⁷, is not detectable in human monocytes (our data). Alternative candidates include the NF- κ B inhibitor zeta (I κ B ζ) that targets Tet2 to *IL6* gene proximal promoter in mouse myeloid cells exposed to lipopolysaccharides¹³, the transcription factor forkhead box O3 (Foxo3a) that interacts with Tet2 to promote the proliferation of mouse neural stem cells⁵⁸, and recently, the transcription factor EGR2, another member of EGR transcription factor family, that acts as an epigenetic pioneer to recruit *TET2* to its binding sites in IL4/GM-CSF-differentiated dendritic cells from primary human monocytes⁷. Here, we show that EGR1 transcription factor recruits *TET2* at the *MIF* promoter in human primary monocytes.

Current evidence from animal models and clinical observations indicate that *TET2* inactivation in hematopoietic stem cells may be an early event in the initiation of myeloid malignancies, and that additional hits are necessary for tumor progression¹⁸. Inflammatory signals may provide these hits, as suggested in a

Discussion

The present study identifies a role for *TET2* protein in the transcriptional regulation of *MIF* gene thanks to EGR1 transcription factor in monocytes. *TET2* deficiency, e.g., as a consequence of truncating mutation, promotes *MIF* gene expression. In turn, the secreted cytokine favors the differentiation of CD34⁺

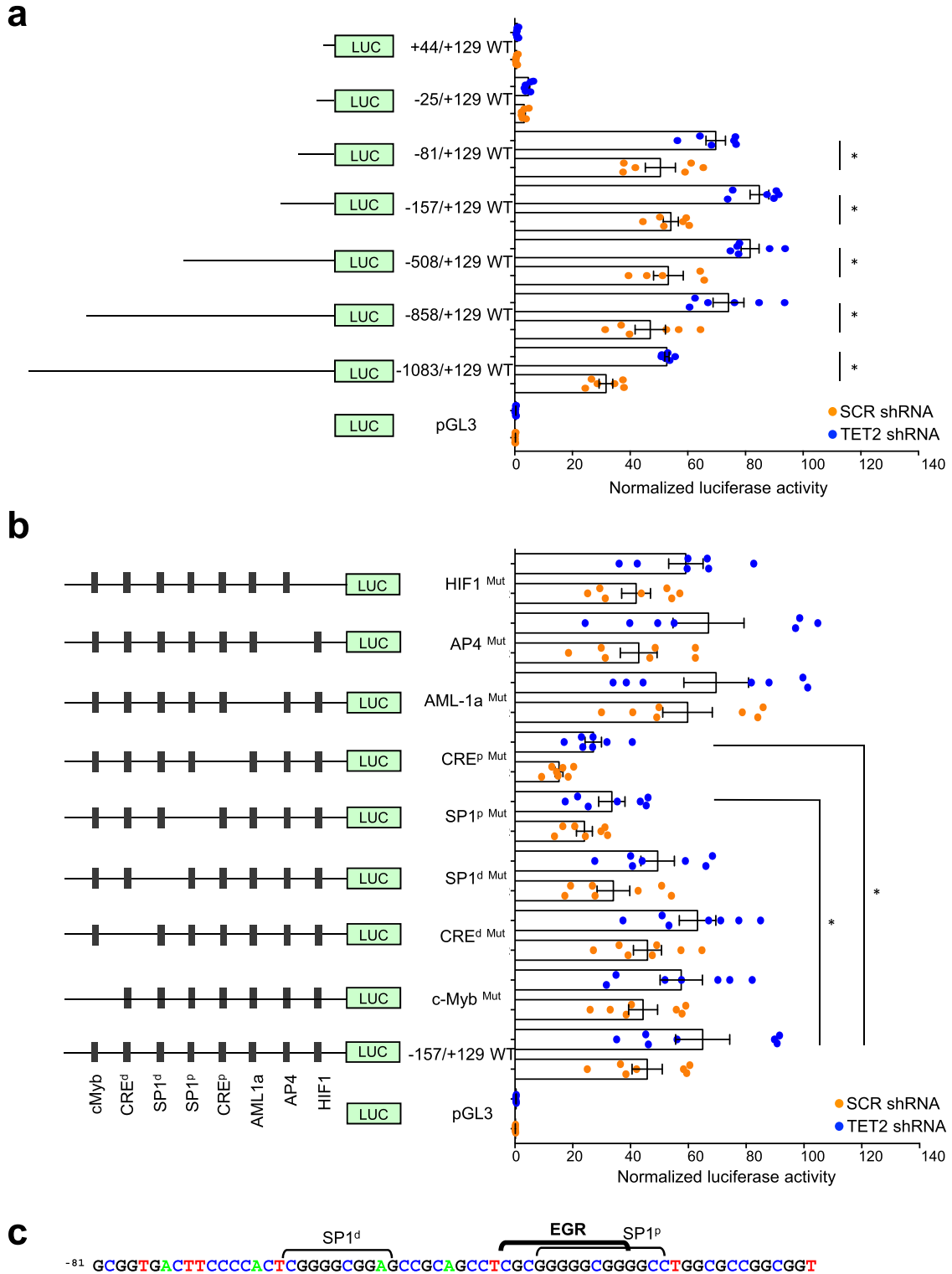


Fig. 4 Identification of a minimal proximal promoter of *MIF* gene. **a** Kasumi-1 cells stably transduced with SCR and TET2 shRNAs were transiently transfected with the pGL3 vector encoding the *Luciferase* gene alone (LUC) or *LUC* gene under control of indicated *MIF* promoter fragments. Cells were co-transfected with a Renilla luciferase construct for normalization and results are the ratio between luciferase and Renilla activities. Data are mean \pm SEM from at least six independent well experiments; Unpaired Students-t test, $*P < 0.05$. **b** The same experiments were performed using the -157/+129 *MIF* promoter sequence in which mutations were induced in the DNA binding sites c-Myb, CRE^d, CRE^p, SP1^d, SP1^p, AML1a, AP4, and HIF1. Data are mean \pm SEM from seven independent well experiments; Unpaired t test, $*P < 0.05$. **c** DNA consensus sequences for SP1^d, SP1^p, and EGR transcription factors in *MIF* proximal promoter.

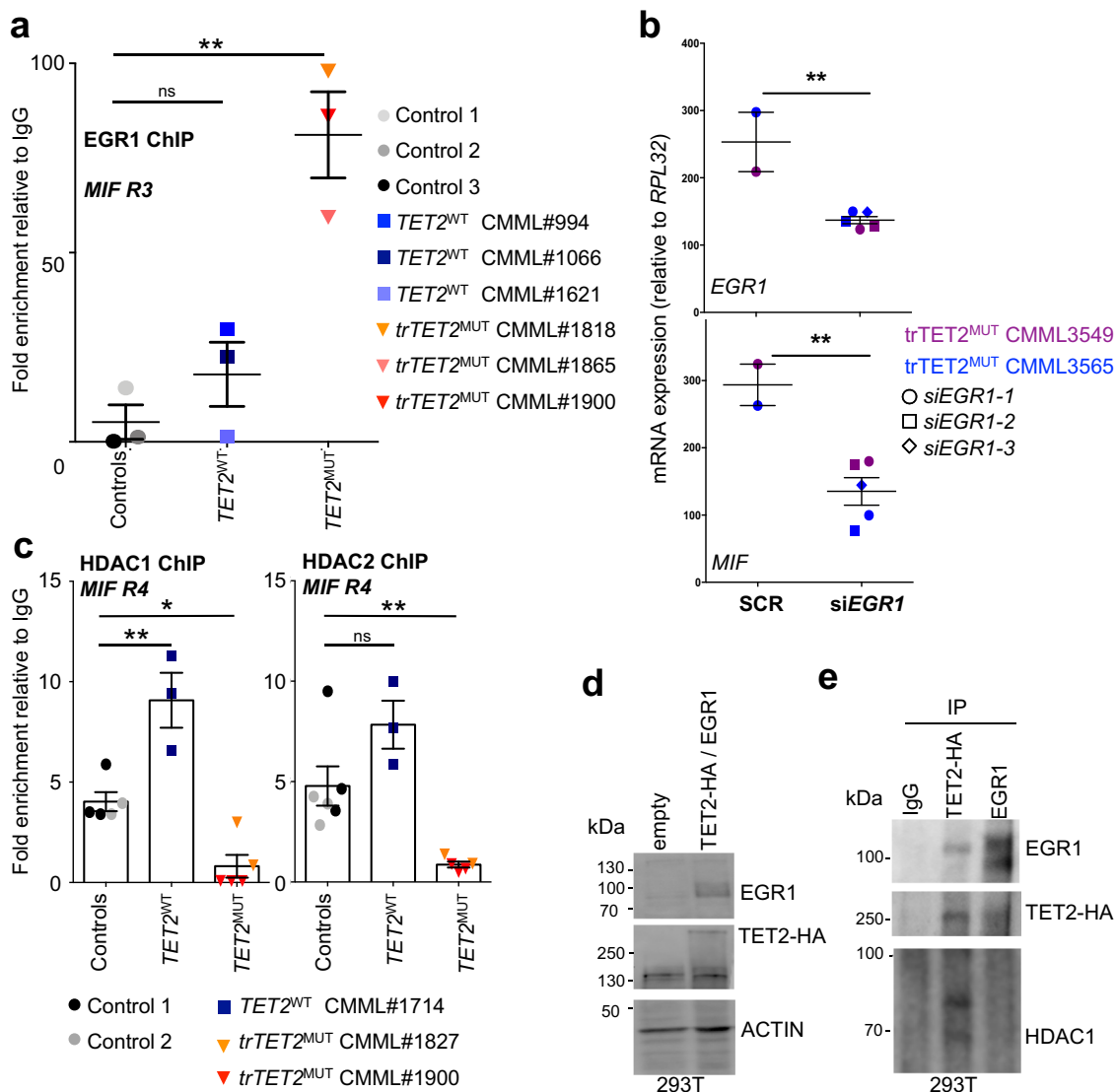


Fig. 5 EGR1 is recruited to the MIF promoter in *trTET2*-mutant CMML monocytes. **a** Quantitative ChIP-PCR analyses of *MIF* promoter (R3) were performed in sorted peripheral blood monocytes collected from three healthy donors (controls, circles) and 6 CMML patients, including 3 *TET2*^{WT} (squares) and 3 *trTET2*^{MUT} (triangles) cases. EGR1-associated DNA was immunoprecipitated. Results are expressed as fold enrichment relative to DNA immunoprecipitated with control immunoglobulin G (IgG). Data are mean \pm SEM of each sample run in triplicate. Dunnett’s multiple comparison test using control monocytes as control, ns, non-significant, $**P < 0.01$. **b** siRNA-mediated downregulation of EGR1 in *trTET2*^{MUT} monocytes isolated from CMML3549 (purple) and CMML3565 (blue) with 2 and 3 sets of *EGR1*-siRNA respectively. RT-qPCR analysis of *EGR1* and *MIF* mRNA expression normalized to *RPL32*. Data are mean \pm SEM of each sample run in triplicate. Unpaired Student’s-t test using control monocytes transfected with SCR as control, $**P < 0.01$. **c** Quantitative ChIP-PCR analyses of *MIF* promoter performed in 2 controls, one *TET2*^{WT} CMML and 2 *trTET2*^{MUT} CMML using antibodies against HDAC1 and HDAC2, focusing on R4 of *MIF* gene. Data are mean \pm SEM of each sample run in duplicate or triplicate. Dunnett’s multiple comparison test using control monocytes as control, ns non-significant, $*P < 0.05$, $**P < 0.01$. **d** 293T cells were transfected using Lipofectamine 2000 with empty vectors or pcDNA3-EGR1 and pcDNA3-HA-TET2. Immunoblot analysis of EGR1 and HA expression in transfected 293T cell. Actin, loading control. **e** Co-immunoprecipitation experiments in 293T-transfected cells. An anti-HA (HA 16B12, Covance), an anti-EGR1 (303-390 A, Bethyl Laboratories) or a control IgG were used for IP, followed by immunoblotting with anti-EGR1, anti-HA, and HDAC1 (#39531, Active Motif).

mouse model of human chronic myelogenous leukemia in which IL-6 secreted by mature myeloid cells contributes to leukemic progenitor cell development^{59,60}. *Tet2* is upregulated by innate immune cells challenged with LPS and its knockdown delays inflammation resolution by precluding the repression of inflammatory cytokines as demonstrated for IL-6^{13,33}. In the context of HIV-1 infection, *TET2* protein degradation via Vpr protein similarly sustains IL-6 production, which enhances virus replication⁵³. We noticed that, in the absence of additional infectious or inflammatory stimulus, MIF is the main cytokine overproduced by *Tet2*-deleted mice, as well as *TET2*

downregulated leukemic cells and *TET2*-mutated CMML monocytes. Many parameters may influence the level of *TET2* gene expression. In CMML patients with a *TET2* variant, truncation of the protein and the VAF of the mutated allele play essential roles. *TET2* gene expression can also be decreased in the absence of gene mutation through poorly understood mechanisms. The multiple effects of MIF on immune cells have been largely investigated⁶¹. However, MIF effects on hematopoietic stem and progenitor cells remain poorly understood. In a mouse retroviral model of AML, MIF produced by a *FLT3*-mutated subclone was observed to favor the expansion of leukemia-

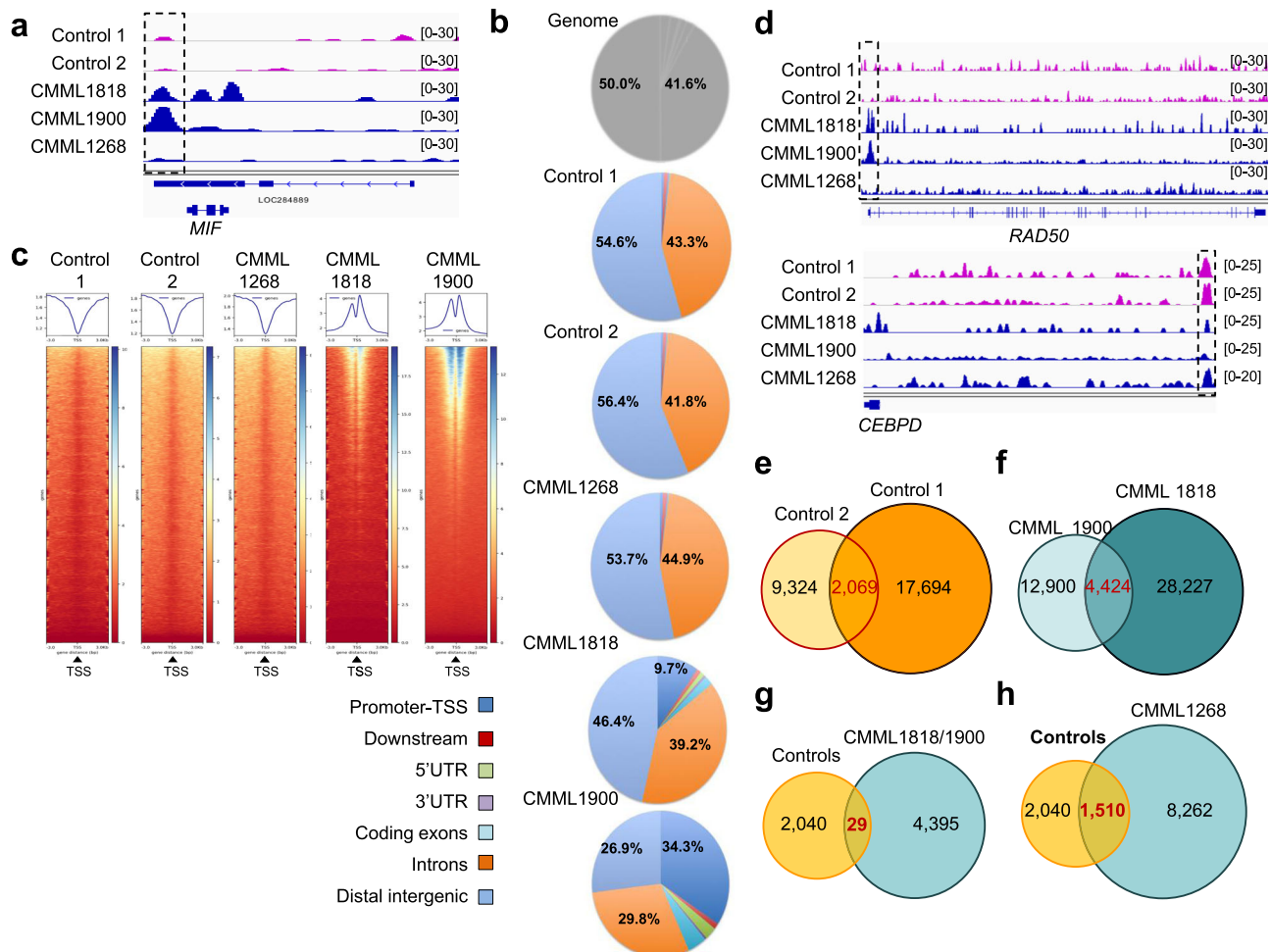


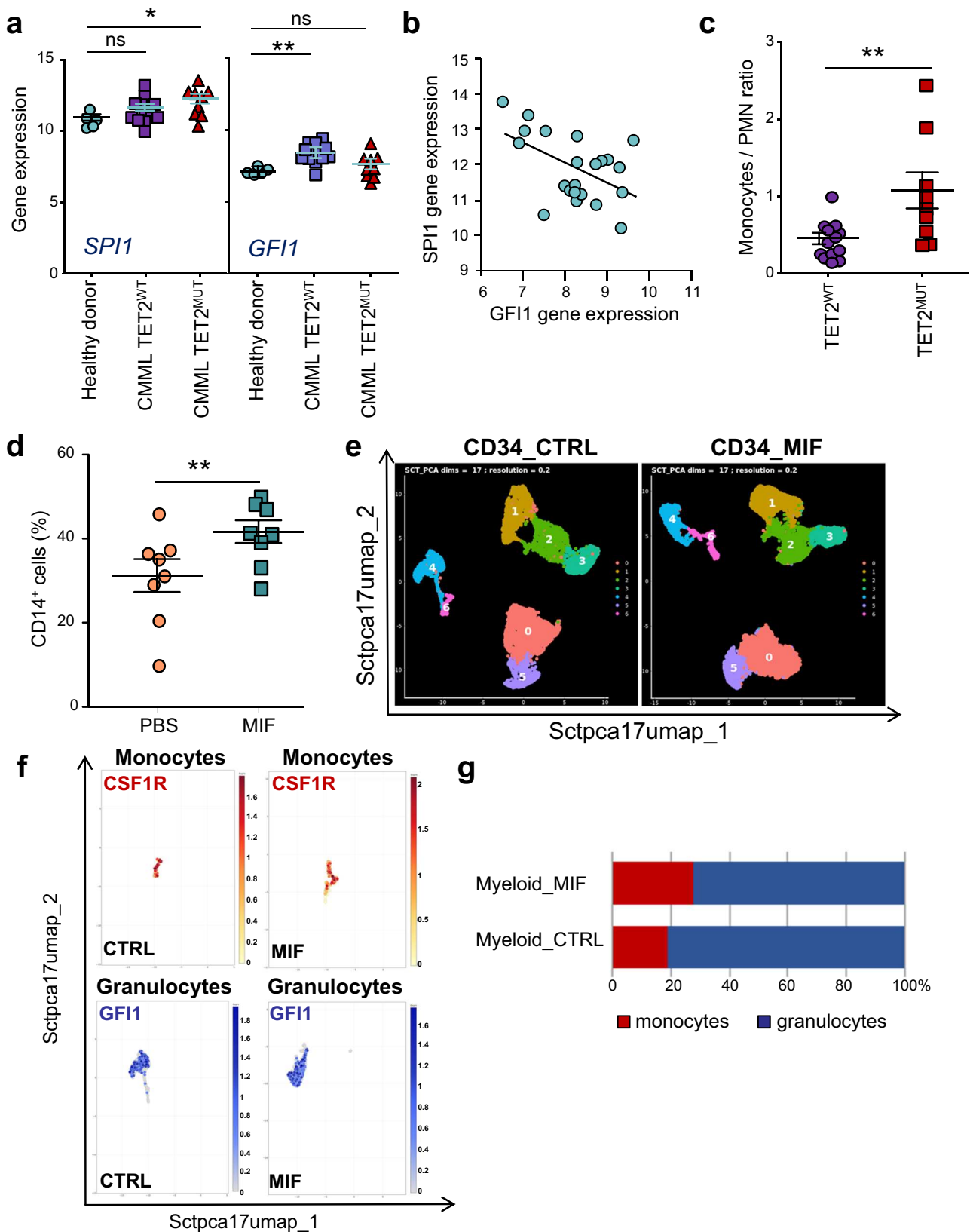
Fig. 6 EGR1 recruitment to DNA is modified by TET2 mutation in monocytes. ChIP-seq experiments were performed using an anti-EGR1 antibody in sorted peripheral blood monocytes from 2 healthy donors (control 1 and 2) and three CMML patients (CMML1268: *TET2* H800fs, VAF = 16%; CMML1818: *TET2* A1241fsX1, VAF = 40% and *TET2* E1513GfsX9, VAF = 49%; CMML1900: *TET2* W564X, VAF = 100%). **a** Peak calling for EGR1 on *MIF* gene in controls (pink) and CMML (dark blue). **b** Repartition of EGR1 peaks on the genome compared to hg19 reference annotation (genome). **c** Ranking heatmaps of EGR1 peaks centered on gene transcription starting sites (TSS). **d** Peak calling for EGR1 in control (pink) and CMML (dark blue) monocytes on *RAD50* and *CEBPD* genes. **e** Venn diagram of peak calling in two healthy donors and **f** two CMML samples with high VAF *trTET2*^{MUT}. **g** Venn diagram of peak calling comparing two controls and two CMML samples with high VAF *trTET2*^{MUT} or **h** with the CMML sample with low VAF *trTET2*^{MUT}.

initiating cells⁶². Here, we show that, when added to human healthy CD34⁺ cells in culture, MIF promotes their differentiation into monocytes, suggesting an autocrine/paracrine feedback loop in which MIF produced by mature clonal cells creates a microenvironment that promotes the development of a myeloproliferative syndrome.

MIF levels rise during infectious, inflammatory, and autoimmune diseases. MIF promotes carcinogenesis and plays a central role in atherosclerosis pathogenesis^{41,63}. The atherogenic process is initiated by endothelial dysfunction followed by an accumulation of oxidized low-density lipoproteins and an inflammatory cell infiltrate in which monocytes dominate⁶⁴. In mouse models, *Tet2* deletion accelerates atherosclerosis through enhanced secretion of cytokines, i.e., IL-8 and IL-1 β ^{30,65} whereas *Mif* deletion reduces the aortic inflammatory response⁴⁴. The overexpression of MIF in *TET2*-mutated monocytes, identified in the present study, could both promote the recruitment of additional inflammatory cells in atherosclerotic lesions and favor the monocyte production of IL-1 β in response to inflammatory stimuli through its direct role in NLRP3 inflammasome activation^{47,61}. Of note, a G/C single-nucleotide polymorphism

(rs755622) at position -173 of human *MIF* gene is associated to a higher susceptibility to develop coronary diseases and cancers^{43,66}. It would be worth determining whether this polymorphism impacts the outcome of *TET2*-mutated CHIP, including the development of coronary diseases and overt myeloid malignancies.

Together, we have shown that *TET2*-truncating mutations allows *MIF* gene expression through a catalytic domain-independent mechanism and provoke a chronic MIF overproduction by peripheral blood monocytes, in the absence of inflammatory signals. MIF could potentially promote the development of a myeloproliferative syndrome while it may accelerate atherosclerosis development. Anti-MIF antibodies were shown to exert protective effects in models of sepsis⁶⁷. Imalumab (BAX69), one of these humanized anti-MIF monoclonal antibodies, has completed phase I and II clinical testing with acceptable toxicity⁶⁸. As *TET2* by itself is hardly druggable, targeting MIF should be considered as an alternative therapeutic strategy for preventing the development of atherosclerotic lesions and chronic myeloid malignancies in individuals with *TET2*-truncating mutations.



Methods

In vitro granulo-monocyte differentiation. Umbilical cord blood samples were collected from healthy newborns with mother consent: AP-HP, Hôpital Saint-Louis, Unité de Thérapie Cellulaire, CRB-Banque de Sang de Cordon, Paris, France - N° d'autorisation: AC-2016-2759. Sorted CD34⁺ cells, using magnetic beads and AutoMacs system (Miltenyi Biotec), were depleted for *TET2* and subjected to monocyte/macrophage differentiation as previously described⁴⁸. Supernatants were

collected at indicated day. For Fig. 7d, sorted CD34⁺ were cultured 24 h at 1 × 10⁶ cells/mL in MEM-alpha medium (Thermo Fisher Scientific) with 10% heat inactivated fetal bovine serum (FBS), 1% penicillin/streptomycin and 2 mM L-Glutamine (Thermo Fisher Scientific), stem cell factor recombinant (SCF, 50 ng/mL; Immunex), interleukin-3 recombinant (IL-3, 10 ng/mL; Novartis), IL-6 recombinant (10 ng/mL; Peprotech), thrombopoietin recombinant (TPO, 10 ng/mL; Peprotech), Fms-like tyrosine kinase 3 recombinant (FLT-3, 50 ng/mL;

Fig. 7 MIF promotes monocyte differentiation. **a** CD34⁺ cells were sorted from the peripheral blood of healthy donor ($n = 5$) and CMML patients, without ($n = 13$) or with TET2 ($n = 9$) mutation, and analyzed by Agilent microarrays. Indicated gene expression was monitored (Log2 intensity). Data are mean \pm SEM of indicated biological samples. Dunnett's multiple comparison test using healthy donor as control, * $P < 0.05$, ns, non-significant. **b** Inverse correlation between *SPI1* and *GFI1* Log2 intensity expression in CD34⁺ samples. $R^2 = 0.24$; $p = 0.02$. **c** Monocytes to polymorphonuclear cell ratio in the peripheral blood of CMML patients without ($n = 13$) or with TET2 ($n = 9$) mutation. Mann-Whitney test: ** $P < 0.005$. **d** Cord blood CD34⁺ cells were induced to differentiate as described in Fig. 1, in the presence or the absence of 20 ng/mL MIF for 5–9 days before measuring the fraction of CD14⁺ cells by flow cytometry. Data are mean \pm SEM from eight independent experiments; Mann-Whitney test: ** $P < 0.005$. **e** Single cell analysis of cord blood CD34⁺ cells induced to differentiate as described in Fig. 1 with or without MIF at day 7. Ummaps of cluster analysis at 17 dimensions with a resolution of 0.2. **f** Ummaps of *CSF1R* and *GFI1* gene expressions in monocyte and granulocyte clusters in CTRL and MIF conditions. Scales indicate the intensity of expression. **g** Percentage of monocytes and granulocytes in the myeloid compartment in CTRL and MIF conditions.

Diaclone), granulocyte colony-stimulating factor recombinant (G-CSF 10 ng/mL; Peprotech), in a 37 °C incubator with 5% CO₂. MIF (289-MF-002, R&D systems; 20 ng/mL every two days) was added to medium for 5–9 days before immunophenotypic analysis (Supplementary Table 5 for antibodies) using a Fortessa flow cytometer (BD biosciences). Analysis was performed using Kaluza Software (Beckman Coulter) with gating strategy indicated in (Supplementary Fig. 11).

Cytokine profiler array and MIF immunoassay. CD34⁺ collected supernatants were analyzed using human cytokine antibody array (panel A; R&D Systems). MIF concentrations were quantified by ELISA (human: MIF Quantikine ELISA Kit, R&D Systems; mouse: Mif USCNK life Science). Supernatants from fresh bone marrow samples were frozen at -80 °C until MIF analysis using mesoscale technology (Meso Scale Diagnostics). CD14⁺ monocyte cells were plated at 500,000 cells/ml in serum free RPMI medium during 18 h and collected supernatants were stored at -80 °C until analysis with human cytokine antibody array (panel A; R&D Systems). Serum samples from age-matched controls and TET2-mutated CMML patients were collected and stored at -80 °C until analysis with human cytokine antibody array (panel A; R&D Systems).

Cell culture and derivation of TET2 deficient cell lines. Kasumi-1 (CRL-2724), M07e (CRL-7442), TF-1 (CRL-2003) cell lines (American Type Culture Collection), and UT-7 (kindly provided by Dr. Patrick Mayeux, Cochin Hospital, Paris, France), which all express wildtype *TET2*, were maintained in RPMI1640 or MEMA medium (Gibco) with 10 mM L-Glutamine, 10 mM penicillin-streptomycin and 20% (v/v) FBS (Hyclone) with or without GM-CSF (100 ng/mL, Peprotech). Modified cell lines with shRNA-TET2 (5'-GGGTAAGCCAAAGAAAGAAA-3') or shRNA-scramble (SCR) (5'-GCCGCGAGCTAGCGACGCCAT-3') were previously described⁴⁸.

Immunoblotting. Cells were lysed for 15 min at 4 °C in lysis buffer (150 mM NaCl, 50 mM TRIS pH 7.8, 1% NP40, 2 mM EDTA, 1 mM PMSF, NAF, orthovanadate, Protease Inhibitor Complete (Roche)). 15 μ g of total proteins were separated on polyacrylamide gel and transferred to nitrocellulose membrane (ThermoFisher Scientific). Membranes were blocked with 5% bovine serum albumin in PBS, with 0.1% Tween-20 (Sigma-Aldrich) for 40 min at RT, incubated overnight at 4 °C with the primary antibodies (dilution 1/1000e), washed in PBS-0.1% Tween-20, incubated further with HRP-conjugated secondary antibody (400 ng/mL) for 1 h at RT and washed again before analysis using Immobilon Western Chemiluminescent HRP Substrate system (Millipore). Actin (A5441, Sigma-Aldrich) and MIF (sc-80191, Santa Cruz Biotechnology) antibodies were used for detection and quantification were performed using Image J software (<http://imagej.nih.gov/ij>).

RT-qPCR analysis. Total RNA was extracted with RNeasy Mini Kit (Qiagen) and reverse transcribed with SuperScript II reverse transcriptase (Thermo Fisher Scientific) with random hexamers (Thermo Fisher Scientific). Real-time quantitative polymerase chain reaction (RT-qPCR) was performed with AmpliTaq Gold polymerase in an Applied Biosystems 7500 thermocycler using the standard SyBR Green detection protocol as outlined by the manufacturer (Thermo Fisher Scientific). Briefly, 12 ng of total cDNA, 50 nM (each) primers, and 1 \times SyBR Green mixture were used in a total volume of 20 μ L. Primers are listed in Supplementary Table 5.

Mice. Animal experiments were conducted according to Gustave Roussy guidelines and authorized by the Direction Départementale des Services Vétérinaires du Val-de-Marne. Animal experiments were performed in accordance with 2010/63/UE European legislation and decree n°2013-118 of French legislation and recorded under protocol number APAFIS# 2012-018-16-540 and 2016-104-7171. Mouse models were previously described²⁰. Blood collection from young mice (1–3 months) was performed under anesthesia with isoflurane at Gustave Roussy animal core facility. Complete blood counts were obtained using MS9-5 hematology analyzer (Melet Schloesing Technologies). Bone marrow cells were flushed and centrifuged at 1500 rpm for 10 min at RT before collecting supernatant that was frozen at -80 °C.

Control and patient sample collection. Peripheral blood samples were collected from healthy donors (Buffy coat, Etablissement Français du Sang, Rungis, France), age-matched donors, and CMML patients with informed consents in compliance with the ethical committee Ile-de-France (MYELOMONO cohort, DC-2014-2091). CMML patients were diagnosed according to the latest 2016 WHO criteria⁶⁹. CD14⁺ monocytes were isolated as previously described and all patient monocyte DNA included in the study were subjected to next generation sequencing (NGS) for a myeloid panel, by previously described methods⁵⁰. CMML patient clinicobiological characteristics, summarized in Tables S2–S4 and S9, were obtained from our CMML database with clinical/biological annotations (DR-2016-256) and NGS analysis of a previously described panel¹⁹.

RNA sequencing and analysis. 10 age-matched healthy CD14⁺/CD16⁻ monocyte and 60 CMML CD14⁺ monocyte RNAs were used to prepare libraries with Illumina TruSeq RNA Sample Prep Kit v2. All controls and CMML patients were subjected to NGS myeloid panel sequencing⁵⁰. RNA-seq libraries were sequenced on a 75 bp pair-end run using NextSeq500 High Output Kit 150 cycles; 400 M flow cell. All transcripts from Gencode V24 were quantified for each library in transcript per million (TPM) units using the Kallisto software. Gene-level expression was then calculated as the sum of TPM values for each gene transcript. Heatmap was generated using Morpheus (<https://software.broadinstitute.org/morpheus/>). For cord blood CD34⁺ cell RNA-seq differentiated in presence or not of MIF, the RNA integrity (RNA Integrity Score ≥ 7.0) was checked on the Agilent Fragment Analyzer (Agilent) and quantity was determined using Qubit (Invitrogen). SureSelect Automated Strand Specific RNA Library Preparation Kit was used according to the manufacturer's instructions with the Bravo Platform. Briefly, 50–100 ng of total RNA sample was used for poly-A mRNA selection using oligo(dT) beads and subjected to thermal mRNA fragmentation. The fragmented mRNA samples were subjected to cDNA synthesis and were further converted into double stranded DNA using the reagents supplied in the kit, and the resulting dsDNA was used for library preparation. The final libraries were bar-coded, purified, pooled together in equal concentrations, and subjected to paired-end sequencing on Novaseq-6000 sequencer (Illumina) at Gustave Roussy. We used CIBERSORT, an analytical tool from the Alizadeh Laboratory developed by Newman et al. to provide an estimation of the abundances of member cell types in a mixed cell population, using gene expression data⁷⁰.

Chromatin immunoprecipitation (ChIP) and ChIP-seq. ChIP experiments were performed using ChIP-IT kit (Active Motif) as previously described⁷¹. 5 μ g of antibodies (Supplementary Table 5) were used. Immunoprecipitated-chromatin was eluted after several washes, reverse cross-linked and stored at -20 °C. ChIP-qPCRs were performed in the same way as RT-qPCR with 2 μ L of ChIP or IgG samples instead of cDNA. Primer sequences are in Supplementary Table 5. Enriched DNA from EGR1-ChIP and input DNA fragments were used to generate libraries as previously described⁷¹. Fifty-cycle single-end sequencings were performed using HiSeq 2000 (Illumina). Reads were aligned using human genome hg19 with BWA (v0.7.5a), peak calling assessed using MACS 2.0, and annotation done with HOMER (v4.7.2), with a p value of 0.01. Integrative Genomics Viewer (IGV 2.1) was used for representation.

Luciferase assays. Kasumi-1 cells were transfected with the reporter plasmid pGL3-MIF-luc and diverse fragments of *MIF* gene promoter⁵¹, with or without mutation in transcription factor binding motifs (Supplementary Table 5) and co-transfected with TK-Renilla reporter for normalization of transfection efficiency. Using dual luciferase assay, luciferase activity was measured with a luminometer (Promega).

Bisulfite DNA treatment and sequencing. Genomic DNA was isolated from monocytes of controls or CMML patients using QIAGEN's standard procedures. Two hundred nanograms of total genomic DNA was modified by bisulfite treatment according to the manufacturer's instructions (MethylIDetector, Active Motif). Converted *MIF* promoter was identified by PCR with converted primers forward

(5'-GGTGATTTAGTGAAAGGATTAAGAA-3') and reverse (5'-CATAATAACA AAAAAACCAAAAACCC-3'), and direct sequencing reaction was performed using standard conditions according to the manufacturer's instructions (Applied Biosystems).

EGR1 knockdown in TET2-truncated mutated CMML monocytes. We used Stealth siRNA duplex (ThermoFisher Scientific) targeting EGR1 and Stealth RNAi™ siRNA negative control (SCR) introduced into CMML monocytes by Lipofectamine 2000 (ThermoFisher Scientific). Briefly, 1×10^6 cells in 500 μ l in a 24-well plate were transfected according to the manufacturer's recommendations with 1 nmol siRNA.

siRNAs targeting EGR1:

siEGR1-1: 5'-UCUCCCAGGACAAUUGAAAUUUGCU-3';

siEGR1-2: 5'-AGCAAUUUCAUUUGCCUGGAGA-3';

siEGR1-3: 5'-GAUCUCUGACCCGUUCGGAUCCUUU-3'.

Knock-down efficacy was performed using RT-qPCR and MIF expression was addressed.

293 T transient transfection. 293T cells were transfected with pcDNA3-HA-TET2 and pcDNA3-EGR1 plasmids using Lipofectamine 2000 according to the manufacturer's recommendation. 72 h after transfection, cells were harvested and lysed 20 min on ice in a buffer containing 50 mM Tris pH 7.4, 150 mM NaCl, 1% NP-40, 10% Glycerol, 1 mM Na₃VO₄, and 1 \times Protease inhibitor cocktail (100 μ l for 10×10^6 cells). Samples were then centrifuged 15 min at 4 °C at 18,000 \times g and the supernatant containing the proteins was collected. 10 μ g of anti-HA or anti-EGR1 antibody or negative control IgG were added and the samples incubated one night at 4 °C with agitation. 100 μ l of Protein A/G PLUS-Agarose (Santa Cruz biotechnology) were incubated with the samples during 2 h at 4 °C with agitation. The complexes were then precipitated with 1 min centrifugation at 2000 \times g and the supernatant was removed. The remaining beads were washed 5 times with 500 μ l of lysis buffer without NP-40. 2 \times Laemmli with 0.1 M DTT and Protease inhibitor cocktail 1 \times was added on the beads and outputs. The samples were then boiled 10 min at 95 °C. Following SDS-PAGE and blotting, the membranes were incubated with either an anti-EGR1 antibody, an anti-HA, or an anti-HDAC1 antibody.

Immunoprecipitation experiment in primary monocytes. CD14⁺ cells were lysed 20 min on ice in a buffer containing 50 mM Tris pH 7.4, 150 mM NaCl, 1% NP-40, 10% Glycerol, 1 mM Na₃VO₄ and 1 \times Protease inhibitor cocktail (100 μ l for 10×10^6 cells). Samples were then centrifuged 15 min at 4 °C at 18,000 \times g and the supernatant containing the proteins was collected. 10 μ g of anti-HDAC1 (#39531, Active Motif) or an anti-HDAC2 (#39533, Active Motif) or negative control IgG were added for 10×10^6 cells and the samples incubated one night at 4 °C under agitation. One hundred μ l of Protein A/G PLUS-Agarose (Santa Cruz Biotechnology) were incubated with the samples during 2 h at 4 °C under agitation. The beads were washed 5 times with 500 μ l of lysis buffer without NP-40. 2 \times Laemmli with 0.1 M DTT and Protease inhibitor cocktail 1 \times was added on the beads and boiled 10 min at 95 °C. Proteins were separated on polyacrylamide gel and transferred to nitrocellulose membrane (ThermoFisher Scientific). Membranes were blocked with 5% bovine serum albumin in PBS, with 0.1% Tween-20 (Sigma-Aldrich) for 40 min at RT, incubated overnight at 4 °C with anti-TET2 antibody (sc-136926, Santa Cruz biotechnology) (dilution 1/1000e), washed in PBS-0.1% Tween-20, incubated further with HRP-conjugated secondary antibody (400 ng/ml) for 1 h at RT and washed again before analysis using Immobilon Western Chemiluminescent HRP Substrate system (Millipore, Molsheim, France).

Gene expression microarray analysis. Gene expression in purified CD34⁺ cells was analyzed with Agilent® SurePrint G3 Human GE 8x60K Microarray (Agilent Technologies, AMADID-28004). After single color hybridization and array scanning, microarray images were analyzed using Feature Extraction software version (10.7.3.1) with default settings. Using LIMMA R package, data were normalized by the quantile method and analyzed (for single value of each transcript, the mean of each replicated probe was taken).

3' Single-cell RNAseq. Sample preparation was done at room temperature. Single-cell suspensions were loaded onto a Chromium Single Cell Chip (10x Genomics) according to the manufacturer's instructions for co-encapsulation with barcoded Gel Beads at a target capture rate of ~10,000 individual cells per sample. Captured mRNAs were barcoded during cDNA synthesis using the Chromium Next GEM Single Cell 3' GEM, Library & Gel Bead Kit v3.1 (10x Genomics) according to the manufacturer's instructions. All samples were processed simultaneously with the Chromium Controller (10x Genomics) and the resulting libraries were prepared in parallel in a single batch. We pooled all of the libraries for sequencing in a single SP Illumina flow cell. All of the libraries were sequenced with an 8-base index read, a 28-base Read1 containing cell-identifying barcodes and unique molecular identifiers (UMIs), and a 91-base Read2 containing transcript sequences on an Illumina NovaSeq 6000.

3' Single-cell RNAseq analysis. Raw BCL-files were demultiplexed and converted to Fastq format using bcl2fastq (version 2.20.0.422 from Illumina). Reads quality control was performed using fastqc (version 0.11.9) and assignment to the expected genome species evaluated with fastq-screen (version 0.14.0). Reads were pseudo-mapped to the Ensembl reference transcriptome v99 corresponding to the homo sapiens GRCh38 build with kallisto (version 0.46.2) using its 'bus' subcommand and parameters corresponding to the 10X Chromium 3' scRNA-Seq v3 chemistry. The index was made with the kb-python (version 0.24.4) wrapper of kallisto. Barcode correction using whitelist provided by the manufacturer (10X Genomics) and gene-based reads quantification was performed with BUStools (version 0.40.0).

Cell barcode by symbol count table was loaded in R (version 4.0.4) using the BUSpaRse package (version 1.5.3). To call real cells from empty droplets, we used the emptyDrops function from the dropletUtils package (version 1.10.3), which assesses whether the RNA content associated with a cell barcode is significantly distinct from the ambient background RNA present within each sample. Barcodes with p -value < 0.001 (Benjamini–Hochberg-corrected) were considered as legitimate cells for further analysis.

The count matrix was filtered to exclude genes detected in less than five cells, cells with less than 1500 UMIs or less than 200 detected genes, as well as cells with mitochondrial transcripts proportion higher than 20%. Cell cycle scoring of each cell was performed using two methods: the CellcycleScoring function from the Seurat package (version 4.0.0), and the cyclone function from Scran (version 1.18.5). Barcodes corresponding to doublets were identified and discarded using the union of two methods: scDblFinder (version 1.4.0) using default parameters, and scds (version 1.6.0) with its hybrid method using default parameters. We manually verified that the cells identified as doublets did not systematically correspond to cells in G2M phase.

Seurat (version 4.0.0) was applied for further data processing. The SCTransform normalization method was used to normalize, scale, select 3000 Highly Variable Genes and regress out bias factors (the number of detected transcripts and the proportion of ribosomal transcripts). The number of PCA dimensions to keep for further analysis was evaluated by assessing a range of reduced PCA spaces using 3 to 49 dimensions, with a step of 2. For each generated PCA space, Louvain clustering of cells was performed using a range of values for the resolution parameter from 0.1 to 1.2 with a step of 0.1. The optimal space was manually evaluated as the one combination of kept dimensions and clustering resolution resolving the best structure (clusters homogeneity and compacity) in a Uniform Manifold Approximation and Projection space (UMAP). For the 2 samples, 17 dimensions were retained with a resolution of 0.2.

Marker genes for Louvain clusters were identified through a «one versus others» differential analysis using the Wilcoxon test through the FindAllMarkers function from Seurat, considering only genes with a minimum log fold-change of 0.5 in at least 75% of cells from one of the groups compared, and FDR-adjusted p -values < 0.05 (Benjamini–Hochberg method). Gene visualization was done using Cerebro (version 1.2.2).

Statistics and reproducibility. All statistical analyses (Paired and unpaired t-tests, Mann–Whitney test, Dunnett's multiple comparison tests, correlation using linear regression) were performed using Graph-Pad Prism software (GraphPad Software, La Jolla, CA, USA). Values of p < 0.05 were considered statistically significant. The used statistical test, the p value, and the number of samples are indicated in the respective Figure legends.

Data availability

Control and CMML monocyte RNA-seq data have been deposited in the NCBI Gene Expression Omnibus database under accession number GSE165305 and GSE188624. ChIP-seq datasets are available in the Array Express database at EMBL-EBI (www.ebi.ac.uk/arrayexpress) under accession number E-MTAB_6305 and E-MTAB_11132. Cord blood bulk RNAseq and scRNAseq have been deposited in the European Genome-Phenome archive (EGA) under accession number EGAS00001005814. The data that support the findings of this study are available within the article and its Supplementary Information and Supplementary Data files. Uncropped western blots are available in (Supplementary Fig. 12). The source data underlying all graphs and charts are provided as Supplementary Data 7.

Received: 22 March 2021; Accepted: 6 January 2022;

Published online: 03 February 2022

References

- Ito, S. et al. Tet proteins can convert 5-methylcytosine to 5-formylcytosine and 5-carboxylcytosine. *Science* **333**, 1300–1303 (2011).
- Lu, F., Liu, Y., Jiang, L., Yamaguchi, S. & Zhang, Y. Role of Tet proteins in enhancer activity and telomere elongation. *Genes Dev.* **28**, 2103–2119 (2014).
- Wu, X. & Zhang, Y. TET-mediated active DNA demethylation: Mechanism, function and beyond. *Nat. Rev. Genet.* **18**, 517–534 (2017).
- Costa, Y. et al. NANOG-dependent function of TET1 and TET2 in establishment of pluripotency. *Nature* **495**, 370–374 (2013).

5. Lio, C. W. et al. Tet2 and Tet3 cooperate with B-lineage transcription factors to regulate DNA modification and chromatin accessibility. *Elife* **5**, e18290 (2016).
6. de la Rica, L. et al. PU.1 target genes undergo Tet2-coupled demethylation and DNMT3b-mediated methylation in monocyte-to-osteoclast differentiation. *Genome Biol.* **14**, R99 (2013).
7. Mendes, K. et al. The epigenetic pioneer EGR2 initiates DNA demethylation in differentiating monocytes at both stable and transient binding sites | Enhanced Reader. *Nat. Commun.* **12**, 1556 (2021).
8. Rampal, R. et al. DNA hydroxymethylation profiling reveals that WT1 mutations result in loss of TET2 function in acute myeloid leukemia. *Cell Rep.* **9**, 1841–1855 (2014).
9. Wang, Y. et al. WT1 recruits TET2 to regulate its target gene expression and suppress leukemia cell proliferation. *Mol. Cell* **57**, 662–673 (2015).
10. Sun, Z. et al. EGR1 recruits TET1 to shape the brain methylome during development and upon neuronal activity. *Nat. Commun.* **10**, 3892 (2019).
11. Williams, K. et al. TET1 and hydroxymethylcytosine in transcription and DNA methylation fidelity. *Nature* **473**, 343–348 (2011).
12. Zhong, J. et al. TET1 modulates H4K16 acetylation by controlling auto-acetylation of hMOF to affect gene regulation and DNA repair function. *Nucleic Acids Res.* **45**, 672–684 (2017).
13. Zhang, Q. et al. Tet2 is required to resolve inflammation by recruiting Hdac2 to specifically repress IL-6. *Nature* **525**, 389–393 (2015).
14. Vella, P. et al. Tet proteins connect the O-linked N-acetylglucosamine transferase Ogt to chromatin in embryonic stem cells. *Mol. Cell* **49**, 645–656 (2013).
15. Chen, Q., Chen, Y., Bian, C., Fujiki, R. & Yu, X. TET2 promotes histone O-GlcNAcylation during gene transcription. *Nature* **493**, 561–564 (2013).
16. Deplus, R. et al. TET2 and TET3 regulate GlcNAcylation and H3K4 methylation through OGT and SET1/COMPASS. *EMBO J.* **32**, 645–655 (2013).
17. Delhommeau, F. et al. Mutation in TET2 in myeloid cancers. *N. Engl. J. Med.* **360**, 2289–2301 (2009).
18. Lio, C. W. J., Yuita, H. & Rao, A. Dysregulation of the TET family of epigenetic regulators in lymphoid and myeloid malignancies. *Blood* **134**, 1487–1497 (2019).
19. Coltro, G. et al. Clinical, molecular, and prognostic correlates of number, type, and functional localization of TET2 mutations in chronic myelomonocytic leukemia (CMML)—a study of 1084 patients. *Leukemia* **34**, 1407–1421 (2020).
20. Quivoron, C. et al. TET2 inactivation results in pleiotropic hematopoietic abnormalities in mouse and is a recurrent event during human lymphomagenesis. *Cancer Cell* **20**, 25–38 (2011).
21. Li, Z. et al. Deletion of Tet2 in mice leads to dysregulated hematopoietic stem cells and subsequent development of myeloid malignancies. *Blood* **118**, 4509–4518 (2011).
22. Ko, M. et al. Ten-eleven-translocation 2 (TET2) negatively regulates homeostasis and differentiation of hematopoietic stem cells in mice. *Proc. Natl Acad. Sci. USA* **108**, 14566–14571 (2011).
23. Moran-Crusio, K. et al. Tet2 loss leads to increased hematopoietic stem cell self-renewal and myeloid transformation. *Cancer Cell* **20**, 11–24 (2011).
24. Hirsch, P. et al. Genetic hierarchy and temporal variegation in the clonal history of acute myeloid leukaemia. *Nat. Commun.* **7**, 12475 (2016).
25. Makishima, H. et al. Dynamics of clonal evolution in myelodysplastic syndromes. *Nat. Genet.* **49**, 204–212 (2017).
26. Kunimoto, H. et al. Cooperative epigenetic remodeling by TET2 loss and NRAS mutation drives myeloid transformation and MEK inhibitor sensitivity. *Cancer Cell* **33**, 44–59.e8 (2018).
27. Busque, L. et al. Recurrent somatic TET2 mutations in normal elderly individuals with clonal hematopoiesis. *Nat. Genet.* **44**, 1179–1181 (2012).
28. Mason, C. C. et al. Age-related mutations and chronic myelomonocytic leukemia. *Leukemia* **30**, 906–913 (2016).
29. Jaiswal, S. et al. Age-related clonal hematopoiesis associated with adverse outcomes. *N. Engl. J. Med.* **371**, 2488–2498 (2014).
30. Fuster, J. J. et al. Clonal hematopoiesis associated with TET2 deficiency accelerates atherosclerosis development in mice. *Science* **355**, 842–847 (2017).
31. Wang, Y. et al. Tet2-mediated clonal hematopoiesis in nonconditioned mice accelerates age-associated cardiac dysfunction. *JCI Insight* **5**, e135204 (2020).
32. Jaiswal, S. & Libby, P. Clonal haematopoiesis: connecting ageing and inflammation in cardiovascular disease. *Nat. Rev. Cardiol.* **17**, 137–144 (2020).
33. Cull, A. H., Snetsinger, B., Buckstein, R., Wells, R. A. & Rauh, M. J. Tet2 restrains inflammatory gene expression in macrophages. *Exp. Hematol.* **55**, 56–70.e13 (2017).
34. Bick, A. G. et al. Genetic interleukin 6 signaling deficiency attenuates cardiovascular risk in clonal hematopoiesis. *Circulation* **141**, 124–131 (2020).
35. Sano, S. et al. Tet2-mediated clonal hematopoiesis accelerates heart failure through a mechanism involving the IL-1 β /NLRP3 inflammasome. *J. Am. Coll. Cardiol.* **71**, 875–886 (2018).
36. David, J. R. Delayed hypersensitivity in vitro: Its mediation by cell-free substances formed by lymphoid cell-antigen interaction. *Proc. Natl Acad. Sci. USA* **56**, 72–77 (1966).
37. Bloom, B. R. & Bennett, B. Mechanism of a reaction in vitro associated with delayed-type hypersensitivity. *Science* **153**, 80–82 (1966).
38. Calandra, T. & Roger, T. Macrophage migration inhibitory factor: A regulator of innate immunity. *Nat. Rev. Immunol.* **3**, 791–800 (2003).
39. Calandra, T. et al. MIF as a glucocorticoid-induced modulator of cytokine production. *Nature* **377**, 68–71 (1995).
40. Calandra, T. et al. Protection from septic shock by neutralization of macrophage migration inhibitory factor. *Nat. Med.* **6**, 164–170 (2000).
41. Bernhagen, J. et al. MIF is a noncognate ligand of CXC chemokine receptors in inflammatory and atherogenic cell recruitment. *Nat. Med.* **13**, 587–596 (2007).
42. Tilstam, P. V., Qi, D., Leng, L., Young, L. & Bucala, R. MIF family cytokines in cardiovascular diseases and prospects for precision-based therapeutics. *Expert Opin. Ther. Targets* **21**, 671–683 (2017).
43. Kang, I. & Bucala, R. The immunobiology of MIF: Function, genetics, and prospects for precision medicine. *Nat. Rev. Rheumatol.* **15**, 427–437 (2019).
44. Burger-Kentischer, A. et al. Reduction of the aortic inflammatory response in spontaneous atherosclerosis by blockade of macrophage migration inhibitory factor (MIF). *Atherosclerosis* **184**, 28–38 (2006).
45. Bozza, M. et al. Targeted disruption of migration inhibitory factor gene reveals its critical role in sepsis. *J. Exp. Med.* **189**, 341–346 (1999).
46. Mitchell, R. A. et al. Macrophage migration inhibitory factor (MIF) sustains macrophage proinflammatory function by inhibiting p53: Regulatory role in the innate immune response. *Proc. Natl Acad. Sci. USA* **99**, 345–350 (2002).
47. Shin, M. S. et al. Macrophage migration inhibitory factor regulates U1 small nuclear RNP immune complex-mediated activation of the NLRP3 inflammasome. *Arthritis Rheumatol.* **71**, 109–120 (2019).
48. Pronier, E. et al. Inhibition of TET2-mediated conversion of 5-methylcytosine to 5-hydroxymethylcytosine disturbs erythroid and granulomonocytic differentiation of human hematopoietic progenitors. *Blood* **118**, 2551–2555 (2011).
49. Itzykson, R. et al. Clonal architecture of chronic myelomonocytic leukemias. *Blood* **121**, 2186–2198 (2013).
50. Merlevede, J. et al. Mutation allele burden remains unchanged in chronic myelomonocytic leukaemia responding to hypomethylating agents. *Nat. Commun.* **7**, 10767 (2016).
51. Roger, T., Ding, X., Chanson, A. L., Renner, P. & Calandra, T. Regulation of constitutive and microbial pathogen-induced human macrophage migration inhibitory factor (MIF) gene expression. *Eur. J. Immunol.* **37**, 3509–3521 (2007).
52. Roger, T., David, J., Glauser, M. P. & Calandra, T. MIF regulates innate immune responses through modulation of Toll-like receptor 4. *Nature* **414**, 920–924 (2001).
53. Lv, L. et al. Vpr targets TET2 for degradation by CRL4 VprBP E3 ligase to sustain IL-6 expression and enhance HIV-1 replication. *Mol. Cell* **70**, 961–970.e5 (2018).
54. Carrillo-Jimenez, A. et al. TET2 regulates the neuroinflammatory response in microglia. *Cell Rep.* **29**, 697–713.e8 (2019).
55. Itoh, H. et al. TET2-dependent IL-6 induction mediated by the tumor microenvironment promotes tumor metastasis in osteosarcoma. *Oncogene* **37**, 2903–2920 (2018).
56. Lugin, J. et al. Histone deacetylase inhibitors repress macrophage migration inhibitory factor (MIF) expression by targeting MIF gene transcription through a local chromatin deacetylation. *Biochim. Biophys. Acta - Mol. Cell Res.* **1793**, 1749–1758 (2009).
57. Ko, M. et al. Modulation of TET2 expression and 5-methylcytosine oxidation by the CXXC domain protein IDAX. *Nature* **497**, 122–126 (2013).
58. Li, X. et al. Ten-eleven translocation 2 interacts with forkhead box O3 and regulates adult neurogenesis. *Nat. Commun.* **8**, 15903 (2017).
59. Reynaud, D. et al. IL-6 controls leukemic multipotent progenitor cell fate and contributes to chronic myelogenous leukemia development. *Cancer Cell* **20**, 661–673 (2011).
60. Welner, R. S. et al. Treatment of chronic myelogenous leukemia by blocking cytokine alterations found in normal stem and progenitor cells. *Cancer Cell* **27**, 671–681 (2015).
61. Harris, J., VanPatten, S., Deen, N. S., Al-Abed, Y. & Morand, E. F. Rediscovering MIF: New tricks for an old cytokine. *Trends Immunol.* **40**, 447–462 (2019).
62. Hyrenius-Wittsten, A. et al. De novo activating mutations drive clonal evolution and enhance clonal fitness in KMT2A-rearranged leukemia. *Nat. Commun.* **9**, 1770 (2018).
63. Morand, E. F., Leech, M. & Bernhagen, J. MIF: A new cytokine link between rheumatoid arthritis and atherosclerosis. *Nat. Rev. Drug Discov.* **5**, 399–410 (2006).

64. Sinitiski, D. et al. Macrophage migration inhibitory factor (MIF)-based therapeutic concepts in atherosclerosis and inflammation. *Thromb. Haemost.* **119**, 553–566 (2019).
65. Jaiswal, S. et al. Clonal Hematopoiesis and risk of atherosclerotic cardiovascular disease. *N. Engl. J. Med.* **377**, 111–121 (2017).
66. Illescas, O., Gomez-Verjan, J. C., García-Velázquez, L., Govezensky, T. & Rodriguez-Sosa, M. Macrophage migration inhibitory factor -173 G/C polymorphism: A global meta-analysis across the disease spectrum. *Front. Genet.* **9**, 55 (2018).
67. Kerschbaumer, R. J. et al. Neutralization of macrophage migration inhibitory factor (MIF) by fully human antibodies correlates with their specificity for the β -sheet structure of MIF. *J. Biol. Chem.* <https://doi.org/10.1074/jbc.M111.329664> (2012).
68. Mahalingam, D. et al. Phase I study of imalumab (BAX69), a fully human recombinant antioxidantized macrophage migration inhibitory factor antibody in advanced solid tumours. *Br. J. Clin. Pharmacol.* **86**, 1836–1848 (2020).
69. Arber, D. A. et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood* **127**, 2391–2405 (2016).
70. Newman, A. M. et al. Robust enumeration of cell subsets from tissue expression profiles. *Nat. Methods* **12**, 453–457 (2015).
71. Bencheikh, L. et al. Dynamic gene regulation by nuclear colony-stimulating factor 1 receptor in human monocytes and macrophages. *Nat. Commun.* **10**, 1935 (2019).

Acknowledgements

This work was supported by INSERM and by the Ligue contre le cancer (labeled team to F.P.). F.D. received a PRTK-16-122 with N.D. as partner. 'Taxe d'apprentissage' program Genomic Core Facilities - TA2012 to R.I. A.I. was supported by PRTK-16-122 and then by a grant from the Ligue Nationale Contre le Cancer. We thank Bastien Job for bulk RNA-seq analysis. M.F. and M.A. are supported by SIRIC SOCRATE INCa-DGOS-Inserm_1255. Part of high-throughput sequencing was performed by the genomic platform of Gustave Roussy, which is supported by Cancéropole Ile de France and by the SIRIC SOCRATE program. The authors thank Margot Morabito and Jeffie Lafosse for collecting clinical and biological annotations on CMML patients, and physicians and patients of the Groupe Francophone des Myélodysplasies for providing samples. We thank for umbilical cord blood samples Hôpital Saint-Louis, Unité de Thérapie Cellulaire, CRB-Banque de Sang de Cordon, Paris, France - N° d'autorisation: AC-2016-2759. The authors are grateful to Gustave Roussy Animal core facility and cytometry core facility for their helpful assistance in experiments. T.R. is supported by the Swiss National Science Foundation (SNSF grant number 310030_173123).

Author contributions

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Competing interests

The authors declare no competing interests.

Additional information

Supplementary information The online version contains supplementary material available at <https://doi.org/10.1038/s42003-022-03057-w>.

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Peer review information *Communications Biology* thanks Reuben Kapur and the other, anonymous, reviewers for their contribution to the peer review of this work. Primary Handling Editors: Vivian Lui and Karli Montague-Cardoso. Peer reviewer reports are available.

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Macrophage migration inhibitory factor is overproduced through EGR1 in *TET2*^{low} resting monocytes

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Supplementary Figures:

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Supplementary Figure 10. Identification of the 7 clusters in CTRL and MIF cultures.

Supplementary Figure 11. Gating strategy for monocyte and granulocyte identification.

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Supplementary Tables:

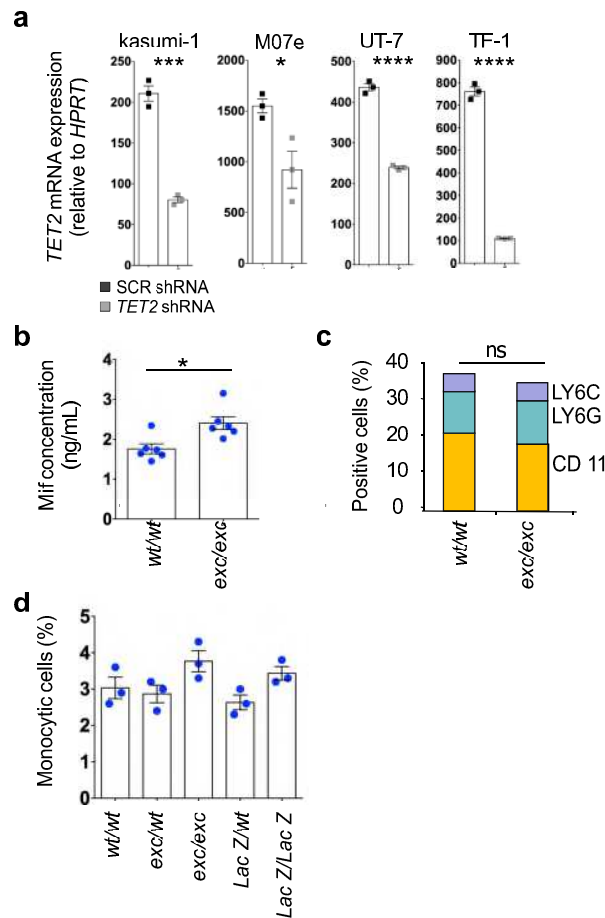
Supplementary Table 1: Characteristic of CMML patients whose sorted monocytes were analyzed by RNA sequencing

Supplementary Table 2: Independent cohort of CMML patients used for RT-qPCR analysis of *MIF* gene expression in sorted peripheral blood monocytes

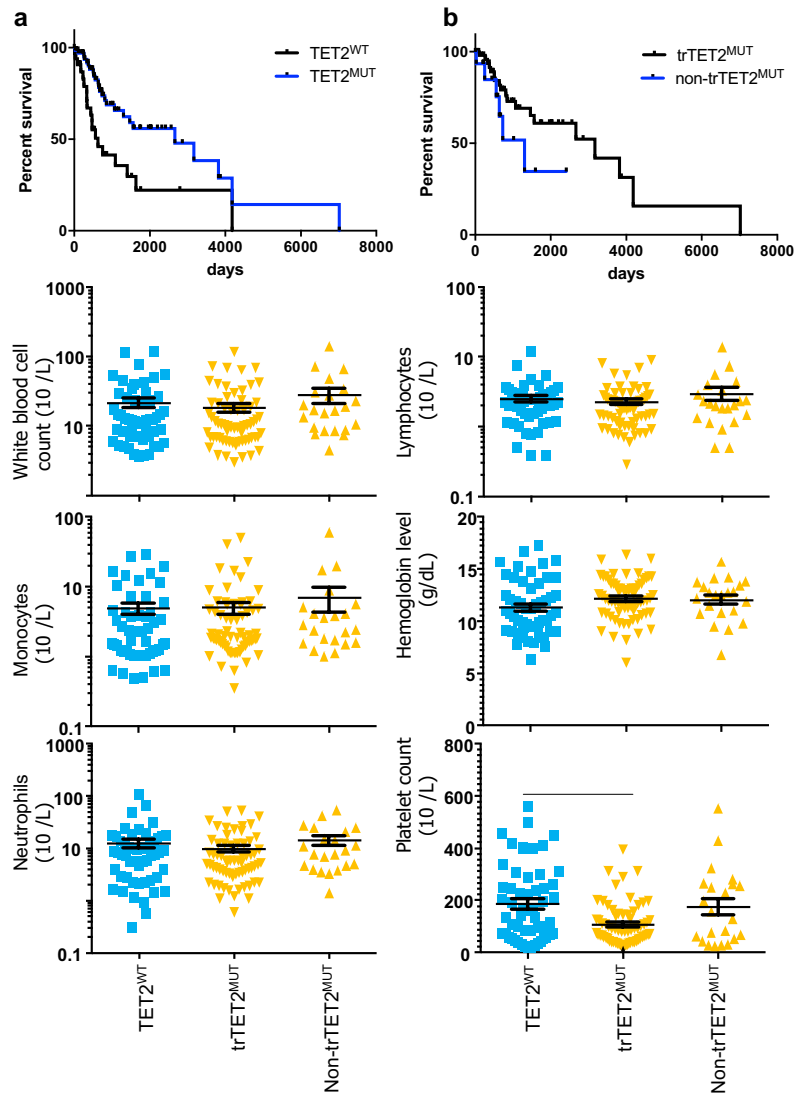
Supplementary Table 3: Characteristic of CMML patients whose bone marrow aspirates were used to quantify MIF

Supplementary Table 4: Cohort of CMML patients whom sorted CD34⁺ cells were analyzed by agilent arrays for gene expression

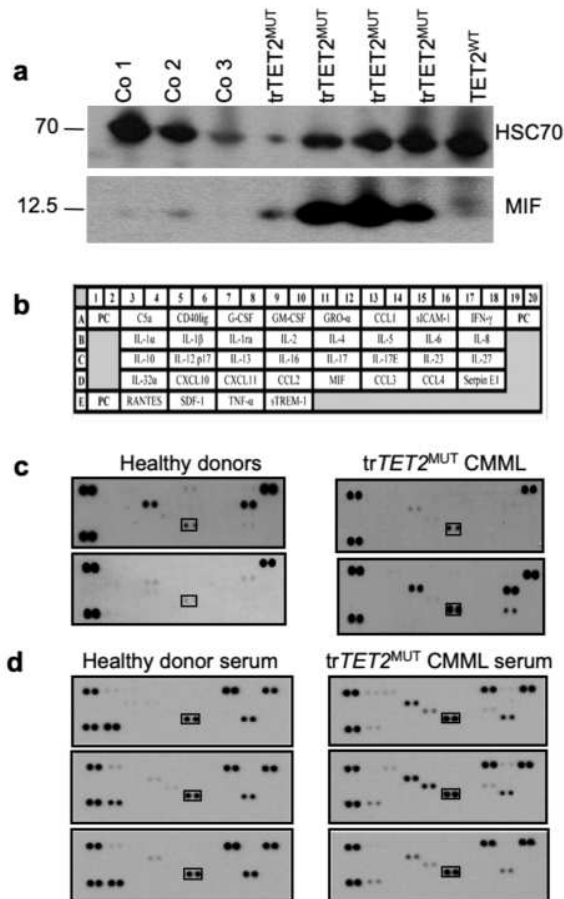
Supplementary Table 5: Primers and antibodies used in this study



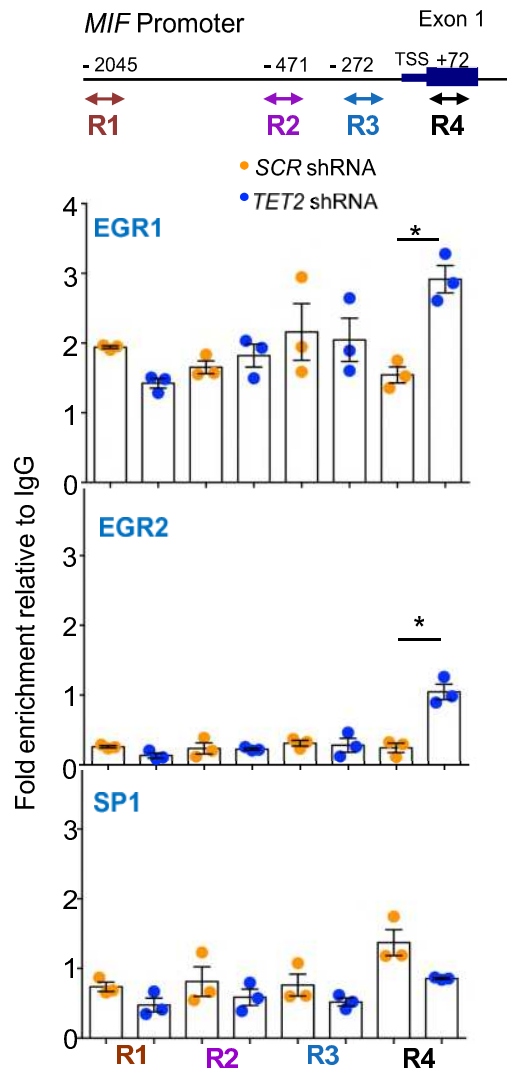
Supplementary Figure 1. MIF increase is detected in *Tet2*-KO mice before any blood cell count change. (a) RT-qPCR analysis of *TET2* mRNA expression in four *TET2*-depleted (*TET2* shRNA, gray bars) and control (SCR shRNA, black bars) human leukemic cell lines. Data are mean +/- SEM of 3 biological replicates. Unpaired t test: * $P < 0.05$; *** $P < 0.001$; **** $P < 0.0001$. (b) ELISA quantification of MIF concentration (ng/mL) in bone marrow of young (1-3 months) *Tet2*-knockout K427 animals (wt/wt or *Tet2*^{+/+} and exc/exc or *Tet2*^{-/-}). Data are mean +/- SEM of 6 different animals. Unpaired t test * $P < 0.05$. (c) Flow cytometry analysis of CD11b⁺ (Yellow), Ly6G⁺ (blue) and Ly6C⁺ (gray) cell fractions in the peripheral blood of young (1-3 months), wildtype and *Tet2*^{-/-} mice. No significant difference between groups of 3 animals in each. (d) Monocyte fraction (%) among white blood cells in the peripheral blood of young (1-3 months) in indicated mouse models. No significant difference between groups of 3 animals in each.



Supplementary Figure 2. *TET2* mutations correlate with a decreased platelet count and better overall survival. (a) Kaplan–Meier estimates of overall survival of ($TET2^{WT}$, n=52) and ($TET2^{MUT}$, n=89) CMML patients; Log-rank (Mantel-Cox test); P=0.0029; (b) Kaplan–Meier estimates of overall survival of CMML patients with $trTET2^{Mut}$ (n=67) and non- $trTET2^{Mut}$ (n=22); Log-rank (Mantel-Cox test); P=0.1262. (c) Peripheral blood parameters in CMML patients without *TET2* mutation ($TET2^{WT}$, n=52, for 4 patients we do not have clinical data) or with ($trTET2^{Mut}$ (n=67, for 1 patient we do not have clinical data), non- $trTET2^{Mut}$ (n=22)). The platelet count was significantly lower in $trTET2^{MUT}$ patients. Data are mean +/- SEM of indicated biological samples. Dunnett’s multiple comparison test using $TET2^{WT}$ as control, *** P<0.001.



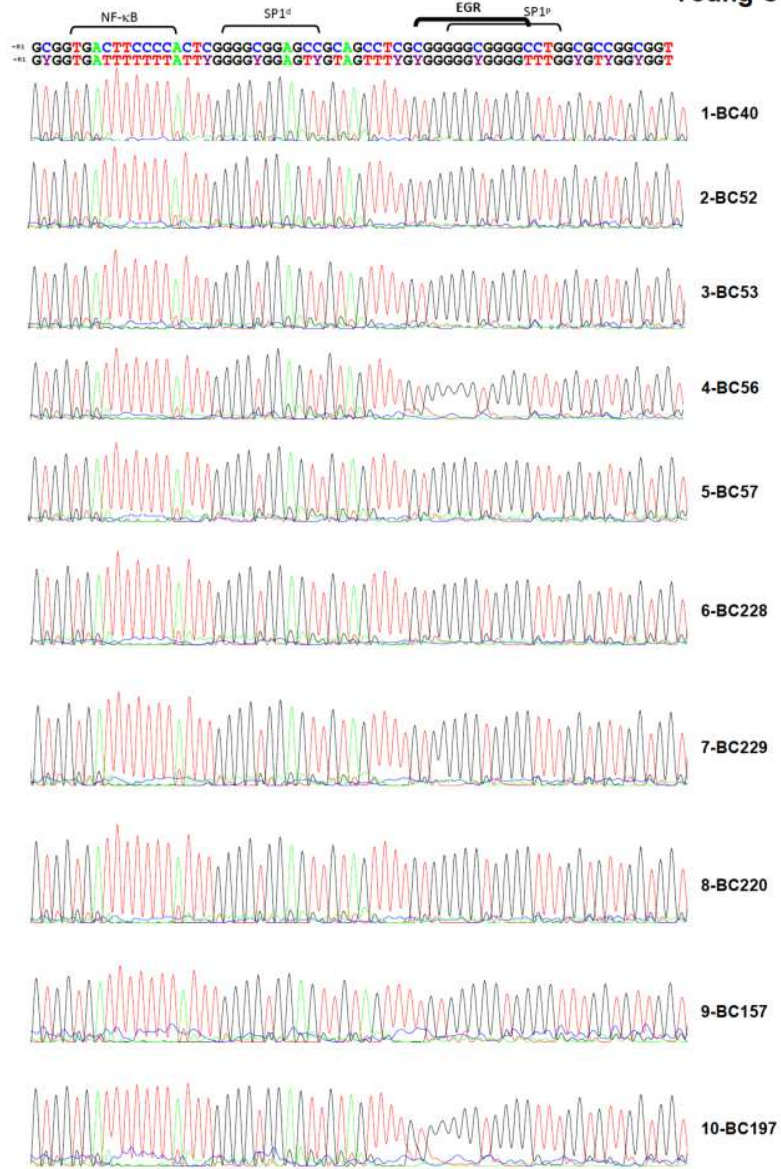
Supplementary Figure 3. Increased expression and secretion of MIF in *TET2*-mutated CMML monocytes. **(a)** Immunoblot analysis of MIF expression in sorted peripheral blood monocytes collected from three age-matched healthy donors (Co), 4 *trTET2*^{MUT} and 1 *TET2*^{WT} CMML patients. HSC70 (sc-7298, Santa Cruz), loading control. **(b)** Cytokine array map; **(c)** Sorted monocytes from 2 age-matched healthy donors and 2 *trTET2*^{MUT} CMML patients were incubated in RPMI medium for 18 hours before cytokine array analysis of culture supernatant. MIF is framed by a rectangle. **(d)** Cytokine array analysis of the serum collected from 3 age-matched healthy donors and 3 *trTET2*^{MUT} CMML patients. MIF is framed by a rectangle.



Supplementary Figure 4. EGR2 and SP1 recruitment on *MIF* gene proximal promoter. Schematic representation of *MIF* gene promoter; quantitative ChIP-PCR analyses were performed in kasumi-1 cells infected by *SCR* (yellow) or *TET2* (blue) shRNAs by using a set of primers targeting regions located in distal (R1), intermediate (R2), proximal (R3) promoter and in exon 1, closed to the TSS (R4). EGR1 (sc-110, Santa Cruz), EGR2 (sc-20690, Santa Cruz) and SP1 (#39058, Active Motif) associated DNA was immunoprecipitated. Results are expressed as fold enrichment relative to DNA immunoprecipitated with control immunoglobulin G (IgG). Data are mean +/- SEM each sample run in triplicate. Unpaired t test, * P<0.05.

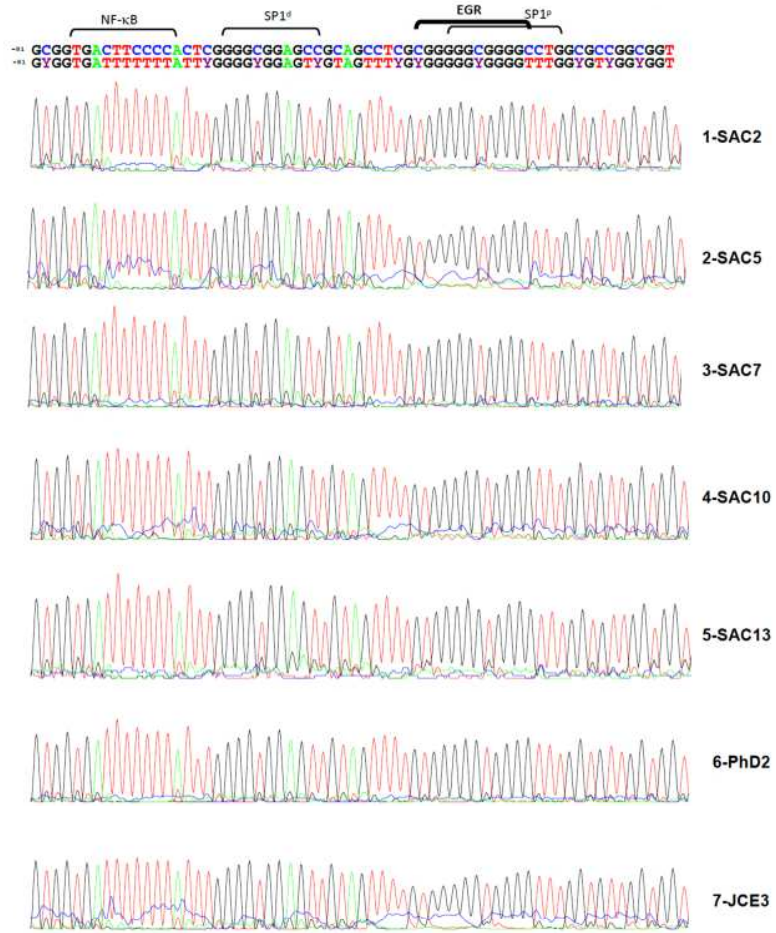
a

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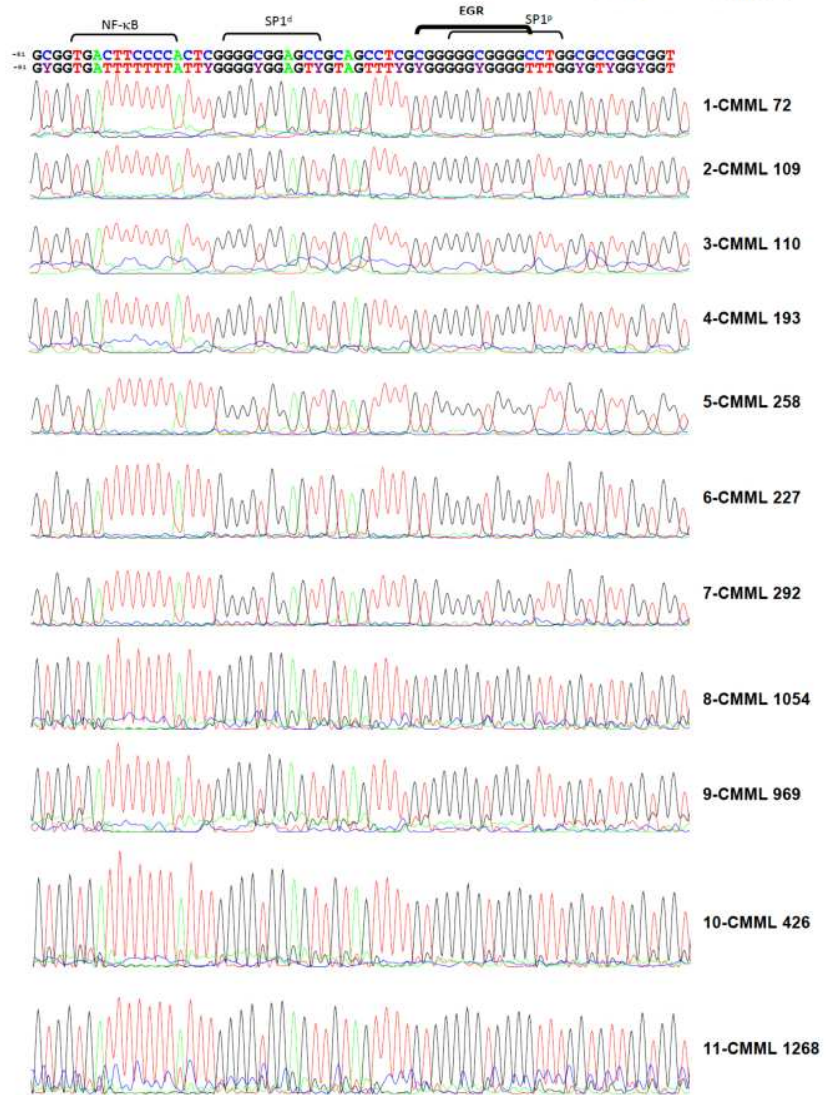
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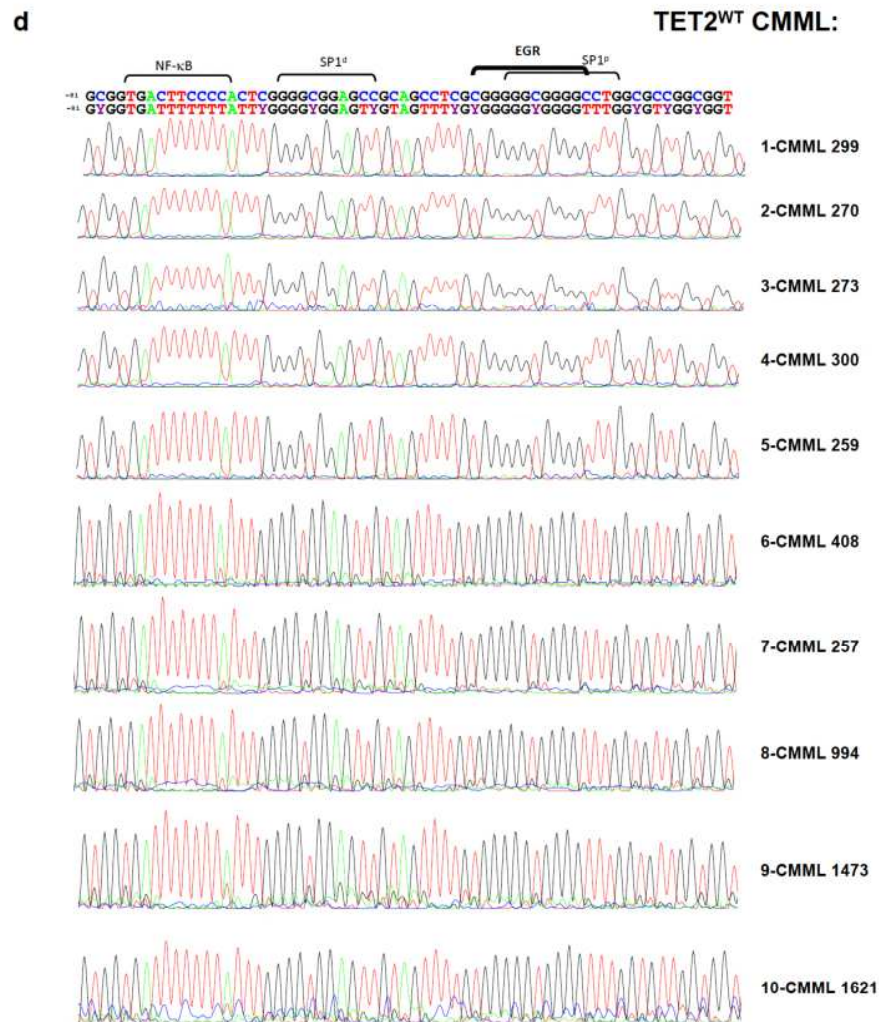
Age-matched CTRL:



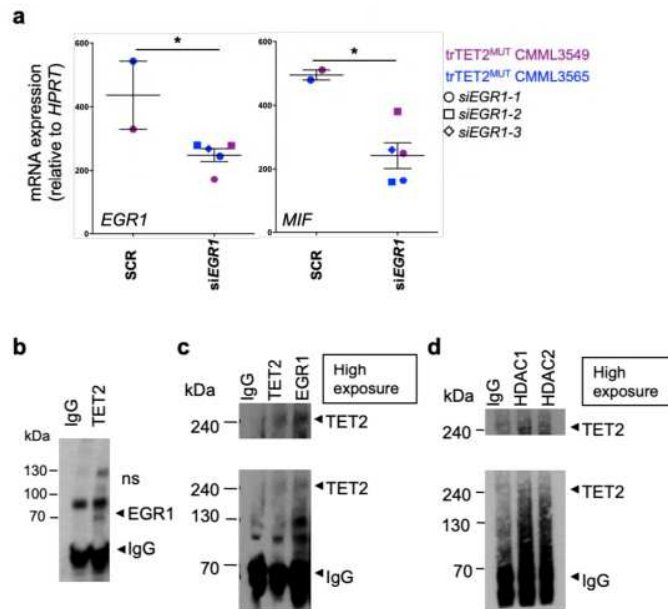
c

TET2^{MUT} CMML:



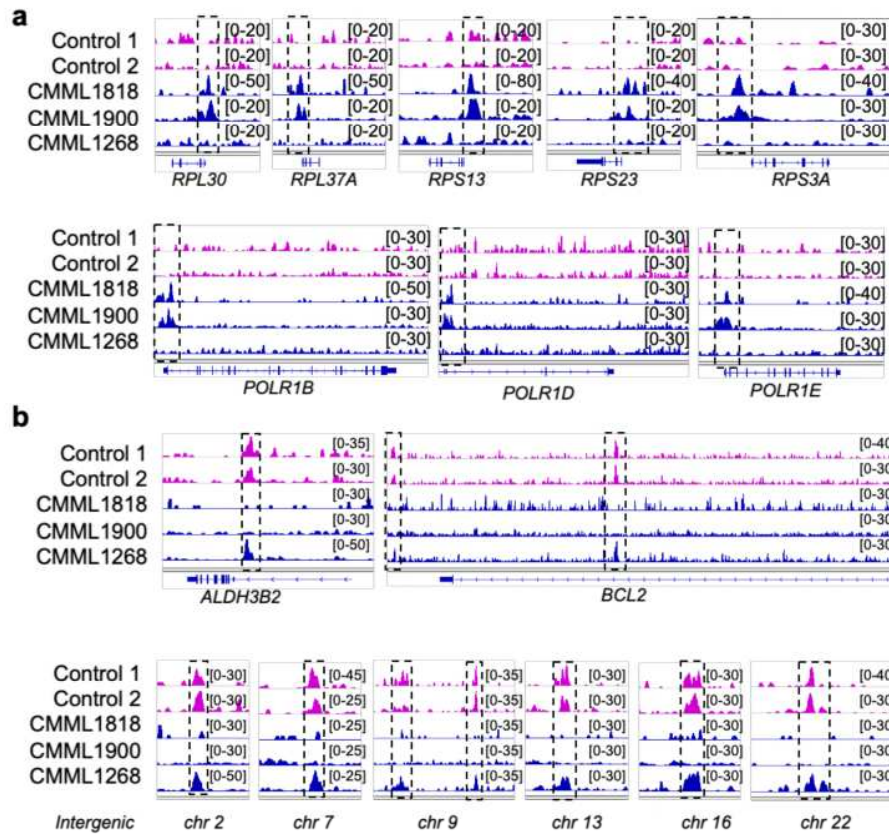


Supplementary Figure 5. MIF promoter is unmethylated in control and CMML monocytes. Sanger sequencing of the bisulfite-modified *MIF* promoter in CD14⁺ monocytes from 10 young controls (a), 7 age-matched donors (b, elderly), 11 wild-type TET2 CMML (c) and 10 mutated TET2 CMML (d).

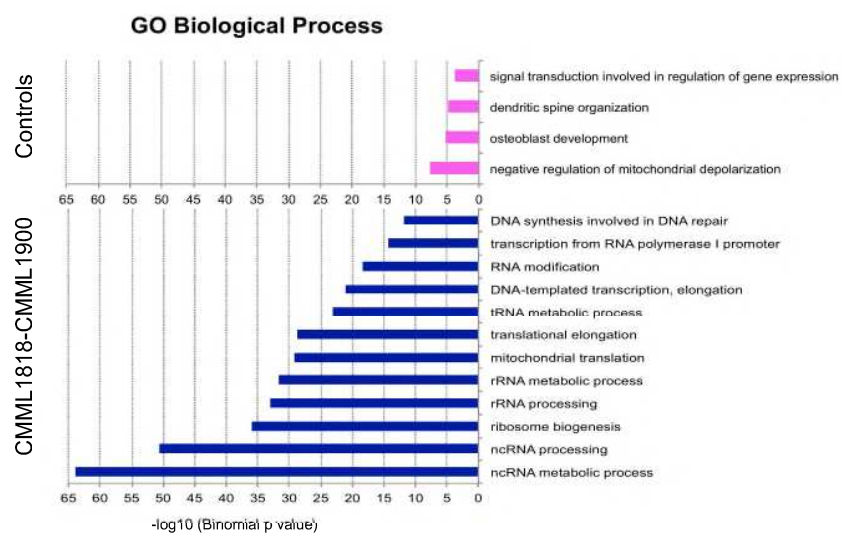


Supplementary Figure 6. TET2 interacts with HDAC1 and HDAC2 in primary monocytes.

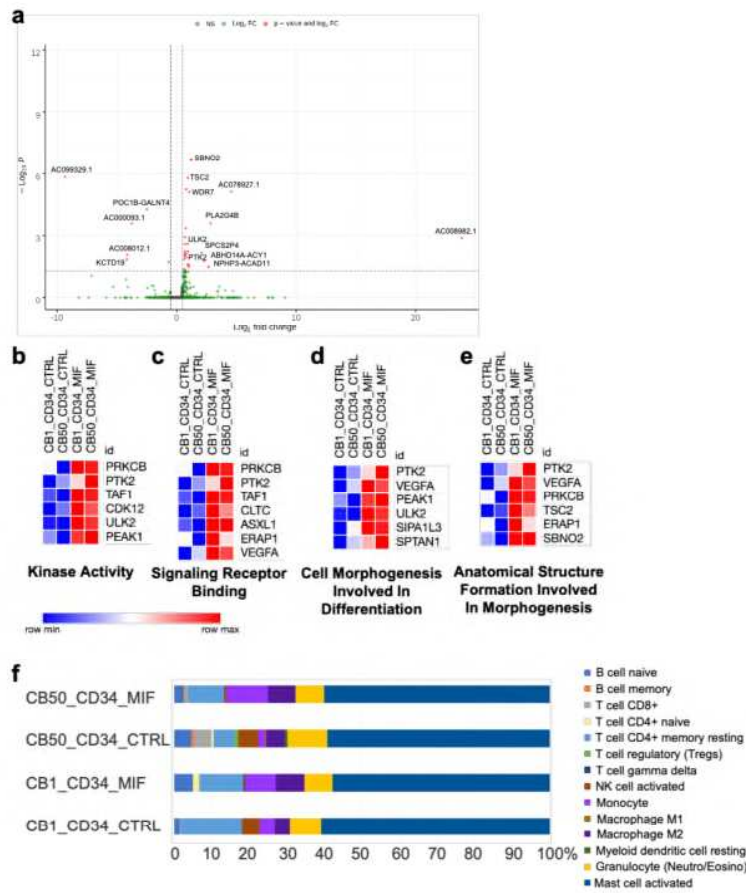
(a) siRNA-mediated down-regulation of *EGR1* in trTET2^{MUT} monocytes isolated from CMML3549 (purple) and CMML3565 (blue) with 2 and 3 sets of *EGR1*-siRNA respectively. RT-qPCR analysis of *EGR1* and *MIF* mRNA expression normalized to *HPRT*, tested in triplicate. Data are mean +/- SEM of each sample run in triplicate. Unpaired Students-t test using control monocytes transfected with SCR as control, * P<0.05. **(b, c)** Co-immunoprecipitation experiments in sorted healthy donor monocytes. **(b)** An anti-TET2 (sc-136926) or a control IgG were used for IP, followed by immunoblotting with anti-EGR1 (sc-110). **(c)** An anti-TET2 (sc-136926), an anti-EGR1 (sc-110) or a control were used for IP, followed by immunoblotting with an anti-TET2 antibody (upper panel is a higher exposure version of the same blot). **(d)** Immunoprecipitation was performed in sorted healthy donor monocytes using an anti-HDAC1 (#39531, Active Motif) or an anti-HDAC2 (#39533, Active Motif) or a control IgG followed by immunoblotting with anti-TET2 antibody (sc-136926).



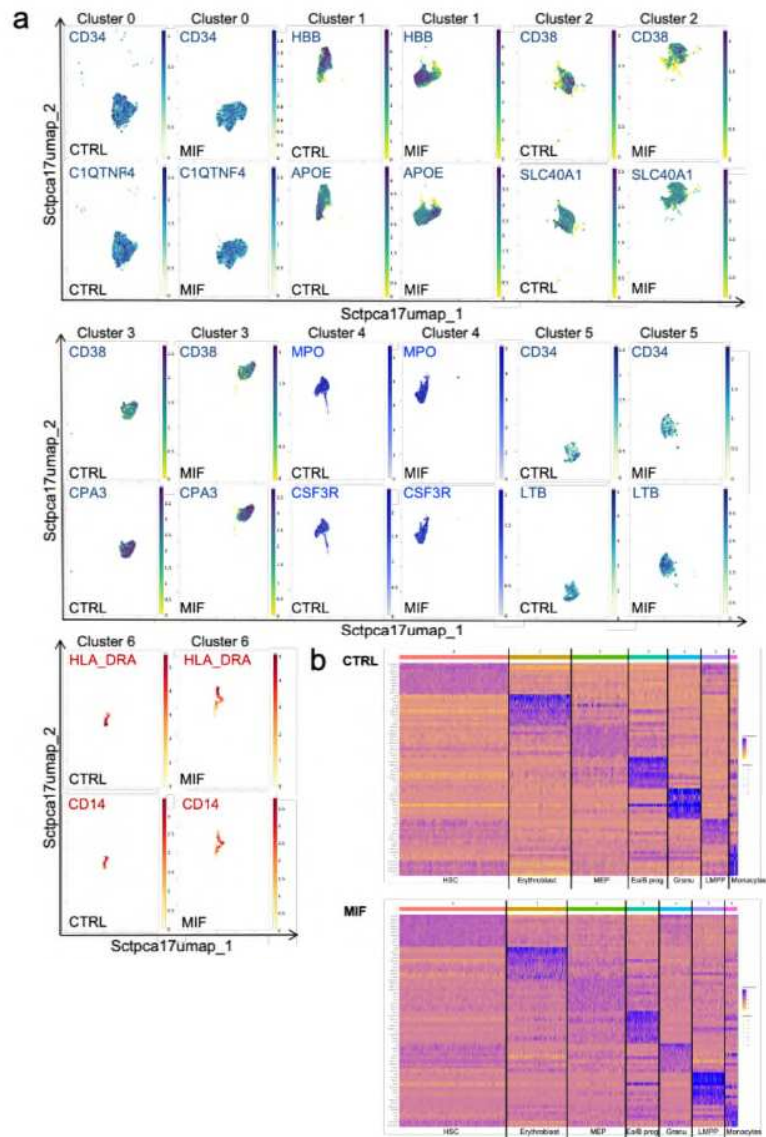
Supplementary Figure 7. EGR1 recruitment to monocyte DNA is modified upon *TET2* mutation. ChIP-seq experiments were performed as described in Figure 6 using an anti-EGR1 antibody in sorted peripheral blood monocytes from 2 healthy donors (controls) and three CMML patients **(a)** Peak calling for EGR1 on indicated genes in controls (pink) and CMML samples (dark blue). **(b)** Peak calling for EGR1 on indicated intergenic regions in controls (pink) and CMML samples (dark blue).



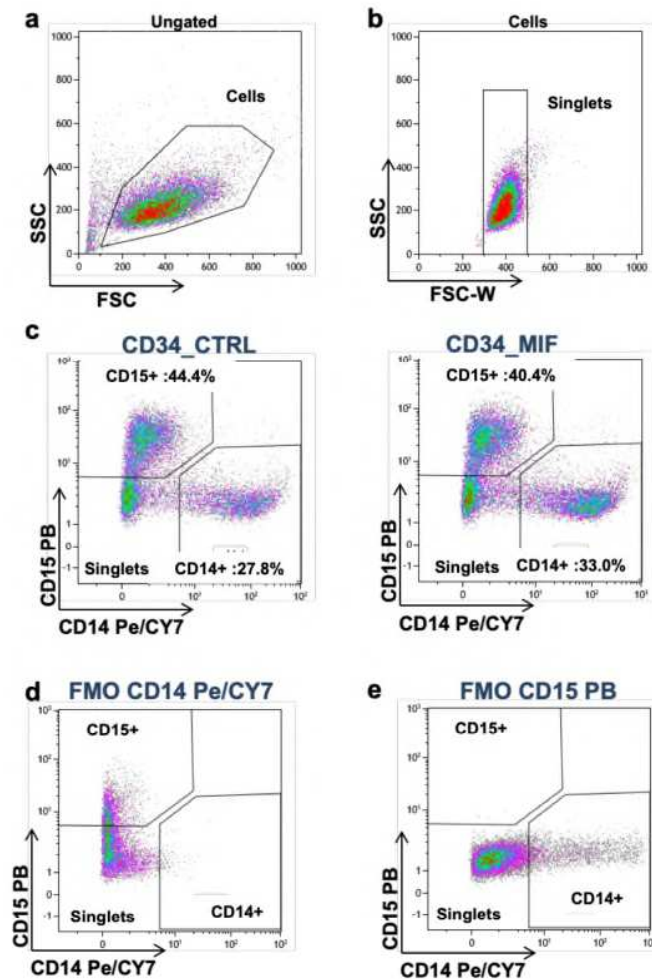
Supplementary Figure 8. GO (Gene Ontology) biological processes detected by analyzing EGR1 ChIP-sequencing data with GREAT (Genomic Regions Enrichment of Annotations Tool: <http://great.stanford.edu/public/html>).



Supplementary Figure 9. MIF strengthens pathway activation toward monocytic differentiation. Cord blood CD34⁺ cells were cultured in medium with SCF, FLT3L, IL-3 and G-CSF with or without MIF during 48h and studied by RNA-seq. **(a)** Volcano plot representation of differentially expressed genes in CTRL versus MIF cultures. The name of differentially deregulated genes is indicated. **(b, c)** GO Molecular Function analysis of differentially deregulated genes represented by heatmap. **(d, e)** GO Biological Process analysis of differentially deregulated genes represented by heatmap. **(f)** CIBERSORT analysis to estimate the abundance of hematopoietic cell types in the mixed cell population, using gene expression data.

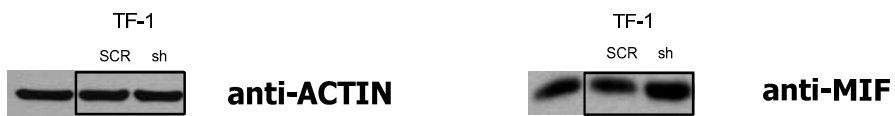
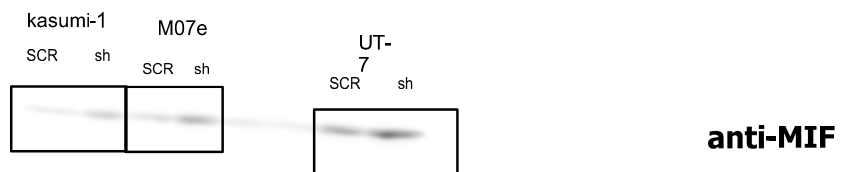
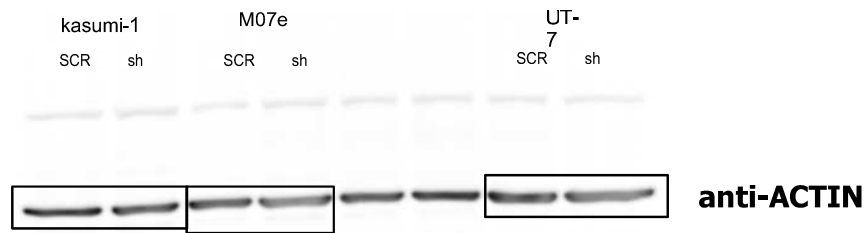
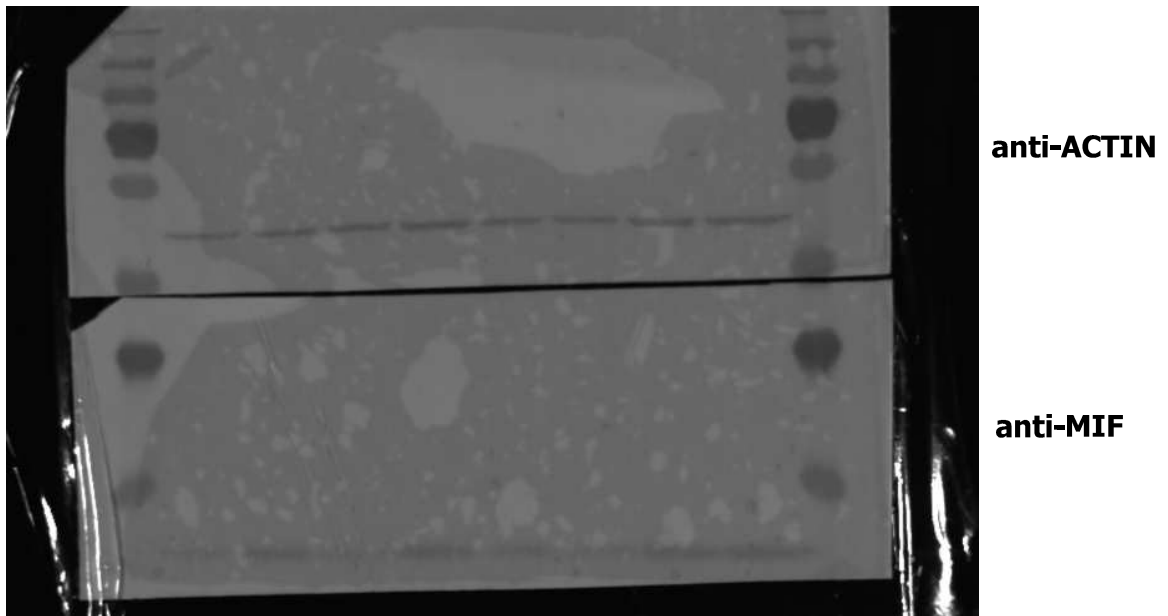


Supplementary Figure 10. Identification of the 7 clusters in CTRL and MIF cultures. Cord blood CD34⁺ cells from CB50 were cultured in medium with SCF, FLT3L, IL-3 and G-CSF with or without MIF during 7 days and studied by scRNA-seq. **(a)** Umaps of 2 discriminant genes in each of the 7 clusters identified in CTRL and MIF cultures. Each cluster was identified thanks to *CD34*, *C1QTNF4*, *HBB*, *APOE*, *CD38*, *SLC40A1*, *CPA3*, *MPO*, *CSF3R*, *LTB*, *HLA-DRA* and *CD14* gene expressions. **(b)** Heatmaps of the 10 top genes in each of the 7 clusters identified in CTRL and MIF cultures. cluster 0: HSC (hematopoietic stem cells); cluster 1: erythroblast; cluster 2: MEP (megakaryocyte–erythroid progenitor cells); cluster 3: Eo/B progenitors (eosinophil/basophil progenitors); cluster 4: granulocytes; cluster 5: LMPP (lymphoid-primed multipotent progenitors) and cluster 6: monocytes.

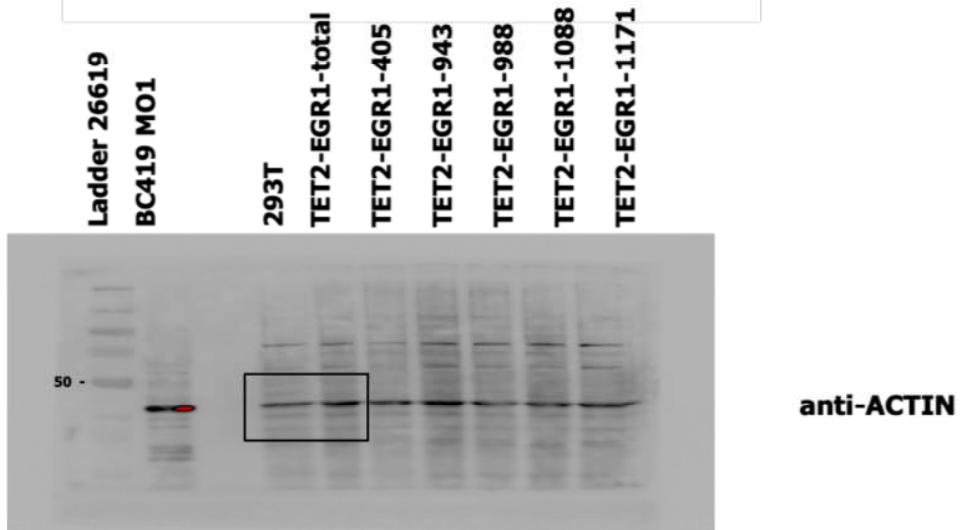
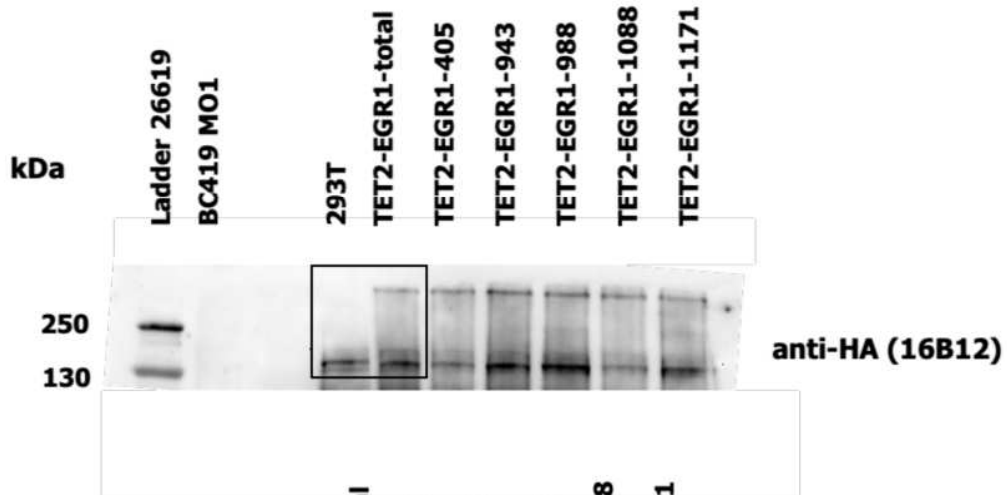
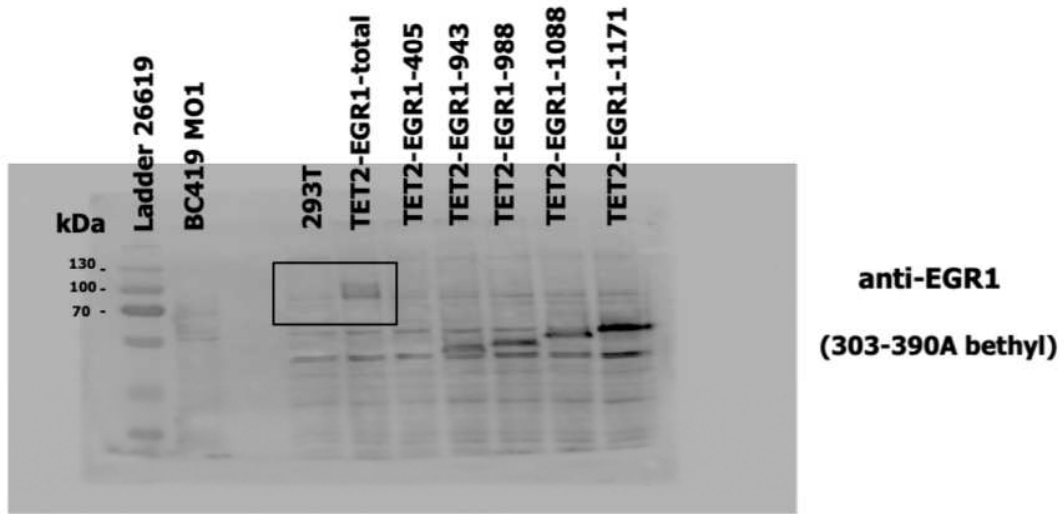


Supplementary Figure 11. Gating strategy for monocyte and granulocyte identification. Differentiation of cord blood CD34⁺ cells during 7 to 9 days with stem cell factor (SCF), interleukin-3 (IL-3), Fms-related tyrosine kinase 3 ligand (FLT3L), granulocyte-colony stimulating factor (G-CSF) in the presence or the absence of 20 ng/mL MIF before flow cytometry analysis. Using forward (FSC) and side scatter (SSC-A) (a, b), dead cells and doublets were eliminated. On the remaining cultured cells, differentiated granulocytic population is identified by CD15⁺ expression, while monocytic population is identified by CD14⁺ expression on a CD14/CD15 dot plot (c). CD15 and CD14 gate positions were determined using Fluorescence minus one (FMO) controls (d, e).

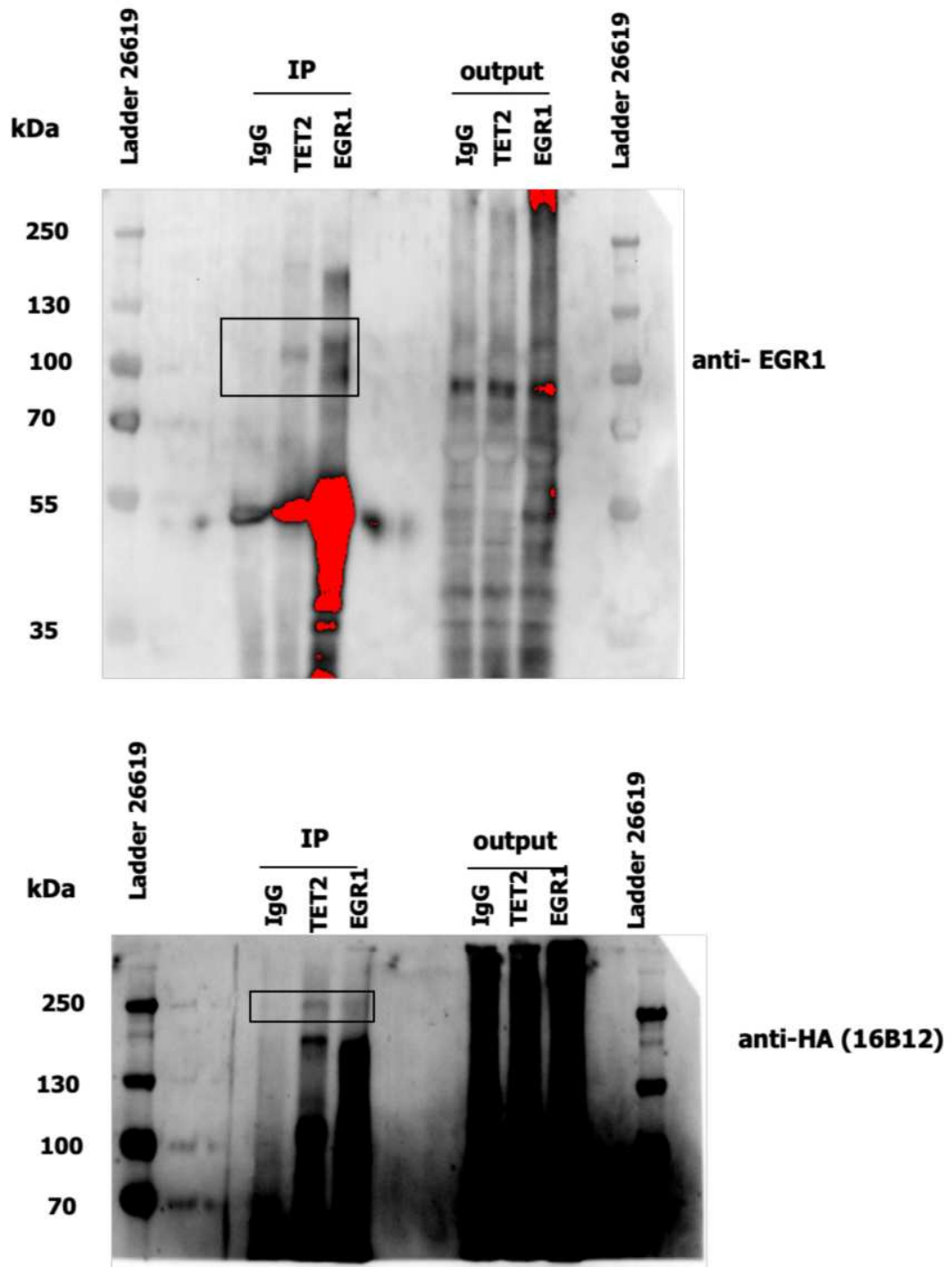
Uncropped blots related to Figure 1e



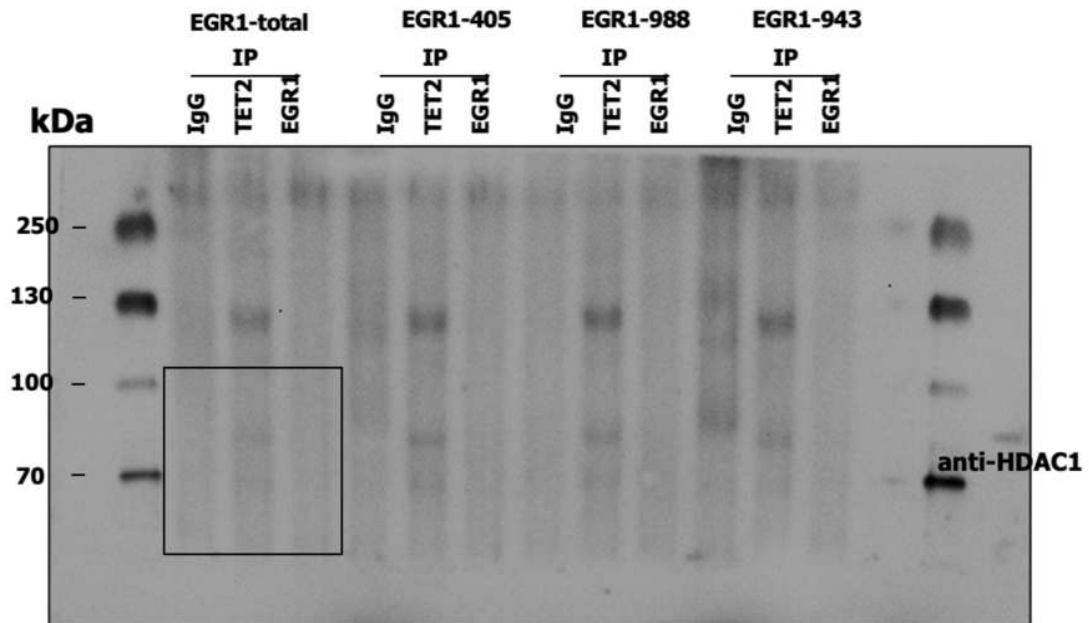
Uncropped blots related to Figure 5d



Uncropped blots related to Figure 5e



Uncropped blots related to Figure 5e



Supplementary Figure 12. Uncropped western blots

Supplementary Table 1: Characteristic of CMML patients whose sorted monocytes were analyzed by RNA sequencing.

| CMML | TET2 wild type | TET2 mutated |
|---|-----------------------|---------------------|
| Number of cases | 17 | 43 |
| Mean age in years (range) | 68.1 (53-79) | 71.3 (45-87) |
| Sex ratio M/F | 10/7/0 | 30/10/3 |
| CMML 0/1/2 | 5/6/5 | 16/11/9 |
| Proliferative/Dysplastic | 11/4 | 29/10 |
| Leucocytes, mean. 10^9 /L (range) | 35.1 (2.7-119) | 27.0 (4.8-110.4) |
| Monocytes, mean. 10^9 /L (range) | 10.4 (1.7-55.2) | 7.9 (1.1-67.0) |
| Platelet count, mean. 10^9 /L (range) | 137.7 (18-417) | 123.4 (25-394) |
| Hemoglobin level, mean g/dL (range) | 9.2 (6.3-13.9) | 11.2 (7.5-14.7) |
| Karyotype (N/A/ND) | 8/4/5 | 27/9/7 |
| Mutations (mutated/analyzed) | | |
| <i>SRSF2</i> | 4/17 (23.5%) | 21/43 (48.8%) |
| <i>ASXL1</i> | 9/17 (52.9%) | 27/43 (62.8%) |
| <i>RUNX1</i> | 2/17 (11.7%) | 9/43 (20.9%) |
| <i>NRAS</i> | 5/17 (29.4%) | 6/43 (13.9%) |
| <i>KRAS</i> | 2/17 (11.7%) | 7/43 (16.3%) |
| <i>CBL</i> | 0/17 (0%) | 3/43 (6.9%) |

Supplementary Table 2: Independent cohort of CMML patients used for RT-qPCR analysis of *MIF* gene expression in sorted peripheral blood monocytes.

| CMML | TET2 wild type | TET2 mutated |
|---|-----------------|------------------|
| Number of patients | 56 | 90 |
| Mean age in years (range) | 68.9 (30-87) | 73.3 (55-90) |
| Sex ratio M/F | 26/20 | 61/29 |
| CMML 0/1/2 | 20/18/9 | 34/39/11 |
| Proliferative/Dysplastic | 23/30 | 39/49 |
| Leucocytes, mean.10 ⁹ /L (range) | 21.5 (3.6-119) | 20.6 (3.0-141.9) |
| Monocytes, mean.10 ⁹ /L (range) | 4.8 (1.0-28.8) | 5.5 (1.0-59.7) |
| Platelet count, mean.10 ⁹ /L (range) | 185.5 (18-560) | 124.1 (13-553) |
| Hemoglobin level, mean g/dL (range) | 11.3 (6.3-17.2) | 12.1 (6.0-16.4) |
| Karyotype (N/A/ND) | 25/12/19 | 54/14/22 |
| Mutations (mutated/analyzed) | | |
| <i>SRSF2</i> | 11/55 (20.0%) | 42/88 (47.7%) |
| <i>ASXL1</i> | 16/56 (28.6%) | 31/90 (34.4%) |
| <i>RUNX1</i> | 7/56 (12.5%) | 15/90 (16.7%) |
| <i>NRAS</i> | 9/56 (16.1%) | 14/90 (15.5%) |
| <i>KRAS</i> | 7/56 (12.5%) | 15/90 (16.7%) |
| <i>CBL</i> | 5/56 (8.9%) | 12/90 (13.3%) |

Supplementary Table 3: Characteristic of CMML patients whose bone marrow aspirates were used to quantify MIF.

| CMML | TET2 wild type | TET2 mutated |
|---|-----------------------|---------------------|
| Number of patients | 12 | 21 |
| Mean age in years (range) | 73.9 (58-84) | 75.1 (59-86) |
| Sex ratio M/F | 7/3/2 | 12/5/4 |
| CMML 0/1/2 | 4/5/0 | 6/2/9 |
| Proliferative/Dysplastic | 6/2 | 12/6 |
| Leucocytes, mean. 10^9 /L (range) | 23.7 (9.4-50.3) | 26.1 (5.7-110.4) |
| Monocytes, mean. 10^9 /L (range) | 6.8 (1.5-27.6) | 8.4 (1-67) |
| Platelet count, mean. 10^9 /L (range) | 176.1 (44-461) | 120.7 (27-394) |
| Hemoglobin level, mean g/dL (range) | 10.7 (7.7-15.5) | 11.0 (7.2-15.2) |
| Karyotype (N/A/ND) | 6/0/6 | 13/4/4 |
| Mutations (mutated/analyzed) | | |
| <i>SRSF2</i> | 3/12 (25%) | 9/21 (42.8%) |
| <i>ASXL1</i> | 8/12 (66.6%) | 13/21 (61.9%) |
| <i>RUNX1</i> | 2/12 (16.6%) | 4/21 (19.0%) |
| <i>NRAS</i> | 3/12 (25%) | 3/21 (14.3%) |
| <i>KRAS</i> | 0/12 (0%) | 4/21 (19.0%) |
| <i>CBL</i> | 3/12 (25%) | 4/21 (19.0%) |

Supplementary Table 4: Cohort of CMML patients whom sorted CD34⁺ cells were analyzed by agilent arrays for gene expression.

| CMML | TET2 wild type | TET2 mutated |
|---|-----------------------|---------------------|
| Number of patients | 13 | 9 |
| Mean age in years (range) | 75.5 (53-87) | 71.3 (60-91) |
| Sex ratio M/F | 10/3 | 7/2 |
| CMML 0/1/ 2 | 5/6/2 | 4/4/1 |
| Proliferative/Dysplastic | 6/7 | 5/4 |
| Leucocytes, mean.10 ⁹ /L (range) | 41.4 (4.3-366.8) | 25.4 (4.0-92.7) |
| Monocytes, mean.10 ⁹ /L (range) | 9.1 (1.1-84.3) | 10.3 (1.3-46) |
| Platelet count, mean.10 ⁹ /L (range) | 241.4 (32-1050) | 156.2 (18-335) |
| Hemoglobin level, mean g/dL (range) | 11.0 (6.8-15.5) | 12.2 (7.1-15.4) |
| Karyotype (normal/abnormal/not done) | 7/6/0 | 8/1/0 |
| Mutations (mutated/analyzed) | | |
| <i>SRSF2</i> | 7/13 (53.8%) | 6/9 (66.7%) |
| <i>ASXL1</i> | 8/13 (61.5%) | 2/9 (22.2%) |
| <i>RUNX1</i> | 1/13 (7.7%) | 0/9 (0%) |
| <i>NRAS</i> | 2/13 (15.4%) | 3/9 (33.3%) |
| <i>KRAS</i> | 1/13 (7.7%) | 1/9 (11.1%) |
| <i>CBL</i> | 2/13 (15.4%) | 1/9 (11.1%) |

Supplementary Table 5: Primers and antibodies used in this study.

| RT-qPCR Primers | Sequences (5' - 3') |
|--|----------------------------|
| RPL32 | |
| Forward | TGTCCTGAATGTGGTCACCTGA |
| Reverse | CTGCAGTCTCCTTGACACCT |
| PPIA | |
| Forward | GTCGACGGCGAGCCC |
| Reverse | TCTTTGGGACCTTGTCTGCAA |
| HPRT | |
| Forward | GGACAGGACTGAACGTCTTGC |
| Reverse | CTTGAGCACACAGAGGGCTACA |
| MIF | |
| Forward | GAACCGCTCCTACAGCAAGCT |
| Reverse | TTGGCCGCGTTCATGTCG |
| EGR1 | |
| Forward | TGATGTCCCCGCTGCAG |
| Reverse | GTCCATGGTGGGCGAGTG |
| TET2 | |
| Forward | CCTGATACCATCACCTCCCATT |
| Reverse | CAGGCAGTGGGCTTCCAT |
| ChIP qPCR Primers | Sequences (5' - 3') |
| MIF prom4 (R1) | |
| Forward | AAGGGTGGCTCGACAGTCAA |
| Reverse | GCCAGAAGCCCCTCTCAAG |
| MIF prom1 (R2) | |
| Forward | CACCTGCCGCGATGACTAC |
| Reverse | CGCATGCGTGAGCTTGTG |
| MIF prom2 (R3) | |
| Forward | GCCTCCTGGCGACTAACATC |
| Reverse | CCACTTGGCGGCTAGAAATC |
| MIF prom3 (R4) | |
| Forward | GCGGGTCTCCTGGTCCTT |
| Reverse | GCACGTTGGTGTTTACGATGA |
| Oligonucleotides Luciferase Assay | Sequences (5' - 3') |
| pGL3 | |
| Forward | CTTTATGTTTTTGGCGTCTTCCA |
| Reverse | CTAGCAAAATAGGCTGTCCC |
| NFκB mut | |
| Forward | GCAGGCGGTGATGGCCCCACTCGGG |
| Reverse | CCCAGTGGGGCCATCACCGCCTGC |

| | |
|--|--|
| EGR1 mut | |
| Forward | AGCCGCAGCCTAATGGCGGCGGGGC |
| Reverse | GCCCCGCCCCCATTAGGCTGCGGCT |
| Sp1^P mut | |
| Forward | TCCCCACTCGGTAGTGAGCCGCAGCC |
| Reverse | GGCTGCGGCTCACTACCGAGTGGGGA |
| Sp1^d mut | |
| Forward | TCGCGGGGGCGACTCCTGGCGCCGG |
| Reverse | CCGGCGCCAGGAGCGCCCCCA |
| Antibodies ChIP experiments | Reference |
| TET2 Ab1 | C2 a gift of Dr Olivier Bernard |
| TET2 Ab2 | sc-136926, Santa Cruz Biotechnology |
| P-S5 polymerase II | #39749, Active Motif |
| H3K4me3 | #39159, Active Motif |
| H3K27me3 | #39155, Active Motif |
| EGR1 | sc-110, Santa Cruz Biotechnology |
| HDAC1 | #39531, Active Motif |
| HDAC2 | #39533, Active Motif |
| Antibodies FACS experiments | Reference |
| CD14-PE-Cy7 | clone MφP9, BD Biosciences (San Diego, CA) |
| CD15-PE-CF594 | clone HI98, BD Biosciences (San Diego, CA) |
| CD16-APC-H7 | clone 3G8, BD Biosciences (San Diego, CA) |
| CD34-FITC | cat 555821, BD Biosciences (San Diego, CA) |

VI. Partie 2 : First draft

CMML patients with low expression of TP53 point to AML transformation with immature classical monocytes

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Résumé de l'article

La protéine p53 exerce un rôle clé dans la suppression tumorale grâce à ses fonctions biologiques en lien avec la régulation de l'apoptose, du cycle cellulaire, de la réparation de l'ADN ou encore de l'autophagie. *TP53* est fréquemment inactivé par mutation dans les tumeurs solides, altérant ainsi les fonctions de p53. Les mutations de *TP53* représentent 10% des SMD et LAM et sont associées à un caryotype complexe et à un mauvais pronostic.

Contrairement à *TET2*, *TP53* est très rarement muté dans la LMMC. L'expression du transcrite et de la protéine *TP53*/p53 est très hétérogène dans les monocytes des patients LMMC. Les analyses de séquençage d'ARN (RNAseq) suggèrent que les patients présentant une expression réduite de *TP53* sont plus susceptibles d'évoluer vers une leucémie myéloïde aiguë et sont plus inflammatoires.

Les monocytes de patients atteints de LMMC exprimant faiblement *TP53* présentent également une expression diminuée du CSF-1R, suggérant un défaut de maturation. Des expériences avec des sh-SCRB et des sh-*TP53* dans les CSH au cours de la monocytopoïèse en présence de CSF1 ont démontré un bloc de différenciation à un stade pré-monocytaire lorsque *TP53* est moins exprimé. La sous-expression de *TP53* a diminué de manière significative la fraction de cellules CD14⁺CD36⁺ avec moins d'expression de CD300E à leur surface, montrant un rôle de p53 dans la maturation terminale des monocytes.

Nous avons mis en place des analyses de cytométrie en flux pour étudier la dernière étape de maturation des monocytes en ajoutant CD300E. Nos données indiquent que les patients présentant une faible expression de *TP53* ont une expression plus faible du marqueur de surface des monocytes CD300E et sont plus immatures. Ce défaut de maturation terminale, lorsque *TP53* est moins exprimé, a également été validé au niveau unicellulaire. Les analyses à l'échelle unicellulaire ont également montré que ces cellules exprimant moins de *TP53* étaient plus inflammatoires.

EGR1 (*Early Growth Response Protein 1*) code pour un facteur de transcription essentiel à la monocytopoïèse et est recruté par le promoteur *TP53* dans les cellules du sang de cordon en cours de différenciation monocytaire. La diminution de l'expression d'*EGR1* par des siARN réduit l'expression de *TP53* dans les monocytes du patient LMMC. Enfin,

CMML patients with low expression of TP53 point to AML transformation with immature classical monocytes

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Keywords

Monocyte, Chronic Myelomonocytic Leukemia (CMML), Tumor protein P53 (TP53), CD300E, Early growth response protein 1 (EGR1).

Abstract

Unlike TET2, TP53 is very rarely mutated in CMML. The expression of the TP53 transcript and protein is very heterogeneous in the monocytes of CMML patients. RNA sequencing (RNAseq) analyzes suggest that patients with reduced TP53 expression are more likely to progress to acute myeloid leukemia. Monocytes from CMML patients weakly expressing TP53 also have decreased CSF-1R expression, suggesting a maturation defect. Experiments with sh-SCRB and sh-TP53 in HSCs during monocytopoiesis in the presence of CSF1 demonstrated a block of differentiation at a pre-monocytic stage when TP53 is less expressed, showing a role for TP53 in monocyte terminal maturation. We implemented flow cytometry analyzes to study the final step of monocyte maturation by adding the surface molecule CD300E. Our data indicate that patients with low TP53 expression have lower expression of the monocyte surface marker CD300E and are more immature. This terminal maturation defect, when TP53 is less expressed, has also been validated at the single-cell level. Decreasing EGR1 expression by siRNA diminished *TP53* expression in patient monocytes. Finally, using the DACOTA cohort, we demonstrated that patients expressing weakly *TP53* had poorer survival when treated with hydroxyurea.

Introduction

Chronic Myelomonocytic Leukemia (CMML) is a myeloid hemopathy, classified as a myelodysplastic syndrome/myeloproliferative neoplasm (MDS/MPN), that results from the accumulation of somatic mutations in hematopoietic stem cells (HSC), generating a clone with proliferative advantage and myeloid differentiation bias. This disease combines cellular dysplasia of one or more myeloid lineages with proliferative features, including monocytosis greater than 0.5 G/L representing more than 10% of white blood cells for a period of ≥ 3 months and signs of dysmyelopoiesis (dysgranulopoiesis in particular) [1], [2]. CMML is a rare disease, affecting approximately 4 out of 100,000 subjects each year, with a male predominance and a median age of 72 year-old.^{3,4} The median survival time is 2 to 3 years after diagnosis and 15-20% of CMML patients progress to acute myeloid leukemia (AML). The treatment of CMML is still not satisfactory and the overall management is to correct cytopenias, prolonge survival, delay AML transformation and improve quality of life [3]. Among cytoreductive therapy, hydroxyurea is the most widely used drug to date and is preferentially recommended as a symptomatic treatment for CMML patients. Hypomethylating agents (HMA), including 5-azacytidine and decitabine, are also ofently used to treat CMML patients with an improvement in the clinical symptoms when the patient responds but there are no effects on the mutation allele burden [4]. Recently, no difference in event-free survival or overall survival has been seen between patients with advanced proliferative CMML treated with decitabine compared with hydroxyurea [5]. To date, HSC allograft still remains the only curative treatment for CMML, but its application is limited due to the age of patients and their comorbidities.

Monocytes represent 5-10% of circulating leukocytes in humans and 2% of circulating leukocytes in mice. In humans, monocytes are divided into three subsets defined by the level of expression of the surface markers CD14 and CD16 with classical CD14⁺⁺CD16⁻ monocytes, accounting for the majority of monocytes in the blood of healthy humans (85% of total monocytes), intermediate CD14⁺⁺CD16⁺ monocytes (5% of monocytes) and non-classical CD14⁻CD16⁺⁺ monocytes (10% of monocytes) [6]. The proportion of classical monocytes is increased in CMML patients, with a percentage of classical monocyte $\geq 94\%$ [7], [8]. This abnormal distribution of classical monocytes in the peripheral blood of CMML patients is

independent of the CMML subtype, karyotype and mutational status and is now a new supporting criterion for CMML diagnosis [2].

Molecular abnormalities found in CMML patients are mutation in genes encoding epigenetic regulators including those involved in DNA methylation (TET2, DNMT3A), RNA splicing (SRSF2, SF3B1, U2AF1, ZRSR2) and histone modification (ASXL1, EZH2); cell signaling pathway (NRAS, KRAS, CBL, JAK2) and transcriptional regulator (RUNX1) [9]. Mutations in the RUNX1 gene and in signaling genes are often found as secondary hits. Most CMML patients have an average of 14 somatic mutations in the coding regions [4]. Whole genome sequencing identify two signatures related to aging (signature 1 and 5, [4]) and some of the identified gene mutations are also frequently observed in age-related clonal hematopoiesis, suggesting a contribution of the hematopoietic aging process to the pathophysiology of CMML [10].

TP53 is a tumor suppressor gene that is altered by deletion and/or point mutation in the majority of solid tumors [11], [12]. *TP53* mutations are often found in solid cancers but their incidence is lower in hematological malignancies (around 10% of *de novo* AML and MDS cases). In MDS, *TP53* mutations have been associated with high-risk disease, with rapid transformation to AML and with resistance to chemotherapies, including low-dose of cytarabine [13], [14]. *TP53* multi-hit mutations are in MDS predominantly associated with complex karyotype, high-risk presentation and poor prognosis [15]. Interestingly, in comparison to MDS (7%) and AML (13%), somatic *TP53* mutations are very rare in CMML (< 3%) [16]–[18]. Gene expression profile by RNAseq study in peripheral blood monocytes from CMML patients demonstrated a heterogeneous expression of *TP53* messenger independent of mutational landscape. This downregulation of *TP53* at the mRNA is also observed at the protein level in monocytes from some CMML patients. Using the two extreme RNAseq groups, this decreased expression of *TP53* correlated with an increased risk of AML transformation and increased inflammatory signatures. Monocytes from CMML patients weakly expressing *TP53* also have decreased CSF-1R expression, suggesting a maturation defect. *TP53* downregulation by shRNA in CD34⁺ cord blood cells delays monocyte maturation during CSF1-induced monocytic differentiation. CMML patients with weak expression of *TP53* expressed less CD300E at the surface of their monocytes. Confirming these results, scRNAseq analysis demonstrate that monocytes expressing weakly *TP53* are less mature and more inflammatory. In 293T cell lines, overexpression of *EGR1* upregulates *TP53* expression and siRNA downregulation of *EGR1* in *TP53*-expressing monocyte diminished its expression. These

results underline an important role of TP53 in the late stage of monocytopoiesis and highlight the involvement of *TP53* mRNA level in AML transformation.

Results

TP53 is rarely mutated in CMML patients and its expression is heterogeneous in CMML monocytes. We investigated TP53 mutational status using a cohort of CMML patients with available molecular and clinical data and classical monocyte phenotype percentage. Using this cohort of 249 CMML patients, we identified 7 (2.8%) patients with TP53 missense alteration (**Fig. 1a**). No difference in classical monocyte percentage (**Fig. 1b**), monocyte, white blood cell, neutrophil, lymphocyte, platelet count and hemoglobin level was observed between the 2 groups (**Supplementary Fig. 1**). Using the DACOTA trial with advanced disease as an independent cohort [9], we identified 2 (1.5%) patients with TP53 missense alteration, validating the low level of TP53 mutations in CMML disease (**Fig. 1c**). To explore the link between mutation status and TP53 expression in human primary samples, we sorted peripheral blood monocytes from 129 CMML patients included in the DACOTA trial and performed bulk RNA sequencing (**Fig. 1c**). Interestingly, we observed an heterogeneous expression of *TP53* messenger in classical monocytes, with some patient expressing weakly or not all *TP53* in their monocytes (**Fig. 1c**). Importantly, we did not detect any significant correlation between *TP53* expression and the mutational profile of these CMML patients. We next sorted human monocytes from elderly healthy donor and CMML patient peripheral blood and quantified p53 protein expression by confocal microscopy. Compared to controls, p53 mean fluorescence intensity was very low in some CMML monocytes (**Fig. 2a**). For example, monocyte from patient 3344 expressed p53 whereas p53 protein was undetectable in monocytes from patient 3336 and 3333 (**Fig. 2a, Supplementary Fig. 2**), confirming p53 protein heterogeneous expression in CMML classical monocytes. We next analyzed p53 expression in classical monocytes by confocal microscopy in a cohort of 43 healthy donors and 120 CMML patients. We observed a decreased expression of p53 in the CMML cohort compared to elderly controls (**Fig. 2c**). Of note, there was no clear group separation but a continuum, suggesting a gradual cellular transition. p53 immunoblotting confirmed that some patient monocytes do not expressed p53 protein (**Fig. 2d**). Finally, we tested p53 functionality in monocyte after etoposide treatment. We selected monocytes from three

elderly controls, three p53-expressing and three non-expressing CMML patients and incubated them during 7 hours with 50 μ M etoposide. p53 is known to transcriptionally regulate genes that encode cell cycle checkpoint regulators (e.g., p21/CDKN1A) and pro-apoptotic factors (e.g., BAX). As expected, *BAX* and *CDKN1A* mRNA expression was decreased in monocytes with no p53 expression (**Fig. 2e**). Together, these results identified a sub-group of CMML patients that do not expressed p53.

CMML patients with diminished *TP53* expression are more likely to progress to acute myeloid leukemia. To better characterize this subgroup of patients, we used monocyte RNAseq data from our 129 CMML patients and compared the two extremes to decipher important pathways that may discriminate these two groups (**Fig. 1c**, 22 first patients against 23 last patients). The two extreme groups were well separated in principal components (**Fig. 3a**) and have different signature (**Fig. 3b**). Based on an adjusted P value of <0.05 and log2 fold change >2 , a total of 8,054 genes (4,928 upregulated and 3,126 downregulated genes) were found to be differentially expressed in TP53-diminished monocytes (**Fig. 3b**). MAplot identified an up-regulation in epigenetic modifiers such as *KDM3A*, *KDM5B*, *TRIM13* whereas *CSF-1R* was down-regulated in p53-diminished group (**Fig. 3c**). Pathway analysis using Gene Set Enrichment Analysis (GSEA) identified 58 pathways in KEGG and 23 pathways in Hallmarks that were significantly different between the two groups. Interestingly, KEGG analysis revealed a highest enrichment as compared with the normal group in acute myeloid leukemia (AML) signature (**Fig. 3d**). GO analysis identified an up-regulation of Hallmark TNFA signaling via NF κ B and inflammatory response whereas a down-regulation of Hallmark DNA repair pathway in p53-diminished group, strengthening the loss of function of p53 (**Fig. 3e**).

***TP53* expression correlate with *CSF-1R* expression.** RNAseq analysis in p53-diminished group pinpointed a down-regulation of *CSF-1R* expression (**Fig. 3c**), a important receptor in monocyte generation. To explore the link between *TP53* and *CSF-1R* expression in human primary samples, we sorted peripheral blood monocytes from 229 CMML patients and from 52 healthy donors and performed RT-qPCR analysis for *CSF-1R*. Again, we observed a continuum in *CSF-1R* expression in CMML monocytes with no clear group separation (**Fig. 4a**). Some patients expressed *CSF-1R* messenger in their monocytes and some not. We next sorted human primary monocytes from healthy donor and CMML patient peripheral blood and quantified

CSF-1R protein expression by confocal microscopy. Compared to controls, CSF-1R mean fluorescence intensity using PA525974 antibody was low in some CMML monocytes (**Fig. 4b and 4c**). For example, monocyte from patient 3285 expressed CSF-1R whereas CSF-1R protein was undetectable in monocytes from patient 3279 (**Supplementary Fig. 3a**), confirming CSF-1R protein heterogeneous expression in CMML classical monocytes as we previously reported [19]. We next analyzed CSF-1R expression in classical monocytes by confocal microscopy in a cohort of 10 healthy donors, 10 CMML patients with p53 expression and 10 with no p53 expression. Interestingly, patients with diminished p53 expression showed significantly lower levels of CSF-1R compared to controls and TP53-expressing patients (**Fig. 4d**). A correlation between TP53 and CSF-1R is also seen at the protein level after confocal image quantification in these two groups (**Fig. 4e**) and at the mRNA level using our 129 CMML monocyte RNAseq (**Fig. 4f**). Using an independent set of RNAseq data [20], we confirmed the correlation between TP53 and CSF-1R (**Supplementary Fig. 3b**). Together, these results identified a correlation between TP53 and CSF-1R expression, suggesting a monocyte maturation defect.

p53 regulates terminal monocyte maturation. We investigated whether *TP53* gene down-regulation could alter monocytopoiesis. Human cord blood CD34⁺ cells, transduced with *TP53* or scrambled (SCR) shRNA lentiviruses, were sorted using GFP and cultured with stem cell factor (SCF) and CSF1 to promote monocytic differentiation. It has been previously shown that maturation toward mature monocytes is associated with upregulation of CD36 and CD14 expression, while CD300E was only found among the more mature monocytes [21]. At day 7 and 8, flow cytometry analysis for CD34, CD36, CD14 and CD300E cell surface markers was performed to characterize these different stages of monocyte maturation. At day 7 and 8, GFP-positive cells were analyzed and CD34 disappearance was verified (**Fig. 5a**). The downregulation of TP53 significantly decreased the fraction of CD14⁺CD36⁺ cells (**Fig. 5b, Supplementary Fig. 4a**) with less CD300E expression at their cell surface (**Fig. 5c**). Compared to sh-SCR-transduced cells, those underexpressing *TP53* generated less CD36⁺CD300E⁺ cells (**Fig. 5d, Supplementary Fig. 4b**), less CD14⁺CD300E^{dim} and less CD14⁺CD300E⁺ cells (**Fig. 5e, Supplementary Fig. 4c**). These experiments suggested that *TP53*-diminishment in cord blood stem cells generate less mature monocytes (**Supplementary Fig. 4d**) and highlight a block of differentiation at a pre-mature monocytic stage regulated by TP53.

CMML patients with diminished *TP53* expression accumulate pre-mature CD300E^{dim} monocytes. We analyzed by flow cytometry the repartition of monocyte subsets in PBMCs from healthy donors and CMML patients by using an exclusion gating strategy (**Supplementary Fig. 5a**) to identify monocytes. We previously shown that CMML is characterized by an increase of classical CD14⁺/CD16⁻ monocytes with less intermediate CD14⁺/CD16⁺ and nonclassical CD14⁻/CD16⁺⁺ monocytes [7]. Compared with control subjects, CMML patients expressing less p53 still demonstrated an increased percentage of classical monocytes and a decreased fraction of intermediate and nonclassical monocyte subsets (**Fig. 6a**). We next gated inside the classical monocyte subset to address CD300E expression level to characterize CD14⁺/CD300E⁻ immature and CD14⁺/CD300E⁺ mature monocytes (**Fig. 6a**). The percentage of CD14⁺/CD300E⁺, assessed in a cohort of 24 CMML patients, was significantly lower in the CMML subgroup with less p53 expression than in the p53 normal group (**Fig. 6b**). In contrast, the percentage of CD14⁺/CD300E⁻ was significantly higher in the CMML group with p53 lower level compared to the normal group (**Fig. 6b**). Compared with control subjects, monocytes from patients with no p53 expression, expressed less CD300E at their surface whereas no difference was observed with the normal group (**Fig. 6b, Supplementary Fig. 5b**). Together, these results suggest that CD300E can be a biomarker to recognize CMML patient with less p53 expression.

Pre-mature monocytes from CMML patients with less p53 are more inflammatory. To better characterize the different stages of monocyte maturation, we performed bone marrow single cell RNA-seq analyses using CD34⁺ and CD14⁺ sorted cells from 2 age-matched controls, 3 TP53-normal, 2 TP53-intermediate and 2 TP53-diminished CMML patients. Single cell analysis of 68,254 cells showed 15 clusters defined by the expression of characteristic genes (**Supplementary Fig. 6a**) and further validated by the ten most expressed genes (**Supplementary Fig. 6b**). We next focused on Promonocytes1, Promonocytes2, Monocytes1 and Monocytes2 clusters, since we demonstrated that p53 is involved in the later stage of maturation (**Fig. 7a**). Interestingly, we observed a TP53 dosage effect regarding the percentage of Promonocytes2, less *TP53* is expressed more you have Promonocytes2 (**Fig. 7b**). Compared to controls, the 3 different CMML subgroups have also more Promonocytes1 (**Fig. 7b**). Monocytes1 were predominant in control samples whereas Monocytes2 were more present in the 3 CMML subgroups (**Fig. 7b**). In Promonocytes1, Promonocytes2, Monocytes1

and Monocytes2 clusters, we observed a TP53 dosage effect regarding the expression of *CEBPD*, *CD14*, *CXCR4*, *CEBPB* and *CD52* (**Fig. 7c**, **Supplementary Fig. 7a**). Together these results confirmed the involvement of p53 in monocyte maturation. In CMML monocytes2 subgroup, *CDKN1A* and *BAX* expression correlated with the level of expression of p53 (**Fig. 7d**). Interestingly, cells with less *TP53* expression have more *REL* expression, suggesting the activation of the NFκB pathway (**Fig. 7d**). Confirming this observation, pathway analysis using Hallmarks identified an up-regulation of Hallmark TNFA signaling via NFκB in TP53^{dim} Monocytes2 cluster compared to control and the TP53 normal group (**Fig. 7e**). An up-regulation of Hallmark TNFA signaling via NFκB and of Hallmark inflammatory response was also seen in TP53^{dim} Promonocytes2 cluster compared to control (**Supplementary Fig. 7b**). Using our 129 bulk monocyte RNAseq, an inverse correlation was also observed between *TP53* and NfκB members *REL* and *RELB* (**Supplementary Fig. 7c**). These data indicate that monocytes expressing less *TP53* are more inflammatory.

EGR1 regulates TP53 expression in CMML monocytes. EGR1 (Early growth response protein 1) encodes a transcription factor that is essential for monocytopoiesis and is recruited to *TP53* promoter in cord blood cells undergoing monocytic differentiation (**Fig. 8a**). EGR1 binds enhancers that regulate monocytic developmental genes such as *CSF-1R* with its FIRE sequence (**Supplementary Fig. 8a**). Using our two extreme groups (**Fig. 3**), we observed that *EGR1* messengers are less expressed in the group expressing less *TP53* (**Fig. 8b**). We next sorted human primary monocytes from healthy donor and CMML patient peripheral blood and quantified EGR1 protein expression by confocal microscopy. For example, monocyte from control SAC154 and CMML patient 3544 expressed EGR1 whereas EGR1 protein was almost not detectable in monocytes from patient 3350 (**Fig. 8c**, **Supplementary Fig. 8b**), confirming EGR1 protein heterogeneous expression in CMML classical monocytes. We next analyzed EGR1 expression in classical monocytes by confocal microscopy in a cohort of 15 healthy donors, 32 p53-expressing CMML patients and 21 CMML with no p53 expression. Patients with diminished p53 expression showed significantly lower levels of EGR1 compared to controls and TP53-expressing patients (**Fig. 8d**). A correlation between TP53 and *CSF-1R* is also seen at the protein level after confocal image quantification in these two groups (**Fig. 8e**). To confirm the regulation of *TP53* by EGR1, we co-transfected 293T cells with an empty vector or pcDNA3-EGR1. In 293T cells, over-expression of EGR1 induces more p53 protein (**Fig. 8f**). To

demonstrate EGR1-dependent *TP53* expression in CMML monocytes, we transfected EGR1-siRNA in monocytes collected from 2 CMML patients. *EGR1* mRNA down regulation was previously validated by RT-qPCR [22]. *EGR1* downregulation prevented *TP53* expression in these monocytes (Fig. 8g, Supplementary Fig. 8c).

CMML patients weakly expressing TP53 in their monocytes have a worst overall survival with hydroxyurea treatment. We next investigated whether p53 expression level may have an impact in CMML treatment. Using the DACOTA trial with advanced disease [5], we analyzed *TP53* expression level impact in the decitabine arm and in the hydroxyurea arm. Whereas no difference in overall survival was seen in the decitabine arm between the two groups (Fig. 9a), we demonstrated that CMML patients expressing weakly TP53 had poorer survival when treated with hydroxyurea (Fig. 9b). Interestingly, monocytes from these CMML patients have a strong downregulation of genes involved DNA repair pathway (Fig. 9c).

Discussion

The present study identifies a role for p53 protein in terminal maturation of monocytes. Classical monocytes detected by flow cytometry in the blood of CMML patients are heterogeneous. When p53 expression is reduced, these classical monocytes have a dysplastic or immature phenotype, in particular they express less CD300E at their surface and they are more inflammatory.

We have previously identified a role for miR-150 through TET3 expression regulation in the generation of intermediate and nonclassical monocyte subsets [23]. In CMML patients who respond to demethylating agents, the restoration of the three monocyte subsets is associated with the reexpression of MIR150 whose promoter is hypermethylated. It will be interesting to check if demethylating agents may also restore *EGR1* and *TP53* expression and induce classical monocyte maturation.

TP53-deficient cells are known to be resistant to apoptosis. We previously shown that classical monocytes from CMML patients can be resistant to apoptosis [24]. This resistance involves MCL-1 protein, an anti-apoptotic member of the BCL2 family, and the MAP kinase pathway.

Indeed, the combined inhibition of MCL1 and MAPK therefore appears to be a potential strategy to slow the progression of CMML by inducing apoptosis of leukemic monocytes. Unfortunately, the toxicity (particularly cardiac) of MCL1 inhibitors, detected during phase 1/2 trials, has currently slowed down their development and the exploration of this approach. It has been shown that loss of p53 function results in down-regulation of the MCL-1 protein levels in keratinocytes [25]. In glioblastoma cells, p53 has been shown to regulate MCL-1 at the transcriptional level [26]. It will be interesting to check if in monocytes *MCL-1* is also a p53-target and whether monocytes with normal expression of p53 are those that express more MCL-1.

The role of p53 in cell death and its function in cell cycle arrest have been extensively studied. Its involvement in cell differentiation is less well known. p53 acts as a negative regulator of autoimmunity, not only by suppressing inflammatory cytokines but also by directly controlling T-cell differentiation [27]. P53 is also involved in normal erythroid differentiation during ribosome biogenesis [28]. Interestingly, in this study, p53 activation in immature erythroblasts was shown to drive erythroid gene expression. Several old publications suggest a role for p53 in the differentiation of granulocytes and in the early and late stages of monocytic differentiation [29]–[32]. Our experiments further suggest a role for p53 in the maturation of classical monocytes through the acquisition of CD300E at the cell surface. To better characterize p53 involvement during monocyte maturation, p53 direct targets should be analyzed by immunoprecipitating p53 on the chromatin followed by sequencing (Cut and Tag).

Interestingly, monocytes with decreased expression of TP53 have enriched pathways related to NF- κ B with TNFA Signaling via NF- κ B and inflammatory response. This observation is consistent with the constitutive activation of NF- κ B that is commonly observed in tumors, which are p53 deficient in the majority [33]. In a mouse model of LPS-induced lung injury, p53-deficient macrophages and neutrophils compared to wild-type cells had increased responses to LPS stimulation, thanks to enhanced NF- κ B DNA binding activity and stronger induction of pro-inflammatory cytokines [34]. All these data indicate that p53 is a negative regulator of inflammation.

The abnormal repartition of monocyte subsets characterizing CMML has provided a diagnostic tool in patients with a monocytosis to rapidly distinguish CMML from a reactive monocytosis. Using the DACOTA trial [5], designed by the European Myelodysplastic Syndromes Cooperative Group, comparing hydroxyurea and decitabine, we observed that CMML patients expressing low level of TP53 in their monocytes have a worst overall survival that the one expressing TP53. In the other hand, in the decitabine arm, there are no differences in the overall survival in both groups. It might be important to determine p53 status before to treat CMML patients with hydroxyurea. The “cut-off” value of the expression of a gene or protein in a sorted cell population is difficult to establish and even more difficult to use in clinical and biological routine. We demonstrated here a role for p53 protein in the final steps of monocytopoiesis. *TP53* expression reduction induces the immaturity/dysplasia of classical monocytes that we could detect by refining the analysis of these monocytes by flow cytometry by adding CD300E.

In conclusion, our data delineate p53 involvement in the generation of mature classical monocytes. Targeting of this pathway may provide new strategies to modulate monocyte maturation and investigate their therapeutic interest in pathological situations.

Methods

Control and patient sample collection. Peripheral blood samples were collected from healthy donors (Buffy coat, Etablissement Français du Sang, Rungis, France), age-matched donors (SAC) and CMML patients with informed consents in compliance with the ethical committee Ile-de-France (MYELOMONO cohort, DC-2014-2091). CMML patients were diagnosed according to the latest 2022 WHO criteria (Leukemia, 2022). CMML patient clinico-biological characteristics, summarized in **Table S1** for p53 immunofluorescence study, were obtained from our CMML database with clinical/biological annotations (DR-2016-256) and NGS analysis of a previously described panel (Coltro G et al, Leukemia, 2020).

Monocyte sorting. Blood samples from healthy donors or CMML patients was separated on Pancoll (Pan-Biotech, Dutscher, Brumath, France) to obtain peripheral blood mononucleated cells (PBMC). Peripheral blood CD14⁺ monocytes were sorted with CD14 magnetic beads and the AutoMacs system (Miltenyi Biotech, Paris, France). Monocytes used for nucleofection experiments were sorted with Monocyte isolation kit II (Miltenyi Biotech). After sorting, the monocytes were used to make slides for immunofluorescence, trizol, dry pellets or cell culture.

RNA sequencing and analysis. 129 CMML CD14⁺ monocyte RNAs were used to prepare libraries with Illumina TruSeq RNA Sample Prep Kit v2. All controls and CMML patients were subjected to NGS myeloid panel sequencing [4], [35]. RNA-seq libraries were sequenced on a 75 bp pair-end run using NextSeq500 High Output Kit 150 cycles; 400M flow cell. Transcripts from Gencode V24 were quantified in transcripts per million using Kallisto software. Gene-level expression was the sum of transcripts per million values for each gene transcript. Raw reads were mapped to the hg19 genome with Tophat2 (v2.0.14)/Bowtie2 (v2.1.0). The number of reads per gene was counted using HTSeq (0.5.4p5) and GENECODE (v24lift37), and differential gene expression analysis was performed by using DESeq2 package (v1.10.1). Genes with an absolute log₂ fold change >1 and P <.05 were reported. Heatmap was generated using Morpheus (<https://software.broadinstitute.org/morpheus/>).

Immunoblotting. Cells were lysed for 15 min at 4 °C in lysis buffer (Laemmli 2x, DTT and Protease Inhibitor Complete (Roche)). 15 µg of total proteins were separated on polyacrylamide gel and transferred to nitrocellulose membrane (ThermoFisher Scientific). Membranes were blocked with 5% bovine serum albumin in PBS, with 0.1% Tween-20 (Sigma-Aldrich) for 1h at RT, incubated overnight at 4 °C with the primary antibodies (dilution 1/1000e), washed in PBS-0.1% Tween-20, incubated further with HRP-conjugated secondary antibody (400 ng/mL) for 1 h at RT and washed again before analysis using Immobilon Western Chemiluminescent HRP Substrate system (Millipore). The chemiluminescent emission was registered by iBright Imaging Systems (Thermofisher scientific). Actin (A5441, Sigma-Aldrich), p53 (2527S, Cell signaling) and EGR1 (A303-390A, Bethyl) antibodies were used for detection and quantifications were performed using Image J software (<http://imagej.nih.gov/ij>).

Confocal microscopy. The monocytes were adhered onto polylysine slides for 1 h at 37°. Then, the cells directly fixed with PFA on the slide for 10 min with 4% paraformaldehyde (PFA). After 3 washes in PBS 1X, cells were incubated with anti-TP53 antibody (2527S, Cell signaling), anti-CSF-1R antibody (sc-692, SantaCruz) or anti-EGR1 antibody (A303-390A, Bethyl) diluted in 0.05% saponin during 1 h at room temperature (RT), followed by several washes. The secondary antibody : goat anti-rabbit Alexa Fluor 488 diluted at 1 :200 with 0.05% saponin during 1h followed by several washes. Slides were mounted using Vectashield mounting medium with Dapi (Vectorlabs, Clinisciences). Images were acquired under a confocal Leica SP8 microscope, with a 63x/1.4 numeric aperture oil objective (Leica Microsystem). Quantification were analyzed using Image J software (<http://imagej.nih.gov/ij>).

Apoptosis experiment. After sorting, monocytes were then seeded at 10⁶/mL in RPMI 1640 Glutamax medium (ThermoFisher Scientific) supplemented with 10% heat inactivated fetal bovine serum (FBS, Lonza, Amboise, France), 1% penicillin/streptomycin, and 2mM L-Glutamine (ThermoFisher Scientific) with ou without Etoposide (50µM) for 7h. After 7hours, cells were washed and stored in Trizol for RT-qPCR experiment.

RT-qPCR analysis. Total RNA was extracted with RNA/DNA/Protein Purification Plus Kits (Norgen) or RNA Extraction Kits (ZYMO research) and reverse transcribed with SuperScript™ IV VILO™ Master Mix with ezDNase™ Enzyme (Thermo Fisher Scientific). Real-time

quantitative polymerase chain reaction (RT-qPCR) was performed with AmpliTaq Gold polymerase in an Applied Biosystems 7500 thermocycler using the standard SyBR Green detection protocol as outlined by the manufacturer (Thermo Fisher Scientific). Briefly, 12 ng of total cDNA, 50 nM (each) primers, and 1× SyBR Green mixture were used in a total volume of 20 µL. Human sequence primers are : HPRT (Forward : 5'-GGACAGGACTGAACGTCTTGC-3'; Reverse : 5'-CTTGAGCACACAGAGGGCTACA-3') ; PPIA (Forward : 5'-GTCGACGGCGAGCCC-3'; Reverse : 5'-TCTTTGGGACCTGTCTGCAA-3'); BAX (Forward : 5'-GTGCCTCAGGATGCGTCC-3'; Reverse : 5'-TCCAGTTCGTCCCGATG-3'); CDKN1A (Forward : 5'-CGCTAATGGCGGGCTG-3'; Reverse : 5'-CGGTGACAAAGTCGAAGTTCC-3'); TP53 (Forward : 5'-TTGGTAGGTGGATTATTTGTTT-3'; Reverse : 5'-CCAATCCAAAAAACATATCAC-3') and CFMS (Forward : 5'-GCCCCCATCACTCACT-3'; Reverse : 5'-GTGTTTTGGAAGGTAGCGTTGTT-3').

3' Single-cell RNAseq: Bone marrow BMNC controls and CMML stored in DMSO were thawed and CD34+ and CD14+ cells were isolated as previously described. Single-cell suspensions were loaded onto a Chromium Single Cell Chip (10x Genomics) according to the manufacturer's instructions for co-encapsulation with barcoded Gel Beads at a target capture rate of ~10,000 individual cells per sample. Captured mRNAs were barcoded during cDNA synthesis using the Chromium Next GEM Single Cell 3' GEM, Library & Gel Bead Kit v3.1 (10X Genomics) according to the manufacturer's instructions. All samples were processed simultaneously with the Chromium Controller (10X Genomics) and the resulting libraries were prepared in parallel in a single batch. We pooled all of the libraries for sequencing in a single SP Illumina flow cell. All of the libraries were sequenced with an 8-base index read, a 28-base Read1 containing cell-identifying barcodes and unique molecular identifiers (UMIs), and a 91-base Read2 containing transcript sequences on an Illumina NovaSeq 6000 at Gustave Roussy.

3' Single-cell RNAseq analysis: Raw BCL-files were demultiplexed and converted to Fastq format using bcl2fastq (version 2.20.0.422 from Illumina). Reads quality control was performed using fastqc (version 0.11.9) and assignment to the expected genome species evaluated with fastq-screen (version 0.14.0). Reads were pseudo-mapped to the Ensembl reference transcriptome v99 corresponding to the homo sapiens GRCh38 build with kallisto (version 0.46.2) using its 'bus' subcommand and parameters corresponding to the 10X

Chromium 3' scRNA-Seq v3 chemistry. The index was made with the kb-python (version 0.24.4) wrapper of kallisto. Barcode correction using whitelist provided by the manufacturer (10X Genomics) and gene-based reads quantification was performed with BUStools (version 0.40.0).

Cell barcode by symbol count table were loaded in R (version 4.0.4) using the BUSpaRse package (version 1.5.3). To call real cells from empty droplets, we used the emptyDrops function from the dropletUtils package (version 1.10.3), which assesses whether the RNA content associated with a cell barcode is significantly distinct from the ambient background RNA present within each sample. Barcodes with p-value < 0.001 (Benjamini-Hochberg-corrected) were considered as legitimate cells for further analysis.

The count matrix was filtered to exclude genes detected in less than 5 cells, cells with less than 1500 UMIs or less than 200 detected genes, as well as cells with mitochondrial transcripts proportion higher than 20%. Cell cycle scoring of each cell was performed using two methods: the CellcycleScoring function from the Seurat package (version 4.0.0), and the cyclone function from Scran (version 1.18.5). Barcodes corresponding to doublets were identified and discarded using the union of two methods: scDbfFinder (version 1.4.0) using default parameters, and scds (version 1.6.0) with its hybrid method using default parameters. We manually verified that the cells identified as doublets did not systematically correspond to cells in G2M phase.

Seurat (version 4.0.0) was applied for further data processing. The SCTransform normalization method was used to normalize, scale, select 3000 Highly Variable Genes and regress out bias factors (the number of detected transcripts and the proportion of ribosomal transcripts). The number of PCA dimensions to keep for further analysis was evaluated by assessing a range of reduced PCA spaces using 3 to 49 dimensions, with a step of 2. For each generated PCA space, Louvain clustering of cells was performed using a range of values for the resolution parameter from 0.1 to 1.2 with a step of 0.1. The optimal space was manually evaluated as the one combination of kept dimensions and clustering resolution resolving the best structure (clusters homogeneity and compacity) in a Uniform Manifold Approximation and Projection space (UMAP). For the 9 samples, 21 dimensions were retained with a resolution of 0.2.

Marker genes for Louvain clusters were identified through a «one versus others» differential analysis using the Wilcoxon test through the FindAllMarkers function from Seurat, considering

only genes with a minimum log fold-change of 0.5 in at least 75% of cells from one of the groups compared, and FDR-adjusted p-values <0.05 (Benjaminin-Hochberg method). Gene visualization was done using Cerebro (version 1.2.2).

In vitro monocyte differentiation. Umbilical cord blood samples were collected from healthy newborns with mother consent: AP-HP, Hôpital Saint-Louis, Unité de Thérapie Cellulaire, CRB-Banque de Sang de Cordon, Paris, France – N° d’autorisation: AC-2016-2759. Sorted CD34+ cells, using magnetic beads and AutoMacs system (Miltenyi Biotech). Sorted CD34+ were cultured 72h at 3×10^5 cells/mL in SFEM II (StemCell technologies) with 1% penicillin/streptomycin and stem cell factor recombinant (SCF, 40 ng/mL; Immunex), thrombopoietin recombinant (TPO, 40 ng/mL; Peprotech), Fms-like tyrosine kinase 3 recombinant (FLT-3, 40 ng/mL; Diaclone), UM729 (StemCell technologies), Stem Regenin1 (StemCell technologies). After, these cells were infected twice with scramble (shCTL) or shTP53 with MOI (Multiplicity of infection) of 12 and sorted 48h later on CD34 and GFP expression. shRNA sequence for p53 knockdown has been previously cloned into the lentiviral vector pRRLsin-PGK-eGFP-WPRE and validated [36]. Cells were cultured in SFEM II (StemCell technologies) with 1% penicillin/streptomycin and a cocktail of human recombinant cytokines containing stem cell factor recombinant (SCF, 40 ng/mL; Immunex) and Monocyte colony-stimulating factor recombinant (M-CSF 10 ng/mL; Peprotech), in a 37 °C incubator with 5% CO₂. At days 4,5,6,7 and 8 of culture, cells were stained with the antibodies for immunophenotypic analysis (Table1 for antibodies) using a Fortessa flow cytometer (BD biosciences). Analysis was performed using Kaluza Software (Beckman Coulter).

Table1 : Antibodies for Flow cytometry analyses and sorting

| Antigen | Clone (Isotype) | Fluorochrome | Source | Catalog nb | Dilution |
|----------------|-----------------|--------------|-----------|------------|----------|
| CD34 | 581 | APC | Sony | 160955 | 1/100 |
| CD14 | M5E2 | PB | BD | 558121 | 1/100 |
| HLA-DR | G46-6 | BV650 | BD | 564231 | 1/100 |
| CD36 | 5-271 | PeCy7 | Biolegend | 336222 | 1/100 |
| CD64 | 10.1 | PerCP5.5 | Biolegend | 305024 | 1/100 |
| CD45 | HI30 | Buv510 | Biolegend | 304036 | 1/100 |
| CD300e (IREM2) | UP-H2 | Pe | Biolegend | 339704 | 1/100 |

Flow cytometry analysis. Human peripheral blood mononuclear cells (PBMCs) were sorted and labeled with antibodies (Table 2 for antibodies) as previously described (Selimoglu-Buet D et al, Blood, 2015). At least 50,000 events in the CD14/CD16 gate were analyzed by flow cytometry (LSR II, BD Biosciences). Flow cytometry standard listmode data were analyzed centrally in a blind fashion using Kaluza software (Beckman Coulter). The monocyte subsets were identified following an exclusion gating strategy ([Supplementary Figure 5](#)).

Table 2 : Antibodies for Flow cytometry analyses and sorting

| Antigen | Clone | Fluorochrome | Source | Catalog nb | Dilution |
|----------------|---------|-----------------|-----------------|------------|----------|
| Cd45 | HI30 | Bv510 | Biolegend | 304036 | 1/100 |
| CD24 | ALB9 | Buv395 | BD | 566221 | 1/100 |
| CD56 | N901 | APC | Beckman Coulter | IM2474U | 1/100 |
| CD16 | 3G8 | PeCy7 | BD | 560195 | 1/100 |
| SLAN | DD-1 | FITC | Miltenyi | 5230308078 | 1/100 |
| CD2 | RPA-210 | R-PE, texas Red | Biolegend | 300228 | 1/100 |
| CD7 | M-T701 | R-PE, texas Red | BD | 562541 | 1/100 |
| CD14 | M5E2 | BV421 | BD | 563743 | 1/100 |
| CD300e (IREM2) | UP-H2 | Pe | Biolegend | 339704 | 1/100 |

293T transient transfection. 293T cells were transfected as previously described (Pronier E et al, Commun Biol, 2022). Culture media were collected containing the proteins. Anti-EGR1 (A303-390A) antibody, were use at 1/1000 : was purchased from Bethyl and anti-TP53 (2527S) antibody, were use ar 1/1000 from Cell Signaling. Detection was performed using HRP-conjugated secondary antibody (400 ng/mL). All membrane were visualized using iBright Imaging Systems (ThermoFisher scientific) and for quantification using Image J software(<http://imagej.nih.gov/ij>).

EGR1 knockdown in CMML patient monocytes. We used Stealth siRNA duplex (ThermoFisher Scientific) targeting EGR1 and Stealth RNAi™ siRNA negative control (SCR) introduced into CMML monocytes by Lipofectamine 2000 (ThermoFisher Scientific). Briefly, 1×10^6 cells in 500 μ l in a 24-well plate were transfected according to the manufacturer's recommendations with 1nmol siRNA.

siRNAs targeting EGR1:

siEGR1-1: 5'-UCUCCCAGGACAAUUGAAAUUUGCU-3';

siEGR1-2: 5'-AGCAAUUUCAAUUGUCCUGGGAGA-3';

siEGR1-3: 5'-GAUCUCUGACCCGUUCGGAUCCUUU-3'.

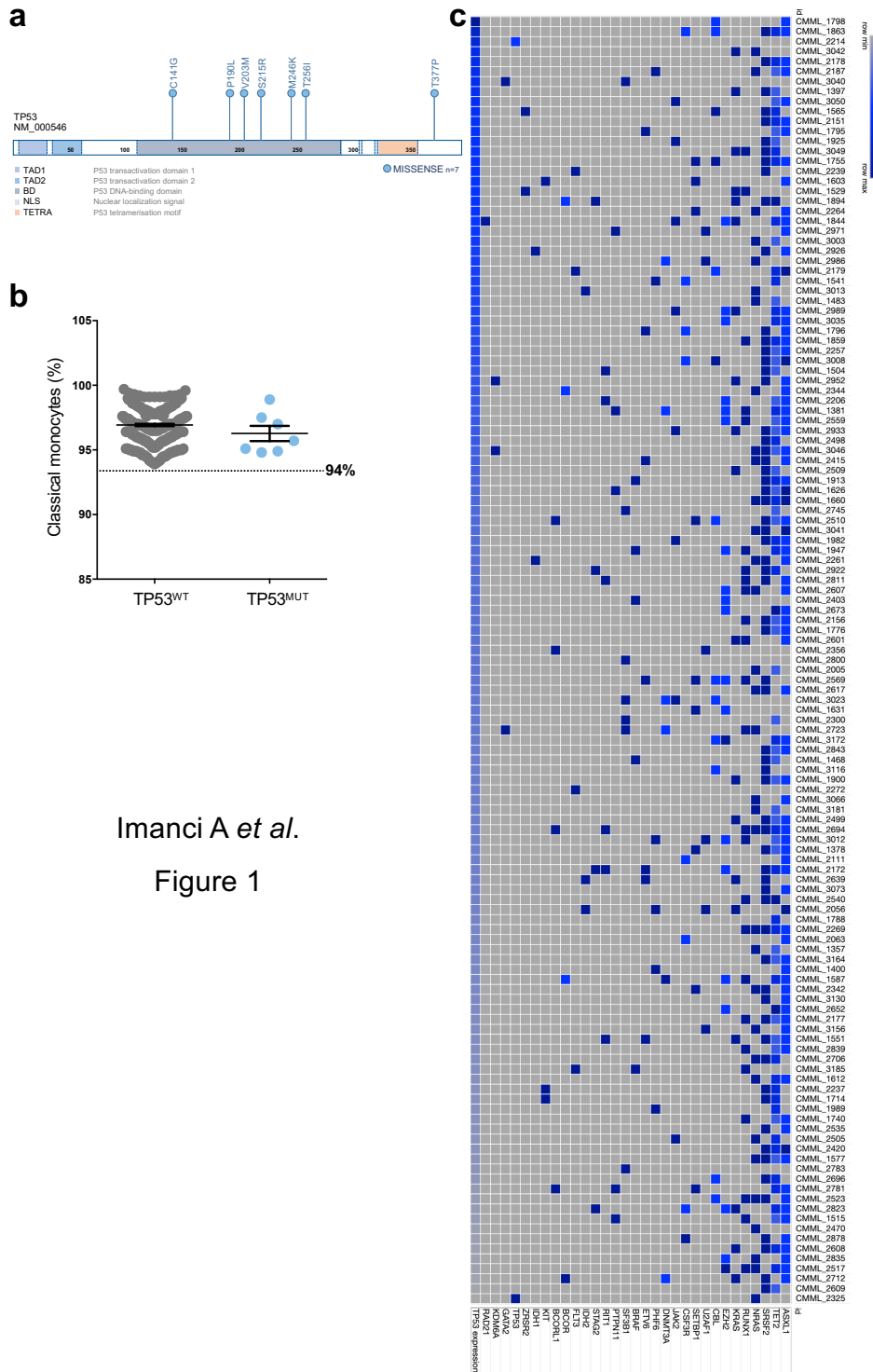
EGR1 knock-down efficacy was previously shown using RT-qPCR (Pronier E et al, Commun Biol, 2022) and p53 mRNA expression was addressed in these monocytes.

References

- [1] X. Calvo *et al.*, « Oligomonocytic and overt chronic myelomonocytic leukemia show similar clinical, genomic, and immunophenotypic features », *Blood Advances*, vol. 4, n° 20, p. 5285-5296, oct. 2020, doi: 10.1182/bloodadvances.2020002206.
- [2] J. D. Khoury *et al.*, « The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Myeloid and Histiocytic/Dendritic Neoplasms », *Leukemia*, vol. 36, n° 7, p. 1703-1719, juill. 2022, doi: 10.1038/s41375-022-01613-1.
- [3] J. Kwon, « Diagnosis and treatment of chronic myelomonocytic leukemia », *Blood Res*, vol. 56, n° S1, p. S5-S16, avr. 2021, doi: 10.5045/br.2021.2020321.
- [4] J. Merlevede *et al.*, « Mutation allele burden remains unchanged in chronic myelomonocytic leukaemia responding to hypomethylating agents », *Nat Commun*, vol. 7, n° 1, p. 10767, févr. 2016, doi: 10.1038/ncomms10767.
- [5] R. Itzykson *et al.*, « Decitabine Versus Hydroxyurea for Advanced Proliferative Chronic Myelomonocytic Leukemia: Results of a Randomized Phase III Trial Within the EMSCO Network », *J Clin Oncol*, vol. 41, n° 10, p. 1888-1897, avr. 2023, doi: 10.1200/JCO.22.00437.
- [6] L. Ziegler-Heitbrock et T. Hofer, « Toward a Refined Definition of Monocyte Subsets », *Frontiers in Immunology*, vol. 4, 2013, Consulté le: 2 octobre 2023. [En ligne]. Disponible sur: <https://www.frontiersin.org/articles/10.3389/fimmu.2013.00023>
- [7] D. Selimoglu-Buet *et al.*, « Characteristic repartition of monocyte subsets as a diagnostic signature of chronic myelomonocytic leukemia », *Blood*, vol. 125, n° 23, p. 3618-3626, juin 2015, doi: 10.1182/blood-2015-01-620781.
- [8] M. M. Patnaik *et al.*, « Flow cytometry based monocyte subset analysis accurately distinguishes chronic myelomonocytic leukemia from myeloproliferative neoplasms with associated monocytosis », *Blood Cancer J*, vol. 7, n° 7, p. e584, juill. 2017, doi: 10.1038/bcj.2017.66.
- [9] R. Itzykson *et al.*, « Prognostic score including gene mutations in chronic myelomonocytic leukemia », *J Clin Oncol*, vol. 31, n° 19, p. 2428-2436, juill. 2013, doi: 10.1200/JCO.2012.47.3314.
- [10] C. C. Mason *et al.*, « Age-related mutations and chronic myelomonocytic leukemia », *Leukemia*, vol. 30, n° 4, Art. n° 4, avr. 2016, doi: 10.1038/leu.2015.337.
- [11] C. Kandoth *et al.*, « Mutational landscape and significance across 12 major cancer types », *Nature*, vol. 502, n° 7471, p. 333-339, oct. 2013, doi: 10.1038/nature12634.
- [12] A. Zehir *et al.*, « Mutational landscape of metastatic cancer revealed from prospective clinical sequencing of 10,000 patients », *Nat Med*, vol. 23, n° 6, Art. n° 6, juin 2017, doi: 10.1038/nm.4333.
- [13] R. Bejar *et al.*, « Clinical effect of point mutations in myelodysplastic syndromes », *N Engl J Med*, vol. 364, n° 26, p. 2496-2506, juin 2011, doi: 10.1056/NEJMoa1013343.
- [14] D. Haase *et al.*, « TP53 mutation status divides myelodysplastic syndromes with complex karyotypes into distinct prognostic subgroups », *Leukemia*, vol. 33, n° 7, p. 1747-1758, juill. 2019, doi: 10.1038/s41375-018-0351-2.
- [15] E. Bernard *et al.*, « Implications of TP53 allelic state for genome stability, clinical presentation and outcomes in myelodysplastic syndromes », *Nat Med*, vol. 26, n° 10, Art. n° 10, oct. 2020, doi: 10.1038/s41591-020-1008-z.
- [16] A. Stengel, W. Kern, T. Haferlach, M. Meggendorfer, A. Fasan, et C. Haferlach, « The impact of TP53 mutations and TP53 deletions on survival varies between AML, ALL,

- MDS and CLL: an analysis of 3307 cases », *Leukemia*, vol. 31, n° 3, p. 705-711, mars 2017, doi: 10.1038/leu.2016.263.
- [17] M. Mei *et al.*, « The mutational landscape in chronic myelomonocytic leukemia and its impact on allogeneic hematopoietic cell transplantation outcomes: a Center for Blood and Marrow Transplantation Research (CIBMTR) analysis », *Haematologica*, vol. 108, n° 1, p. 150-160, janv. 2023, doi: 10.3324/haematol.2021.280203.
- [18] M. Gurney *et al.*, « Somatic TP53 single nucleotide variants, indels and copy number alterations in chronic myelomonocytic leukemia (CMML) », *Leukemia*, juill. 2023, doi: 10.1038/s41375-023-01964-3.
- [19] L. Bencheikh *et al.*, « Dynamic gene regulation by nuclear colony-stimulating factor 1 receptor in human monocytes and macrophages », *Nat Commun*, vol. 10, n° 1, p. 1935, avr. 2019, doi: 10.1038/s41467-019-09970-9.
- [20] A. Franzini *et al.*, « The transcriptome of CMML monocytes is highly inflammatory and reflects leukemia-specific and age-related alterations », *Blood Adv*, vol. 3, n° 20, p. 2949-2961, oct. 2019, doi: 10.1182/bloodadvances.2019000585.
- [21] F. V. Mello *et al.*, « Maturation-associated gene expression profiles along normal human bone marrow monopoiesis », *Br J Haematol*, vol. 176, n° 3, p. 464-474, févr. 2017, doi: 10.1111/bjh.14467.
- [22] E. Pronier *et al.*, « Macrophage migration inhibitory factor is overproduced through EGR1 in TET2^{low} resting monocytes », *Commun Biol*, vol. 5, n° 1, Art. n° 1, févr. 2022, doi: 10.1038/s42003-022-03057-w.
- [23] D. Selimoglu-Buet *et al.*, « A miR-150/TET3 pathway regulates the generation of mouse and human non-classical monocyte subset », *Nat Commun*, vol. 9, n° 1, p. 5455, déc. 2018, doi: 10.1038/s41467-018-07801-x.
- [24] M. Sevin *et al.*, « Cytokine-like protein 1-induced survival of monocytes suggests a combined strategy targeting MCL1 and MAPK in CMML », *Blood*, vol. 137, n° 24, p. 3390-3402, juin 2021, doi: 10.1182/blood.2020008729.
- [25] V. Chaturvedi *et al.*, « Knockdown of p53 levels in human keratinocytes accelerates Mcl-1 and Bcl-xL reduction thereby enhancing UV-light induced apoptosis », *Oncogene*, vol. 24, n° 34, Art. n° 34, août 2005, doi: 10.1038/sj.onc.1208650.
- [26] K. E. Tagscherer, A. Fassl, T. Sinkovic, S. E. Combs, et W. Roth, « p53-dependent regulation of Mcl-1 contributes to synergistic cell death by ionizing radiation and the Bcl-2/Bcl-XL inhibitor ABT-737 », *Apoptosis*, vol. 17, n° 2, p. 187-199, févr. 2012, doi: 10.1007/s10495-011-0664-3.
- [27] J.-S. Park *et al.*, « p53 controls autoimmune arthritis via STAT-mediated regulation of the Th17 cell/Treg cell balance in mice », *Arthritis Rheum*, vol. 65, n° 4, p. 949-959, avr. 2013, doi: 10.1002/art.37841.
- [28] S. Le Goff *et al.*, « p53 activation during ribosome biogenesis regulates normal erythroid differentiation », *Blood*, vol. 137, n° 1, p. 89-102, janv. 2021, doi: 10.1182/blood.2019003439.
- [29] S. Soddu *et al.*, « Wild-type p53 gene expression induces granulocytic differentiation of HL-60 cells », *Blood*, vol. 83, n° 8, p. 2230-2237, avr. 1994.
- [30] D. Banerjee *et al.*, « Transfection of wild-type but not mutant p53 induces early monocytic differentiation in HL60 cells and increases their sensitivity to stress », *Cell Growth Differ*, vol. 6, n° 11, p. 1405-1413, nov. 1995.
- [31] D. Ronen, D. Schwartz, Y. Teitz, N. Goldfinger, et V. Rotter, « Induction of HL-60 cells to undergo apoptosis is determined by high levels of wild-type p53 protein whereas

- differentiation of the cells is mediated by lower p53 levels », *Cell Growth Differ*, vol. 7, n° 1, p. 21-30, janv. 1996.
- [32] D. Matas, M. Milyavsky, I. Shats, L. Nissim, N. Goldfinger, et V. Rotter, « p53 is a regulator of macrophage differentiation », *Cell Death Differ*, vol. 11, n° 4, p. 458-467, avr. 2004, doi: 10.1038/sj.cdd.4401379.
- [33] K. V. Gurova *et al.*, « Small molecules that reactivate p53 in renal cell carcinoma reveal a NF-kappaB-dependent mechanism of p53 suppression in tumors », *Proc Natl Acad Sci U S A*, vol. 102, n° 48, p. 17448-17453, nov. 2005, doi: 10.1073/pnas.0508888102.
- [34] G. Liu, Y.-J. Park, Y. Tsuruta, E. Lorne, et E. Abraham, « p53 Attenuates lipopolysaccharide-induced NF-kappaB activation and acute lung injury », *J Immunol*, vol. 182, n° 8, p. 5063-5071, avr. 2009, doi: 10.4049/jimmunol.0803526.
- [35] G. Coltro *et al.*, « Clinical, molecular, and prognostic correlates of number, type, and functional localization of TET2 mutations in chronic myelomonocytic leukemia (CMML)—a study of 1084 patients », *Leukemia*, vol. 34, n° 5, p. 1407-1421, mai 2020, doi: 10.1038/s41375-019-0690-7.
- [36] E. Mahfoudhi *et al.*, « P53 activation inhibits all types of hematopoietic progenitors and all stages of megakaryopoiesis », *Oncotarget*, vol. 7, n° 22, p. 31980-31992, mars 2016, doi: 10.18632/oncotarget.7881.



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 Figure 1

Figure 1. p53 is rarely mutated in CMML patients. **a**) Molecular spectrum of TP53 missense mutations in a cohort of 249 CMML patients. **b**) percentage of classical monocytes using the cohort of 249 patients in TP53 wild type (TP53^{WT}) and TP53 mutated (TP53^{MUT}) groups. **c**) Heatmap of the mutations and TP53 expression in the monocytes of the CMML patients included in the DACOTA trial at inclusion.

Imanci A *et al.* Figure 2

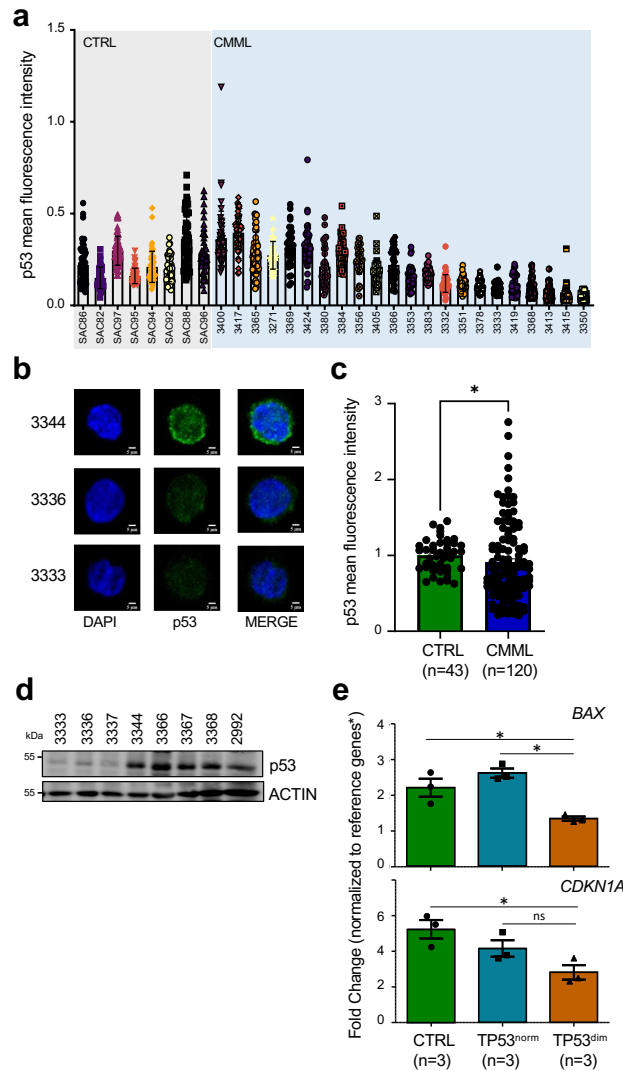


Figure 2. p53 is abnormally expressed in CMML monocytes. **a)** Peripheral blood classical monocytes were fixed and stained for p53 (Ab: 2527s) followed by ImageJ analysis in each cell (Ctrl n= 8, CMML n= 22). Each point on the graph represents a cell. **b)** Peripheral blood monocytes were fixed and stained for p53 (Ab: 2527s, green) and Dapi (blue) followed by confocal imaging in one cell from CMML patient 3344, 3336 and 3333 (scale: 5 μ m). **c)** p53 mean fluorescence intensity in classical monocytes from age-matched controls (SAC, n=43) and CMML patients, (n=120). Mean +/- SEM from 13 experiments independent **d)** Total proteins were extracted from isolated CMML monocytes and analyzed by immunoblotting with antibodies that recognize p53 and actin. **e)** RT-qPCR analysis of *BAX* and *CDKN1A* mRNA expression in monocytes after 7h of treatment with 50 μ M etoposide from controls (CTRL, green), CMML p53-expressing patients (TP53^{norm}, blue) and CMML patients with no p53 expression (TP53^{dim}, orange). The results have been normalized against the genes of reference (*HPRT*, *PPIA* and *GUS*) and reported to untreated conditions. Mean +/- SEM.

Imanci A *et al.* Figure 3

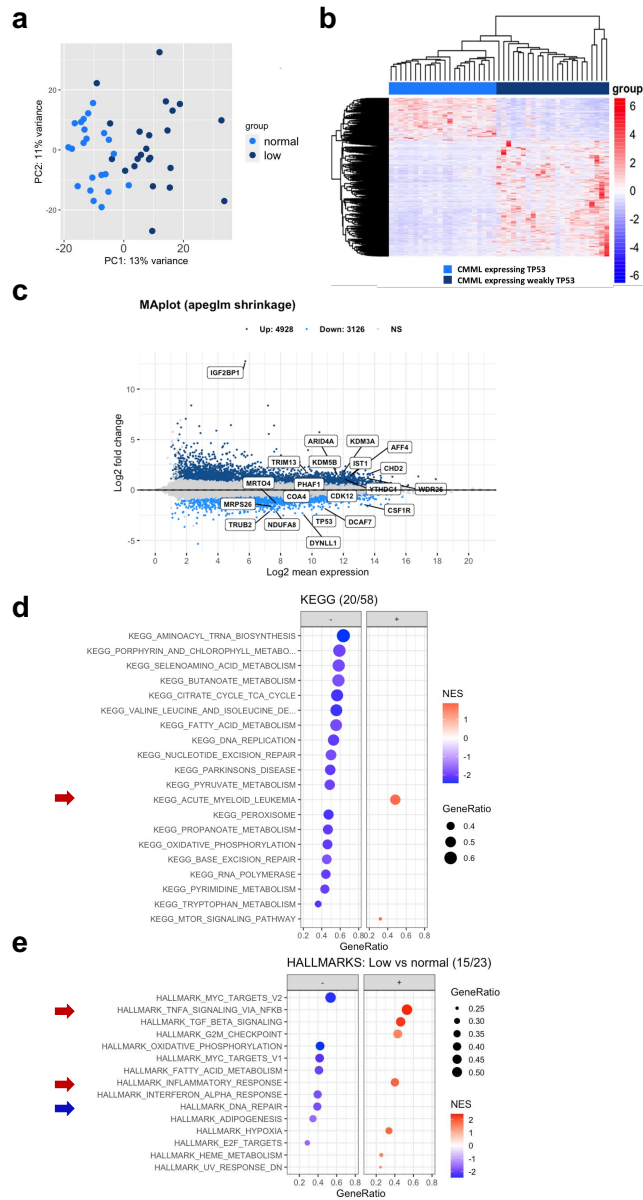


Figure 3. CMML TP53-diminished monocytes have an AML signature and are more inflammatory. **a**) PCA plot of RNA-seq data from TP53-expressing monocytes (normal) and TP53-diminished monocytes (low). **b**) Heatmap of RNA-seq results showing the relative expression levels of differential gene expression in the two subgroups. **c**) MA-plot of the DEGs between TP53-expressing monocytes and TP53-diminished monocytes. The vertical axis represents the log₂ fold change (FC) and the horizontal axis the $-\log_2$ mean expression. Significantly ($p_{adj} < 0.05$) upregulated (dark blue) and downregulated (light blue) genes are shown. **d-e**) GSEA analysis using KEGG and Hallmark gene sets. Significant ($p_{adj} < 0.05$) gene sets between TP53-expressing monocytes and TP53-diminished monocytes.

Imanci A *et al.* Figure 4

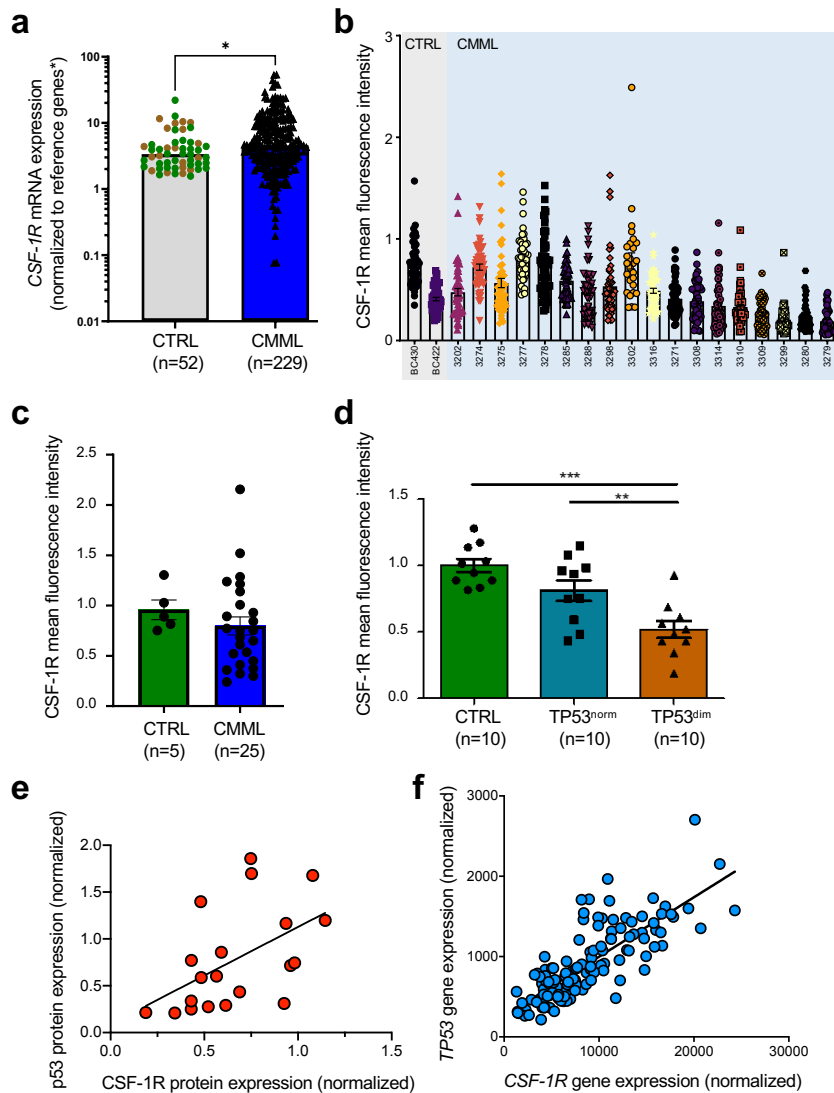


Figure 4. CSF-1R is less expressed in CMML monocytes expressing weakly TP53. **a)** RT-qPCR analysis of *CSF-1R* mRNA expression in monocytes from young controls (n = 17; brown), age-matched controls (n = 52; green) and CMML monocyte (n = 229; blue). The results have been normalized against the genes of reference (*HPRT*, *PPIA* and *GUS*). Mean +/- SEM. **b)** Peripheral blood classical monocytes were fixed and stained for CSF-1R (ab, PA525974) followed by ImageJ analysis (Ctrl n= 2, CMML n= 18). Each point on the graph represents a cell. **c)** CSF-1R (ab, PA525974) expression in CD14⁺ monocytes from healthy subjects (age-matched, SAC, n=5) and CMML patients (n=25). Mean +/- SEM from 2 independent experiments. **d)** CSF-1R (ab, sc-692) expression in CD14⁺ monocytes from healthy subjects (CTRL, n=10), CMML patients with p53 expression (TP53^{norm}, n=10) and CMML patients with no p53 expression (TP53^{dim}, n=10). Mean +/- SEM. **e)** Correlation between p53 and CSF-1R protein expression in CD14⁺ monocytes. **f)** Correlation between *TP53* and *CSF-1R* mRNA expression in 129 CD14⁺ monocytes from the DACOTA trial.

Imanci A *et al.* Figure 5

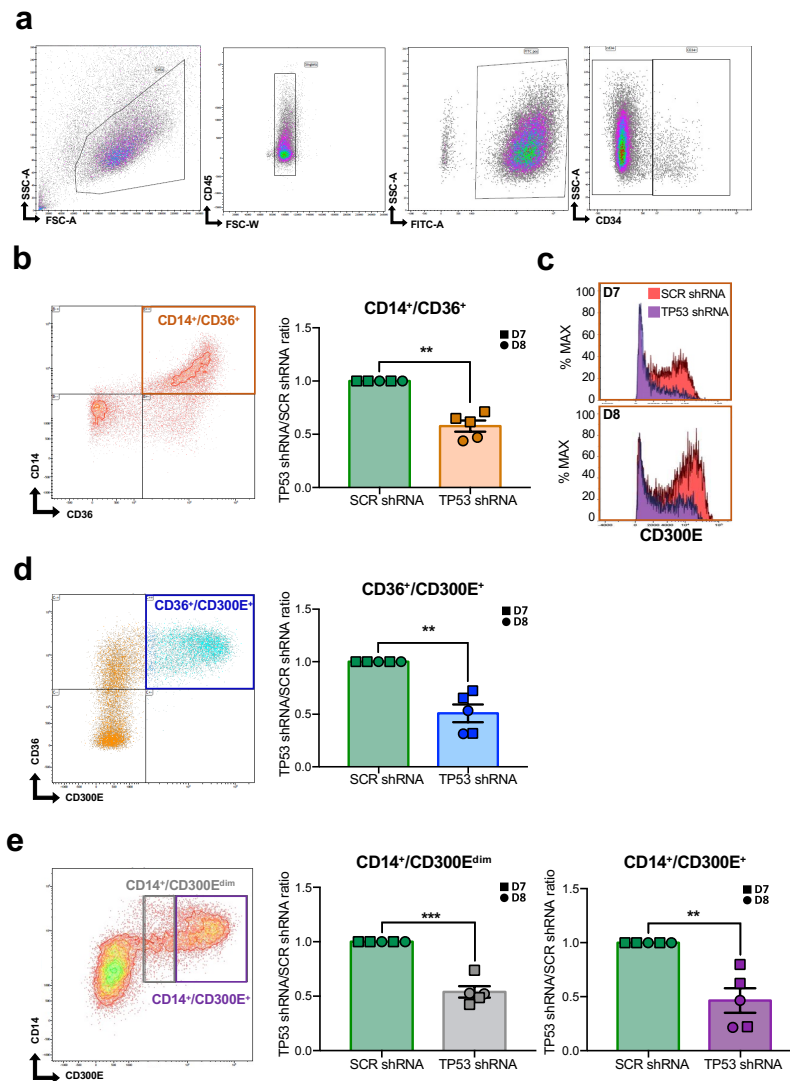


Figure 5. TP53 is involved in terminal monocyte maturation. Cord blood CD34⁺ cells were induced to differentiate using SCF and CSF1 in presence (SCR shRNA) or absence (TP53 shRNA) of *TP53* during 7 and 8 days. **a**) Gating strategy to identify monocyte fraction. Forward (FSC) and side scatter (SSC-A) are used to discard debris and dead cells, doublet cells are eliminated with CD45 and FSC-W parameters. GFP positive cells were selected on a SSC/CD45 plot. CD34 negative cells were selected on a SSC-A/CD34 plot. **b**) CD14⁺/CD36⁺ population and ratio of the CD14⁺/CD36⁺ fraction reported to control cells. **c**) Histogramm of CD300E expression in CD14⁺/CD36⁺ population from SCR shRNA (red) and TP53 shRNA (purple) at day 7 and 8. **d**) CD36⁺/CD300E⁺ population and ratio of the CD36⁺/CD300E⁺ fraction reported to control cells. **e**) CD14⁺/CD300E^{dim} and CD14⁺/CD300E⁺ populations and ratio of the CD14⁺/CD300E^{dim} and CD14⁺/CD300E⁺ fractions reported to control cells.

Imanci A *et al.* Figure 7

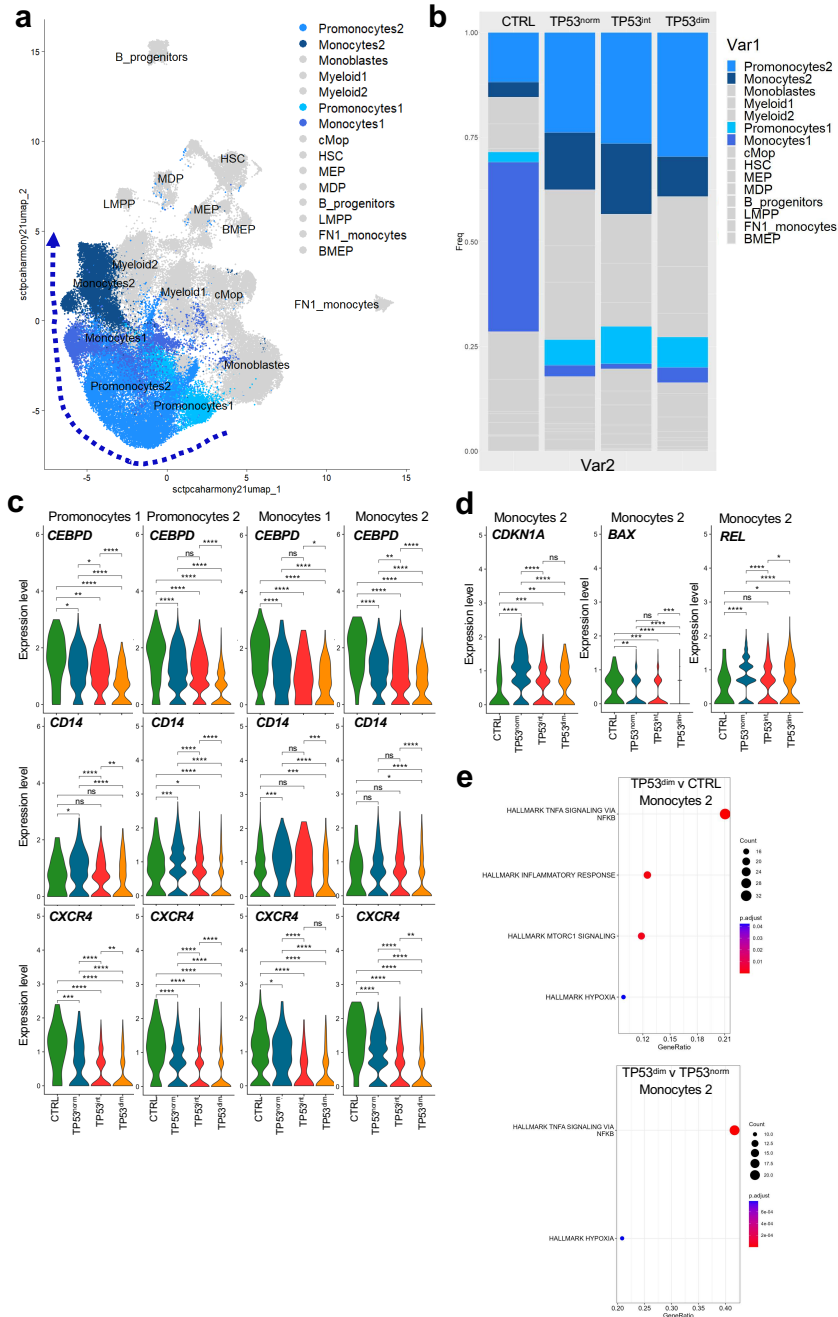


Figure 7. scRNAseq demonstrates that immature monocytes from CMML patients with less p53 expression are more inflammatory. Bone marrow single cell RNA-seq analyses using CD34⁺ and CD14⁺ sorted cells from 2 age-matched controls (CTRL), 3 TP53-normal (TP53^{norm}), 2 TP53-intermediate (TP53^{int}) and 2 TP53-diminished (TP53^{dim}) CMML patients. **a**) UMA plot showing 15 clusters with a focus on Promonocytes1, Promonocytes2, Monocytes1 and Monocytes2. Arrow the sense of maturation. **b**) Proportion of Promonocytes1, Promonocytes2, Monocytes1 and Monocytes2 clusters among the total cell per subgroup. **c**) Violin plots in Promonocytes1, Promonocytes2, Monocytes1 and Monocytes2 clusters in the 4 subgroups for *CEBPD*, *CD14* and *CXCR4* expression. **d**) Violin plots in Monocytes2 cluster in the 4 subgroups for *CDKN1A*, *BAX* and *REL* expression. **e**) GSEA Hallmark pathway analysis in Monocytes2 cluster in TP53^{dim} versus CTRL (upper panel) and in TP53^{dim} versus TP53^{norm} (lower panel).

Imanci A *et al.* Figure 8

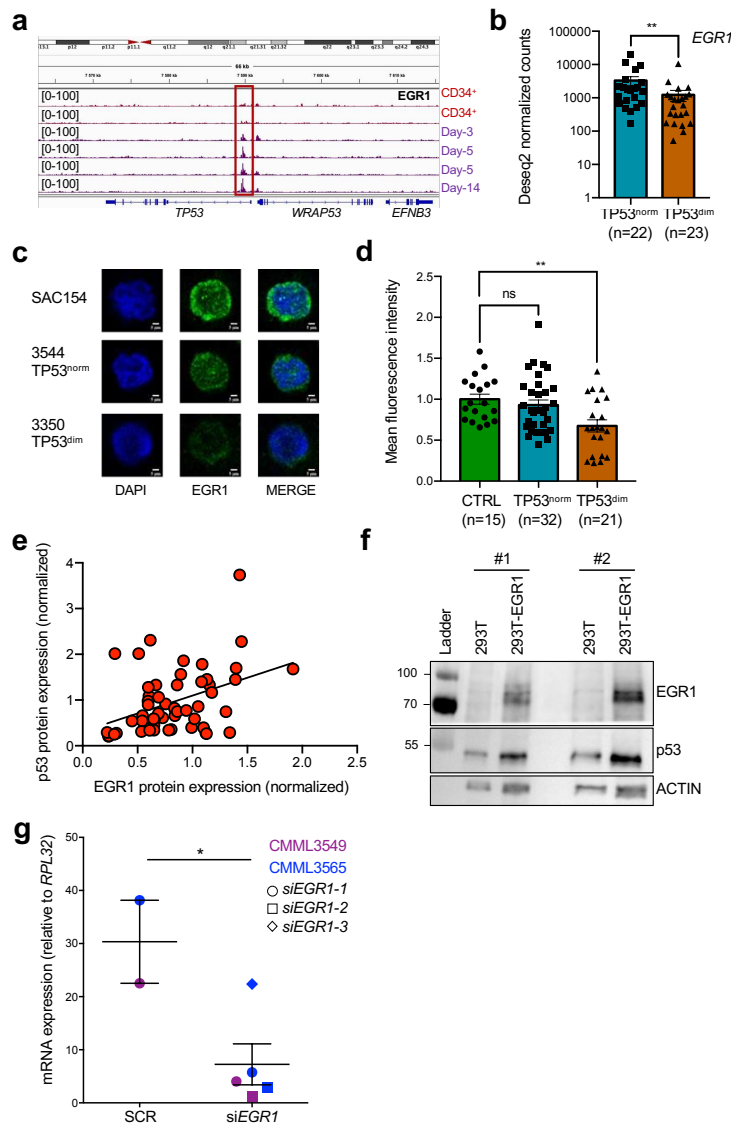


Figure 8. TP53 gene expression is regulated by EGR1. **a)** EGR1 CUT&RUN (from Trizzino *et al.*, 2021) on *TP53* gene during monocyte differentiation using cord blood stem cells. **b)** *EGR1* mRNA expression assessed by RNAseq from monocytes with normal TP53 expression ($TP53^{norm}$, $n=22$) and low TP53 expression ($TP53^{dim}$, $n=23$). **c)** Peripheral blood monocytes were fixed and stained for EGR1 (ab, A303-390A, green) and Dapi (blue) followed by confocal imaging in one cell from SAC154 and patients 3344 and 3350 (scale: 5 μ m). **d)** EGR1 (ab, A303-390A) expression in $CD14^+$ monocytes from healthy subjects (CTRL, $n=15$), CMML patients with p53 expression ($TP53^{norm}$, $n=32$) and CMML patients with no p53 expression ($TP53^{dim}$, $n=21$). Mean \pm SEM from 5 experiments independent. **e)** Correlation between p53 and EGR1 protein expression in $CD14^+$ monocytes (** $P < 0.01$). **f)** 293T cells were transfected using Lipofectamine 2000 with empty vector or pcDNA3-EGR1. Immunoblot analysis of EGR1 (Ab, A303-390A) and p53 (Ab, 2527S) expression in transfected-293T cells. Actin, loading control. **g)** siRNA-mediated downregulation of EGR1 in monocytes isolated from CMML3549 (purple) and CMML3565 (blue) with 2 or 3 sets of EGR1-siRNA, respectively. RT-qPCR analysis of *TP53* mRNA expression normalized to *RPL32*. Data are mean \pm SEM of each sample run in triplicate. Unpaired Students-t test using control monocytes transfected with SCR control, * $P < 0.05$.

Imanci A *et al.* Figure 9

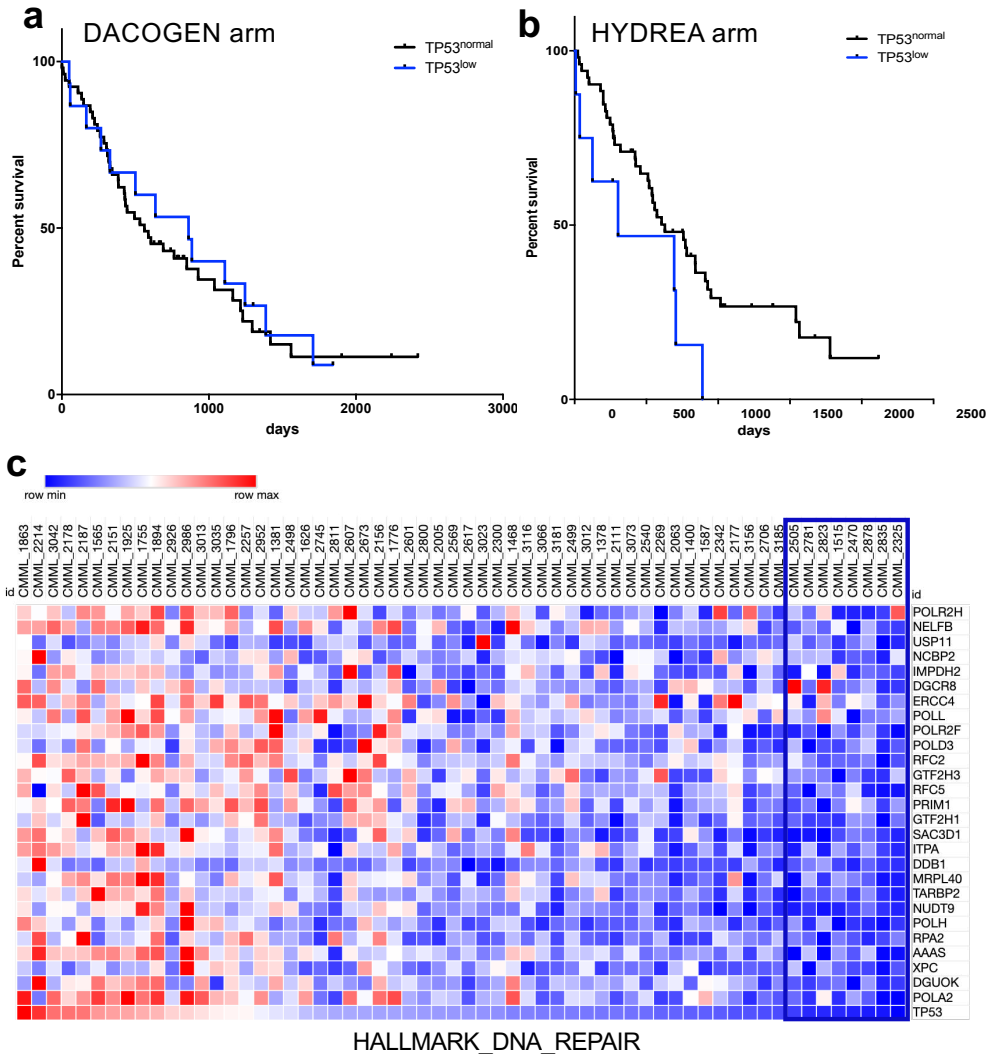


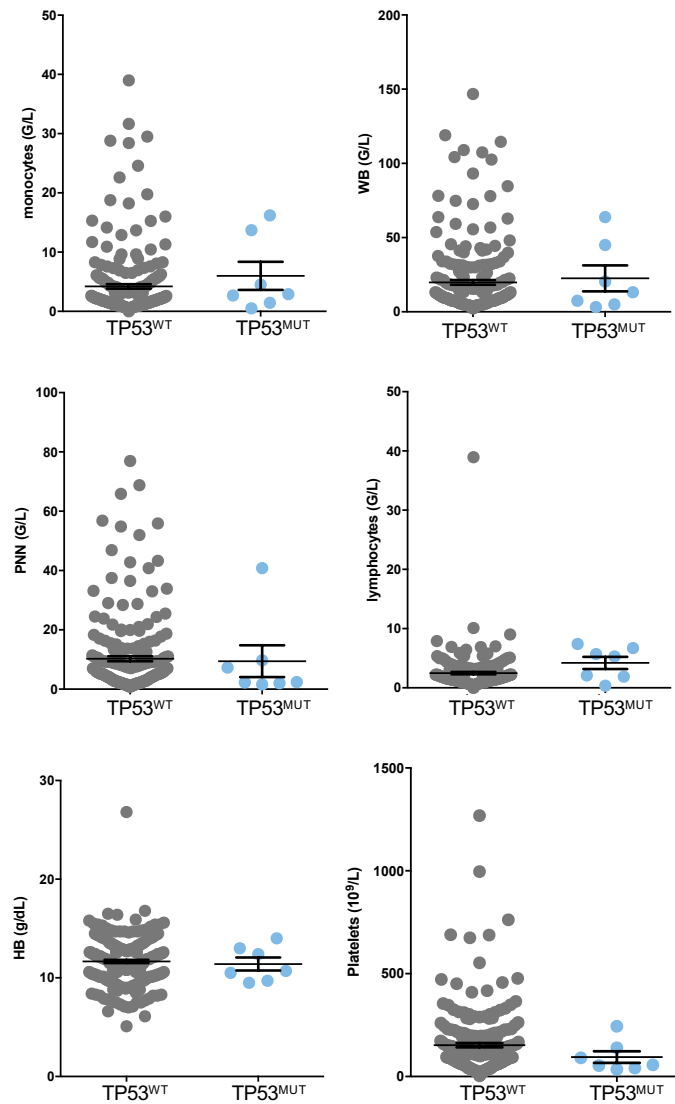
Figure 9. CMML patients with weak expression of TP53 in their monocytes have a worst overall survival with hydroxyurea treatment. **a)** Overall survival of CMML patients treated with decitabine according to *TP53* mRNA expression measured by RNAseq in their monocytes (cut-off 500). **b)** Overall survival of CMML patients treated with hydroxyurea according to *TP53* mRNA expression measured by RNAseq in their monocytes (cut-off 500). **c)** Heatmap of Hallmark DNA repair genes in monocytes from CMML patients included in hydroxyurea arm from DACOTA trial.

Supplementary Table 1.

Clinical data from age-matched controls and CMML patients according to their p53 expression level in their monocytes analyzed by immunofluorescence.

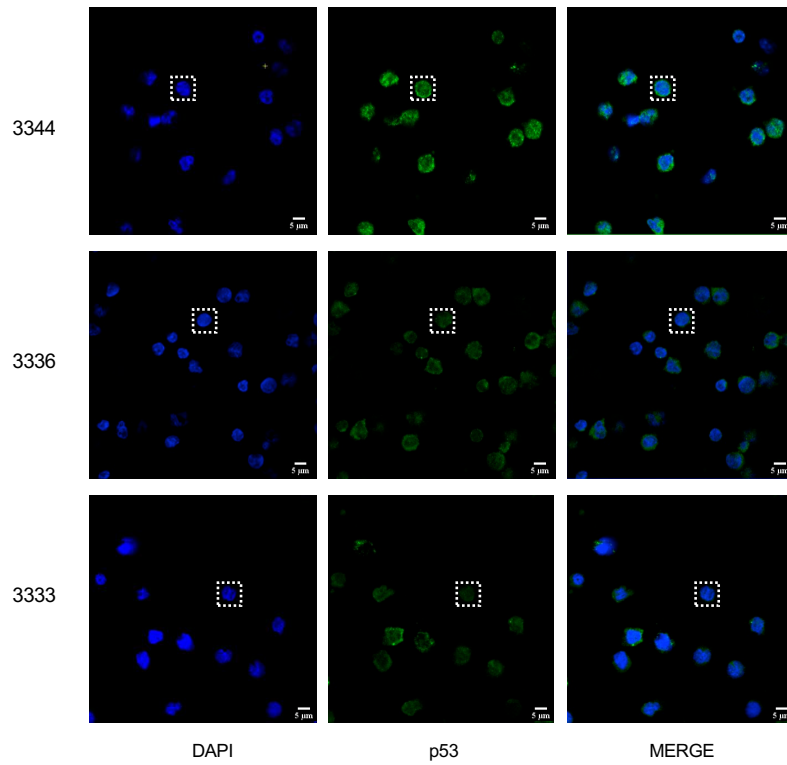
| CMML | Age-matched CTRL | CMML-TP53 ^{norm} | CMML-TP53 ^{dim} |
|-----------------------------|-------------------|---------------------------|--------------------------|
| Number of patients | 43 | 86 | 34 |
| Mean age in years | 77 (68-90) | 74 (45-94) | 72 (51-95) |
| Sex ratio M/F | 28/15 | 46/36 | 18/15 |
| Hemoglobin level, mean g/dL | 14.02 (13.4-16.7) | 12.06 (6.7-17.3) | 12.08 (7.3-14.7) |
| Platelet count, mean.109/L | 236.9 (158-374) | 169.15 (13-980) | 166,375 (29-342) |
| Leucocytes, mean.109/L | 6.99 (4.7-9.4) | 11.98 (2.84-74.36) | 12.33 (2.86-126.1) |
| Lymphocytes, mean.109/L | 1.7 (1.1-2.4) | 2.13 (0.41-28.3) | 1.83 (0.44-5) |
| Monocytes, mean.109/L | 0.59 (0.4-0.9) | 2.85 (0.2-21.49) | 2.94 (0.5-32.7) |
| Monocytes, % | 8.5 (5-12) | 61.5 (2-57.7) | 35.62 (3-53) |

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Supplementary Figure 1



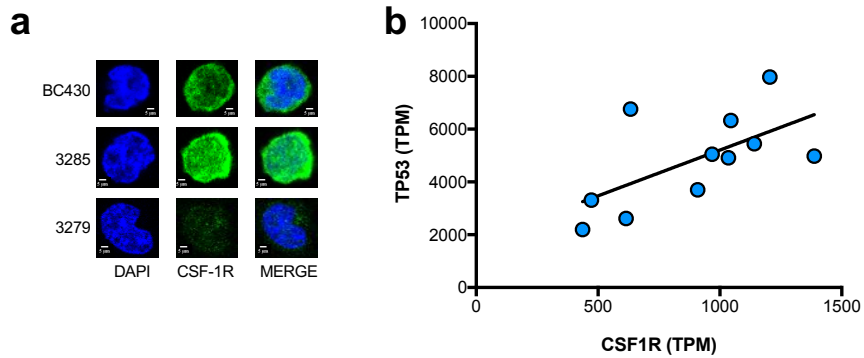
Supplementary Figure 1. No difference in clinical data between TP53 wild-type and mutated-CMML patients. Peripheral blood parameters in CMML patients according to p53 mutation. Monocytes, White blood cells (WB), neutrophils (PNN), lymphocytes, hemoglobin (Hb), platelets.

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Supplementary Figure 2



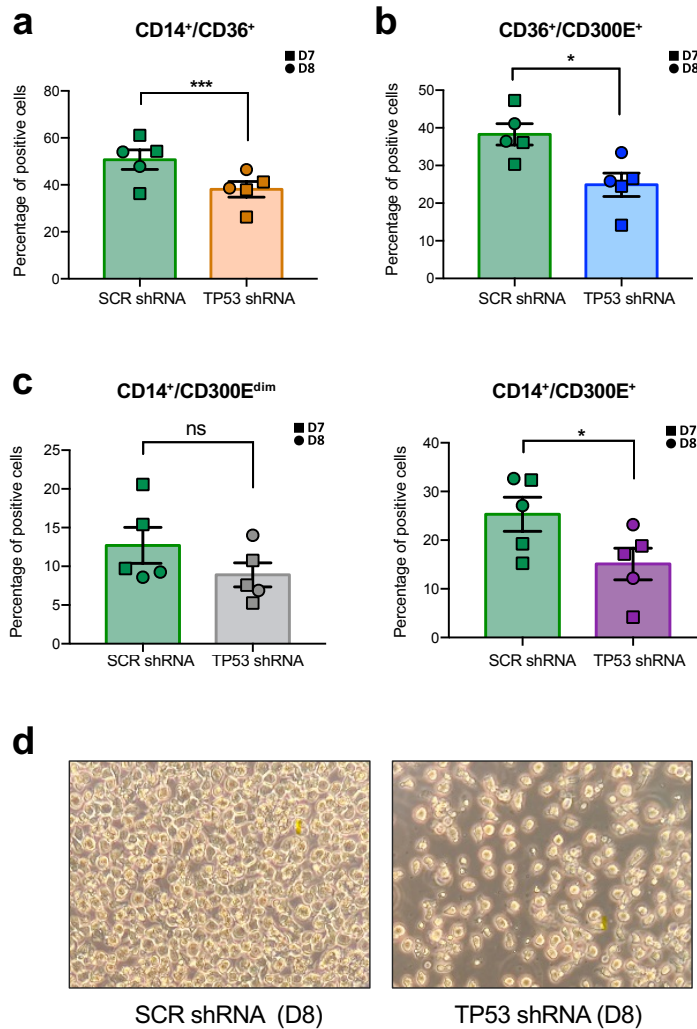
Supplementary Figure 2. p53 is abnormally expressed in some CMML monocytes. Peripheral blood monocytes were fixed and stained for p53 (Ab: 2527s, green) and Dapi (blue) followed by confocal imaging (scale: 5 μm). Cell from CMML patient 3344, 3336 and 3333 shown in **Figure 2b** is framed (dotted line).

Supplementary Figure 3



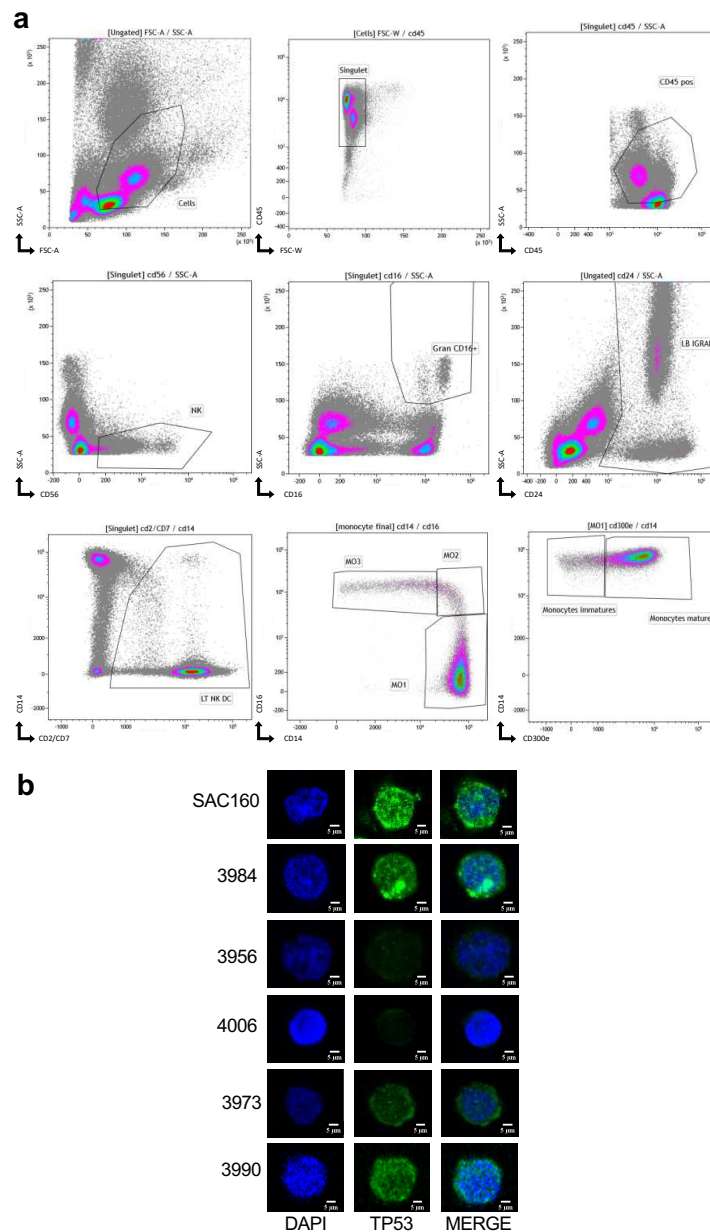
Supplementary Figure 3. CSF-1R is abnormally expressed in some CMML monocytes. a) Peripheral blood monocytes were fixed and stained for CSF-1R (ab, PA525974, green) and Dapi (blue) followed by confocal imaging in one cell from BC430 and patients 3285 and 3279 (scale: 5 μ m). **b)** Correlation between *TP53* and *CSF-1R* mRNA expression in CD14⁺ monocytes using RNAseq data from Franzini A et al, Blood Adv, 2019.

Supplementary Figure 4



Supplementary Figure 4. Involvement of TP53 in terminal monocyte maturation. Cord blood CD34⁺ cells were induced to differentiate using SCF and CSF1 in presence (SCR shRNA) or absence (TP53 shRNA) of *TP53* during 7 and 8 days. **a**) Percentage of CD14⁺/CD36⁺ population in SCR shRNA and TP53 shRNA conditions. **b**) Percentage of CD36⁺/CD300E⁺ population in SCR shRNA and TP53 shRNA conditions. **c**) Percentage of CD14⁺/CD300E^{dim} and CD14⁺/CD300E⁺ populations in SCR shRNA and TP53 shRNA conditions. **d**) Culture picture at day 8 in SCR shRNA and TP53 shRNA conditions.

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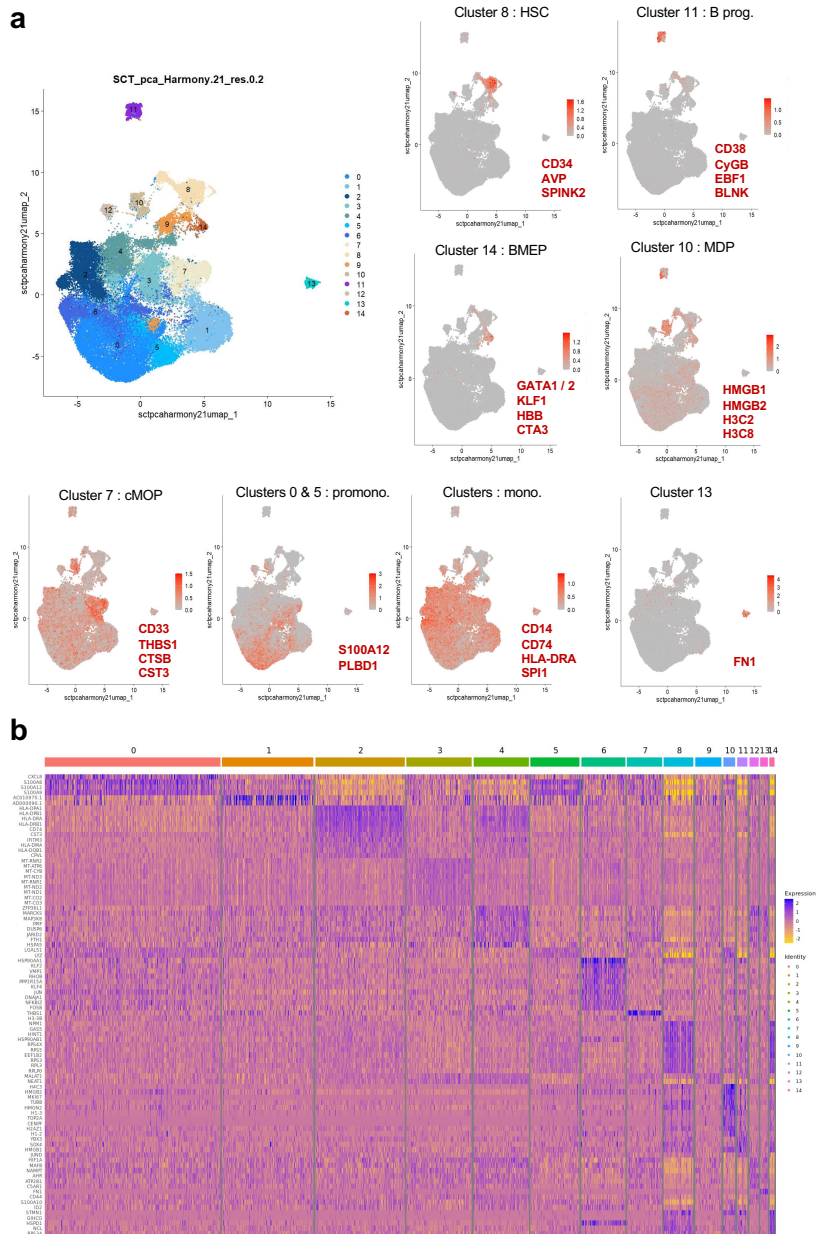


Supplementary Figure 5. CD300E^{dim} monocytes are more immature and expressed less p53.

a) PBMCs from controls and CMML patients were labeled with anti-CD45, -CD24, -CD14, -CD16, -CD56, -CD2, -CD7, -CD300E antibodies. Monocytes were identified using an exclusion gating strategy. Forward (FSC) and side scatter (SSC-A) are used to discard debris and dead cells, doublet cells are eliminated with CD45 and FSC-W parameters. CD45 positive cells were selected on a SSC/CD45 plot. NK CD56-positive, granulocyte CD16-positive, lymphocyte B and immature granulocyte CD24-positive and lymphocyte CD2/CD7 positive cells were excluded. Monocyte subsets were separated on CD14 and CD16 expression. In classical monocyte subset (MO1) gate, immature and mature monocytes were identified according to CD300E expression.

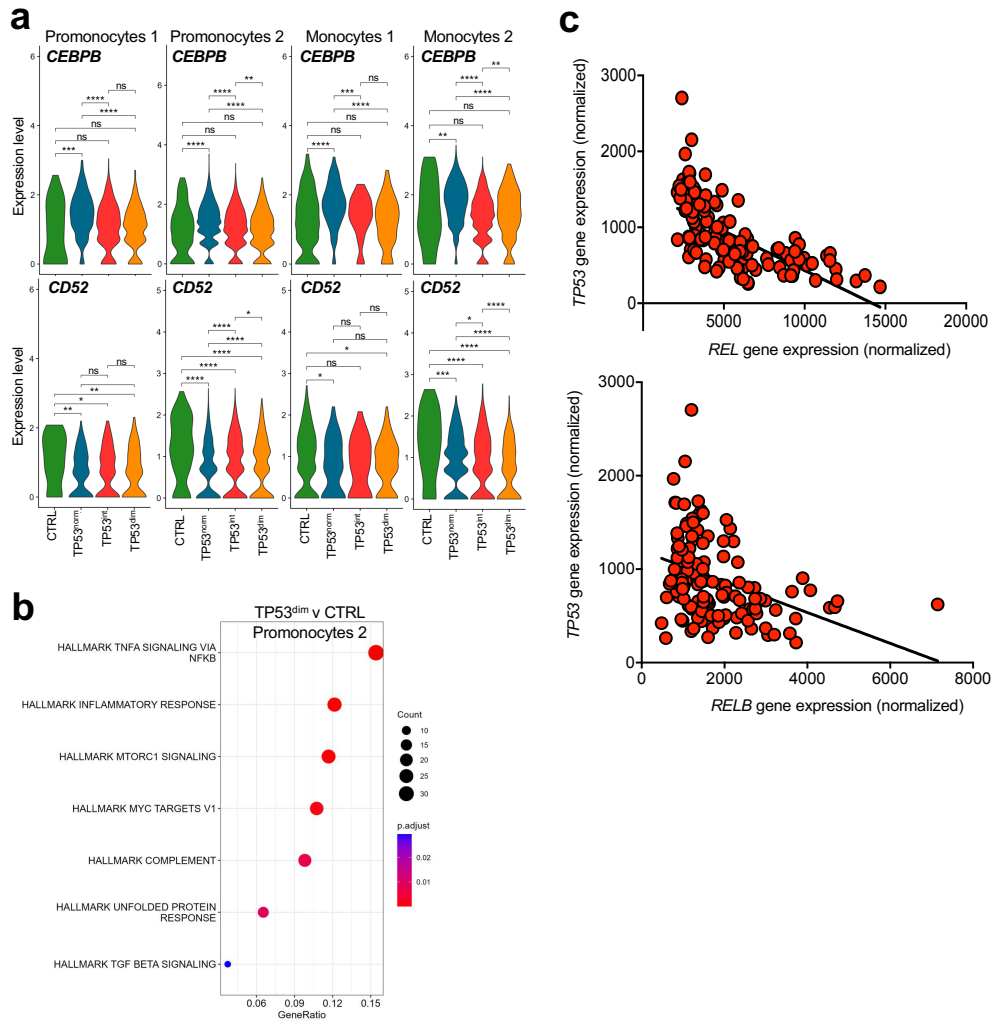
b) Peripheral blood monocytes were fixed and stained for p53 (Ab: 2527s, green) and Dapi (blue) followed by confocal imaging in one cell from SAC160 and CMML patient 3984, 3956, 4006, 3973 and 3990 (scale: 5 μ m).

Imanci A *et al.* Supplementary Figure 6



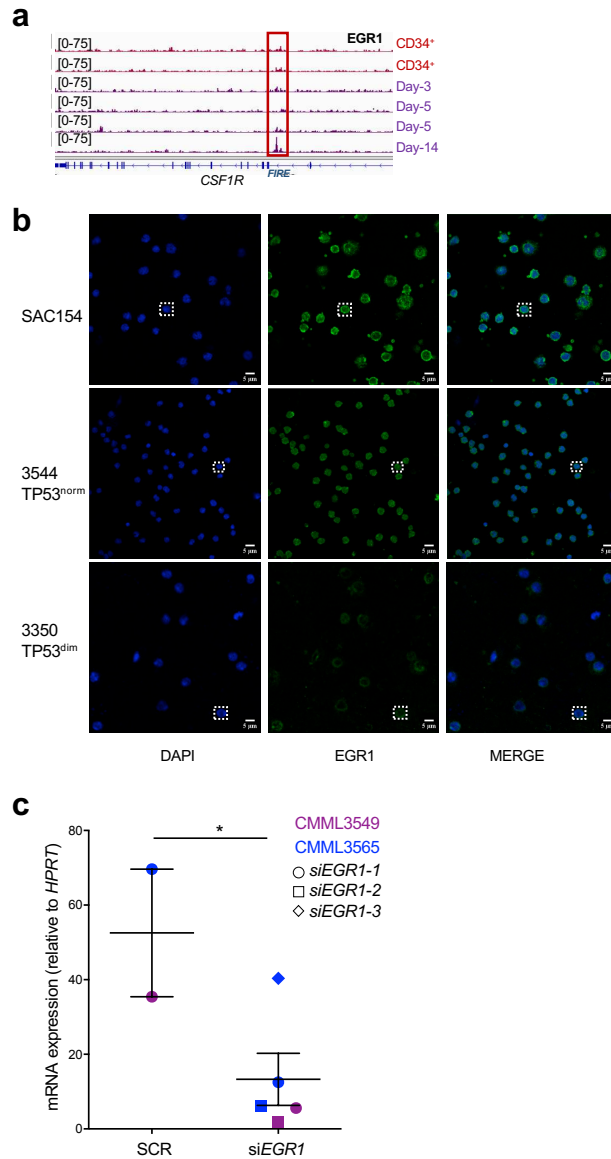
Supplementary Figure 6. Characterization of the different clusters at the single cell level. Bone marrow single cell RNA-seq analyses using CD34⁺ and CD14⁺ sorted cells from 2 age-matched controls (CTRL), 3 TP53-normal (TP53^{norm}), 2 TP53-intermediate (TP53^{int}) and 2 TP53-diminished (TP53^{dim}) CMML patients. **a**) UMA plot showing 15 clusters. Each cluster was identified thanks to *CD34*, *AVP*, *SPINK2*, *CD38*, *CyGB*, *EBF1*, *BLNK*, *GATA1/2*, *KLF1*, *HBB*, *CTA3*, *HMGB1*, *HMGB2*, *H3C2*, *H3C8*, *CD33*, *THBS1*, *CTSB*, *CST3*, *S100A12*, *PLBD1*, *CD14*, *CD74*, *HLA-DRA*, *SPI1* and *FN1* gene expressions. **b**) Heatmap of the 10 top genes in each of the 15 clusters.

Imanci A et al. Supplementary Figure 7



Supplementary Figure 7. Immature monocytes from CMML patients with less p53 expression are more inflammatory. Bone marrow single cell RNA-seq analyses using CD34⁺ and CD14⁺ sorted cells from 2 age-matched controls (CTRL), 3 TP53-normal (TP53^{norm}), 2 TP53-intermediate (TP53^{int}) and 2 TP53-diminished (TP53^{dim}) CMML patients. **a**) Violin plots in Promonocytes1, Promonocytes2, Monocytes1 and Monocytes2 clusters in the 4 subgroups for *CEBPB* and *CD52* expression. **b**) GSEA Hallmark pathway analysis in Promonocytes2 cluster in TP53^{dim} versus CTRL. **c**) Correlation between *TP53* and *REL* mRNA expression (top) and between *TP53* and *RELB* mRNA expression (bottom) in 129 CD14⁺ monocytes from the DACOTA trial.

Imanci A *et al.*
Supplementary Figure 8



Supplementary Figure 8. TP53 expression is regulated by EGR1. **a**) EGR1 CUT&RUN (from Trizzino *et al.*, 2021) on *CSF1R* gene during monocyte differentiation using cord blood stem cells. **b**) Peripheral blood monocytes were fixed and stained for EGR1 (ab, A303-390A, green) and Dapi (blue) followed by confocal imaging (scale: 5 μ m). Cell from from SAC154 and patients 3344 and 3350 shown in **Figure 8c** is framed (dotted line). **c**) siRNA-mediated downregulation of EGR1 in monocytes isolated from CMML3549 (purple) and CMML3565 (blue) with 2 or 3 sets of EGR1-siRNA, respectively. RT-qPCR analysis of *TP53* mRNA expression normalized to *HPRT*. Data are mean \pm SEM of each sample run in triplicate. Unpaired Students-t test using control monocytes transfected with SCR control, *P < 0.05.

VII. Discussion et perspectives : Partie 1

Les altérations génétiques de *TET2* sont fréquentes dans les hémopathies malignes humaines où elles contribuent à la progression tumorale et sont présentes dans d'autres formes de cancer solide où elles peuvent jouer un rôle dans la croissance cellulaire. Les altérations géniques de *TET2* ne sont pas uniquement observées dans les contextes pathologiques, elles ont également été identifiées dans les cellules saines au cours du vieillissement, favorisant un stade pré-leucémique.

L'étude actuelle met en évidence un rôle important de la protéine TET2 dans la régulation transcriptionnelle du gène *MIF* (*Macrophage Migration Inhibitory Factor*) via le facteur de transcription EGR1 au sein des monocytes. Lorsque *TET2* est moins exprimé, par exemple suite à une mutation tronquante, cela conduit à une augmentation de l'expression du gène *MIF*. Cette accumulation du messager a pour conséquence une sécrétion augmentée de la cytokine MIF qui va favoriser la différenciation des cellules souches CD34⁺ en monocytes.

Le recrutement de monocytes hyperinflammatoires a été identifié comme l'événement initiateur du développement des plaques d'athérosclérose. Étant donné l'importance des monocytes et de MIF dans le développement de l'athérosclérose, il est possible que la surproduction de MIF puisse expliquer le risque cardiovasculaire élevé observé chez les patients présentant des mutations clonales de *TET2* dans les cellules souches au cours du vieillissement.

Cela met en évidence un rôle pathogénique de la signalisation de MIF dans le cadre de l'hématopoïèse clonale (CHIP) avec des mutations de *TET2* et désigne des inhibiteurs de MIF comme candidats thérapeutiques pour ces CHIP et autres maladies avec des mutations de TET2.

A. Le rôle clé de TET2 dans la régulation des cytokines

Plusieurs études ont montré que TET2 joue un rôle dans la régulation des cytokines pro-inflammatoires. Par exemple, lorsque les cellules myéloïdes innées telles que les cellules dendritiques et les macrophages résolvent une réaction inflammatoire, la présence de TET2 conduit à une inhibition active de la transcription de l'interleukine-6 (IL-6). Ce processus de répression de l'IL-6 est orchestré par TET2, qui recrute Hdac2 sur le promoteur du gène [320]. Dans le contexte du VIH-1, l'inactivation de TET2 maintient l'expression de la cytokine pro-inflammatoire IL-6 [321]. De plus, il a été

démontré que TET2 est également capable de réprimer l'expression de l'interleukine-1B pendant la phase de résolution de l'inflammation [322], [323].

Dans nos résultats, nous mettons en évidence qu'en absence de *TET2*, il se produit une réorganisation de la structure de la chromatine au niveau du promoteur du gène *MIF*. Cette réorganisation conduit à un changement dans le recrutement des HDAC (histones désacétylases) qui ne sont plus présentes au niveau du promoteur de *MIF*. En conséquence, cette modification de la structure chromatinienne rendant cette dernière plus accessible favorise une augmentation de l'expression du gène *MIF* via le recrutement du facteur de transcription EGR-1. De manière intéressante, il a été montré que les inhibiteurs d'HDAC, en particulier les trichostatines A, affectent la régulation de *MIF* en perturbant le recrutement de l'ARN polymérase II et des facteurs de transcription SP1 et CREB, qui sont requis pour l'expression basale de *MIF* [324]. Il serait donc intéressant de réaliser des études *in vitro* avec des inhibiteurs d'HDAC sur les différentes lignées leucémiques afin de voir si cela va avoir un effet sur l'expression de *MIF*. Il serait aussi intéressant d'étudier le niveau de *MIF* chez les patients présentant une inactivation de *TET2*, et ayant reçu un traitement avec des inhibiteurs de HDAC.

B. MIF une cytokine pro-inflammatoire

MIF est une petite glycoprotéine de 12,5 kDa, localisée sur le chromosome 22, elle est exprimée par divers types cellulaires tels que les cellules immunitaires, endocriniennes et épithéliales [325]. Elle a été la première cytokine à être caractérisée chez l'Homme et a initialement été associée à l'inhibition de la migration des macrophages. Cependant, des découvertes ultérieures ont révélé son rôle dans plusieurs pathologies, notamment la polyarthrite rhumatoïde, le diabète, les maladies inflammatoires de l'intestin, le choc septique et plusieurs types de cancers [326]. La surexpression de cette cytokine a été liée à la progression tumorale dans de nombreux cancers de la prostate et dans l'endométriose. Dans plusieurs types de cancers, une expression élevée de *MIF* a été identifiée comme un indicateur pronostique négatif [327].

Dans notre étude, nous présentons pour la première fois une surexpression de *MIF* chez les souris n'exprimant pas *Tet2* ainsi que dans les monocytes de patients atteints de leucémie myélomonocytaire chronique avec une mutation tronquante de *TET2*. Les effets de *MIF* sur les cellules immunitaires ont été largement étudiés. Nous démontrons ici que l'ajout de *MIF* à des cellules CD34⁺ humaines saines en culture favorise leur différenciation en monocytes. Ceci suggère l'existence d'une boucle de

rétroaction autocrine/paracrine dans laquelle MIF, produit par des cellules clonales matures, crée un microenvironnement propice au développement d'un syndrome myéloprolifératif.

Une fois sécrété, MIF peut activer des voies de signalisation en se liant à des récepteurs transmembranaires, déclenchant ainsi des cascades signalétiques [328]. Il existe trois récepteurs capables de reconnaître MIF, à savoir le récepteur CD74 et l'hétérodimère CXCR2/CXCR4. La liaison de MIF au CD74 en dehors de la cellule initie la transmission du signal via la cascade de kinases ERK/MAP, ce qui entraîne la prolifération cellulaire [329]. Le récepteur CD74 est présent à la surface des monocytes, des cellules B et des fibroblastes [330]. Il serait donc intéressant d'étudier si MIF interagit avec le récepteur CD74 ou le complexe CXCR2/CXCR4 à la surface des cellules souches pour induire la différenciation en monocytes. L'inhibition expérimentale de ces récepteurs (anticorps bloquants ou shRNA) pourrait permettre de trouver lequel de ces récepteurs est responsable de la différenciation monocyttaire dépendante de MIF et ainsi obtenir une meilleure compréhension de ces mécanismes. Des études antérieures ont rapporté que la neutralisation du récepteur CD74 génère un ensemble de caractéristiques biologiques similaires à celles observées lors de l'immunoneutralisation de MIF [331], [332].

Il serait aussi particulièrement intéressant d'explorer les effets des anticorps anti-MIF. Cette idée trouve sa justification dans des études antérieures où l'injection intrapéritonéale d'anticorps neutralisants anti-MIF a entraîné une augmentation du taux de survie, passant de 31% à 81% en cas de choc septique [333]. D'autres études ont montré que l'imalumab, l'un de ces anticorps monoclonaux humanisés anti-MIF, a été soumis à des essais cliniques de phase I et II et a montré une tolérance acceptable chez les patients [330]. Par conséquent, l'utilisation de ces anticorps dans des modèles murins porteurs de la délétion de *Tet2* pourrait être envisagée afin de déterminer si la réduction de l'expression de cette cytokine pourrait influencer le développement de la LMMC, étant donné le rôle connu de MIF dans le processus de différenciation des CSH en monocytes. Il serait aussi intéressant d'examiner par cytométrie en flux la quantité de monocytes générée en réponse au traitement par les anticorps anti-MIF et voir s'il y a une diminution. Si les résultats des modèles murins sont prometteurs, on pourrait envisager des études cliniques pour évaluer l'efficacité des anticorps anti-MIF chez les patients atteints de LMMC ayant des mutations tronquantes de *TET2*. Cela pourrait représenter une nouvelle approche thérapeutique pour cette maladie où il n'existe pas de traitement curateur actuellement.

C. La D-dopachrome tautomérase, MIF-2

Pendant longtemps, MIF a été considéré comme une protéine unique en termes de structure. Cependant, une découverte récente a révélé l'existence d'une deuxième protéine similaire à MIF, appelée la D-dopachrome tautomérase (D-DT ou MIF-2), partageant une similarité de séquence d'acides aminés de 34% et présentant une structure 3D presque identique [334]. De manière similaire, MIF-2 est fortement exprimé chez les patients souffrant de sepsis, et ses niveaux dans la circulation sanguine sont en corrélation avec la gravité de la maladie mesurée par le score APACHE II [334], [335].

Les deux protéines, MIF et MIF-2, sont libérées par les monocytes/macrophages activés et agissent via les récepteurs CD74 et CD44. En conditions inflammatoires, la possibilité d'une approche thérapeutique combinée, ciblant à la fois MIF et MIF-2, pourrait se révéler efficace sur le plan thérapeutique [334]. L'exploration de cette approche est favorisée par la tenue actuelle d'essais cliniques sur des médicaments anti-CD74 [336] et des antagonistes de MIF sous forme de petites molécules pour le traitement de diverses affections inflammatoires [337].

Des études ont révélé qu'en l'absence du récepteur CD74, les souris présentent un effet deux fois plus important que les souris n'exprimant pas *Mif* (*Mif*^{-/-}), suggérant un mécanisme de compensation qui pourrait être via *Mif2* [338]. Par conséquent, il serait intéressant de mener des études sur l'expression de l'ARNm et de la protéine *Mif2*/MIF-2 chez la souris, ainsi que dans les cellules leucémiques et dans le contexte de la LMMC. Si MIF2 est également accumulé, il serait intéressant d'étudier la différenciation monocyttaire en présence de MIF et MIF2 pour déterminer si la présence simultanée des deux MIF influence de manière significative le rapport entre le nombre de monocytes et de granulocytes, notamment en utilisant des techniques de séquençage de cellules individuelles (single cell RNAseq).

D. Les mutations dans la LMMC

Les patients atteints de LMMC sont principalement mutés pour TET2 (60%), ASXL1 (40%), SRSF2 (50%) et la voie RAS (30%). Il serait intéressant d'étudier les cytokines spécifiquement produites en fonction des différentes mutations observées comme cela a été fait avec les mutations tronquantes de TET2. Cela pourrait permettre de mieux comprendre pourquoi certains patients développent des formes plus agressives de la maladie. De plus, ces cytokines identifiées pourraient représenter des cibles thérapeutiques cruciales pour la LMMC, une maladie caractérisée par une grande

hétérogénéité et l'absence d'un traitement spécifique. Il serait particulièrement intéressant d'examiner les combinaisons de mutations pour évaluer comment cette double mutation influence la production des cytokines.

Dans cette étude, il a été observé que l'absence de *TET2*/*TET2* entraîne le déplacement des HDAC et que le facteur de transcription *EGR1* est recruté, suggérant une réorganisation de la chromatine. Il est possible que d'autres facteurs, en dehors des mutations de *TET2*, contribuent également à cette réorganisation de la chromatine, notamment les mutations d'*ASXL1*. Il serait intéressant de caractériser plus en détail la structure chromatinienne avec les mutations d'*ASXL1* et de voir si cela favorise l'expression d'une cytokine particulière.

VIII. Discussion et perspectives : Partie 2

Dans la deuxième partie de mes recherches, j'ai étudié les répercussions de la diminution de l'expression du gène *TP53* sur les monocytes de patients souffrant de leucémie myélomonocytaire chronique (LMMC). L'objectif central de cette partie était d'élucider les mécanismes à l'origine de la réduction de l'expression du gène *TP53* et de comprendre le rôle de p53 dans ce sous-groupe de patient.

En ce qui concerne le mécanisme de sous-expression de *TP53*, nous avons identifié le facteur de transcription *EGR-1* comme un élément clé régulant *TP53* au sein des monocytes, tant chez les individus normaux que chez les patients atteints de LMMC. Cette régulation a été confirmée à la fois par des études d'immunofluorescence et des expériences d'interférence ARN ciblant *EGR-1* dans ces monocytes. L'analyse des transcrits à l'échelle unicellulaire a révélé que chez les patients *TP53*^{Dim}, les monocytes se trouvent dans un état pré-monocytaire, juste avant la phase terminale de maturation des monocytes. Des analyses en cytométrie en flux ont confirmé ce blocage au niveau des monocytes de patients exprimant peu *TP53* via la diminution du marqueur de surface CD300E. Enfin, la caractérisation de ce sous-groupe de patient via la diminution de CD300E pourrait orienter le choix des traitements pour ce groupe de patients.

Mes résultats démontrent pour la première fois la participation de *TP53* dans la maturation des monocytes.

A. Les altérations de l'expression de *TP53* dans les monocytes de patients atteints de LMMC

En absence de stress, la protéine p53 est maintenue à un niveau bas, sa durée de vie étant de quelques minutes seulement [339]. Toutefois, face à différentes formes de stress, p53 est stabilisée et activée, lui permettant de réguler l'expression d'une multitude de gènes. Les protéines MDM2 et MDM4 sont reconnues comme étant les principaux régulateurs négatifs de p53 [340]. D'autres régulateurs de p53 existent, tels que la protéase HAUSP [341], COP1 et TRIM28 [342]. Bien que les mécanismes de régulation post-traductionnelle de p53 aient fait l'objet d'études approfondies, il est également établi que p53 peut être régulée au niveau post-transcriptionnel. Un exemple de cette régulation est l'action de PARN, une polyA-ribonucléase, qui pourrait avoir un rôle dans la modulation des niveaux d'ARNm de p53 par le biais de microARN [343]. Il a aussi été observé que la protéine ribosomale RPL26 peut se lier à la région 5'

non traduite de l'ARNm de *TP53*, induisant une régulation positive de l'expression de p53 en influençant directement son processus de traduction [344].

La recherche dans la base de données GenBank™ a permis d'identifier le gène *TP53* comme étant un candidat potentiel de régulation par EGR1. Ce gène *TP53* possède deux sites de liaison connus pour EGR1, nommés EBS-1 et EBS-2, qui sont situés dans la région promotrice du gène p53. Des études faites dans des lignées cellulaires dérivées de mélanomes humains (A374-C6) ont montré que le facteur de transcription EGR1 peut se lier directement au promoteur du gène *TP53* et activer son expression. Les chercheurs ont aussi entrepris l'identification des domaines essentiels de la protéine EGR1 nécessaires pour induire l'expression de p53. À cette fin, des expériences de cotransfection ont été réalisées en introduisant des variants mutés d'EGR1 présentant des délétions spécifiques dans le domaine de transactivation ou le domaine de liaison à l'ADN. Les résultats obtenus ont montré une réduction significative de l'expression de *TP53*, suggérant que ces 2 domaines d'EGR1 sont requis pour l'expression de p53 [345].

Nous rapportons ici, pour la première fois, une baisse de l'expression de TP53 dans les monocytes chez 30% des patients atteints de LMMC. L'étude de la méthylation du promoteur de TP53 n'a pas donné de résultat concluant. Nos expériences sur des lignées cellulaires et sur des cellules primaires ont montré une régulation de l'expression de TP53 par EGR1. Plus précisément, nos observations ont mis en évidence que les patients présentant une faible expression de p53 manifestaient également une diminution de l'expression d'EGR1.

Une étude menée sur des souris génétiquement modifiées présentant une suppression de l'expression de *Trp53*, conjointement avec une haplo-insuffisance d'EGR1, a révélé une augmentation du risque de formation de néoplasmes hématologiques [346]. Induire une réduction d'expression de *Trp53* et d'*Egr1*, individuellement ou simultanément, pourrait constituer une approche prometteuse pour analyser l'impact de ces protéines sur la gravité de la maladie, le but étant de voir comment leur combinaison peut aggraver ou moduler les aspects pathologiques.

Sachant que *TP53* est régulé par EGR1 dans les monocytes de patients atteints de LMMC, il serait intéressant de comprendre les causes de la sous-expression d'*EGR1* dans les monocytes de ces patients. EGR1 est un facteur de transcription qui peut être régulé par de nombreux éléments régulateurs qui comprend les hormones, les facteurs de croissance, les cytokines, l'hypoxie, le stress et les signaux mécaniques. Dans de

nombreux types de tumeur, *EGR1* est faible ou absent, indiquant un rôle suppresseur de tumeur [346] [347]. Il serait essentiel d'étudier dans un premier temps les marques d'histones autour du promoteur d'*EGR1*, par des expériences de Cut&Tag, pour voir s'il y a des changements de conformation de la chromatine qui pourraient expliquer sa sous-expression. Dans un second temps, il serait également important d'étudier la méthylation du promoteur par des expériences de bisulfite. Trouver le mécanisme de la sous-expression d'*EGR1* sera essentiel pour le re-exprimer et analyser l'impact sur la restauration de l'expression de TP53 et sur la maturation des monocytes.

B. L'altération de TP53 dans les monocytes de patients atteints de LMMC déclenche le développement d'un environnement inflammatoire

p53 joue également un rôle dans les réponses inflammatoires [348], [349]. Il est important de rappeler que l'inflammation chronique peut créer un environnement potentiellement propice au développement du cancer [350], [351]. En effet, dans les tissus enflammés, les cytokines ou les médiateurs inflammatoires peuvent activer divers facteurs de transcription tels que NF- κ B et STAT3 qui jouent un rôle essentiel dans l'initiation du cancer [352].

Dans cette étude, nous démontrons que les patients LMMC avec une faible expression de *TP53* dans leurs monocytes sont plus inflammatoires. Les résultats de RNAseq ont montré une régulation positive des voies de TNFA_signaling_via_ NF- κ B et de la réponse à l'inflammation. Des études ont démontré le rôle de la protéine p53 dans la suppression de l'inflammation [353]. Les souris génétiquement modifiées pour ne pas exprimer le gène *Trp53* (souris p53 null) ont montré que l'absence de p53 peut conduire à une inflammation sévère et à des maladies inflammatoires. Ces souris p53 null semblent être plus susceptibles de développer des abcès, une gastro-entérite ou une myocardite, qui sont tous des signes d'inflammation [354]. Des études antérieures ont démontré que l'absence de la protéine p53 favorise l'activation de gènes pro-inflammatoires à travers une voie qui dépend du facteur de transcription NF- κ B en réponse à une infection [355], confirmant que la faible expression de *TP53* peut contribuer à une réponse inflammatoire accrue.

Il a également été démontré que la diminution des niveaux de p53 a des répercussions sur le stress oxydatif, représenté par les espèces réactives de l'oxygène (ROS), dans diverses populations de cellules sanguines et dans divers tissus. Les souris n'exprimant pas *Trp53* (KO) présentent des niveaux de ROS plus élevés que les souris ayant une expression normale de *Trp53* (WT) [356]. Il serait intéressant d'explorer les niveaux de

ROS chez nos patients atteints de LMMC à l'aide de techniques telles que CellroX et la cytométrie en flux. L'objectif serait de déterminer si les patients ayant une faible expression de *TP53* affichent effectivement des niveaux de ROS accrus. Ces résultats pourraient potentiellement servir à identifier les niveaux de ROS comme un biomarqueur permettant de détecter les patients présentant une faible expression de *TP53* de manière plus rapide et précise. Il est connu que les ROS endommagent les composants cellulaires. En réponse à ces dommages, les cellules activent souvent des voies de signalisation inflammatoires qui incluent la production de cytokines pro-inflammatoires, l'activation de facteurs de transcriptions tels que NF- κ B [357], [358]. En plus d'étudier les ROS, il serait également important de caractériser le profil cytokinique chez les patients avec une faible expression de *TP53* par rapport aux patients avec une expression basale de *TP53* et de voir s'il y a une augmentation du TNF comme suggéré par les analyses hallmark. L'utilisation de cytokines arrays pourrait être un outil de mesure idéal pour une analyse complète et détaillée du profil inflammatoire des patients. L'analyse des cytokines en parallèle avec l'étude des ROS peut fournir une image plus complète de la réponse inflammatoire dans la LMMC.

C. Le risque de transformation en LAM des patients avec une faible expression de TP53 : « Une menace latente »

Les mutations ou les altérations de *TP53* sont couramment associées à divers types de cancer, y compris les syndromes myélodysplasiques (SMD) et la leucémie aiguë myéloïde (LAM). Les mutations *TP53* sont relativement rares dans les SMD et la LAM, représentant environ 5 à 10% de ces maladies. Cependant, bien que ces mutations soient rares dans les SMD/LAM avec un caryotype normal, elles sont plus fréquentes dans les cas secondaires de LAM et les LAM liées aux traitements, représentant 40 à 50% de ces cas [359]–[361] et 80% de ces mutations sont fréquentes chez les patients avec un caryotype complexe [362].

Notre étude met en évidence qu'une altération de l'expression de *TP53* par *EGR1* dans les monocytes d'environ 30% des patients atteints de LMMC pourrait être un facteur de risque pour la transformation en LAM. En effet, les données cliniques des patients inclus dans une étude préliminaire a montré que les patients avec une faible expression de *TP53* ont transformé en LAM (9/11) par rapport aux patients exprimant *TP53* (1/11). Les résultats du RNAseq ont montré une surexpression des gènes impliqués dans la leucémie aiguë myéloïde chez les patients avec une faible expression de *TP53* par rapport aux patients exprimant *TP53*. Il a été montré que l'haploinsuffisance du gène *Egr1*, suite à une délétion 5q et du gène *Ttp53*, augmente le taux de développement des néoplasmes hématologiques et influence le spectre de la maladie, suggérant une

coopération entre les voies *Egr1* et *Trp53*. Dans la même étude, il a été montré que la perte de *Trp53* dans les HSPC haploinsuffisantes pour *Egr1* et *Apc* crée un environnement d'instabilité génétique favorable au développement de la LAM chez la souris [346]. Cette étude confirme nos résultats quant à une augmentation du risque de transformation en LAM des patients présentant une faible expression de *TP53*. Est-ce que la perte de *TP53*, dans les monocytes de patients LMMC, induit une instabilité génétique qui est à l'origine de la transformation en LAM ? Pour aborder cette question, il serait intéressant d'utiliser des techniques d'analyse chromosomique, telles que la cytogénétique et la FISH. De plus, l'analyse des voies de signalisation a montré une diminution des gènes impliqués dans la réparation de l'ADN chez les patients avec une faible expression de *TP53*. Ces constatations amènent à se demander si l'instabilité génétique favorise la progression vers la LAM chez ces patients, et soulignent la nécessité d'approfondir ces études.

D. Rôle de TP53 dans la maturation des monocytes en condition physiologique et dans une maladie liée au vieillissement, la LMMC.

La formation des monocytes implique une expression graduée de plusieurs facteurs de transcription, récepteurs de surface et cytokines. Le microenvironnement joue un rôle déterminant dans la destinée de ces cellules, car les signaux extracellulaires émis par divers types cellulaires orientent la transformation des CSH vers des monocytes matures. La coopération de tous ces éléments est cruciale pour parvenir à la maturation cellulaire. Toute altération dans l'expression des facteurs de transcription ou autres peuvent perturber ce processus et potentiellement contribuer au développement ou à la progression de la maladie. Il a été démontré que la présence de monocytes immatures $CD14^{\text{faible}}$ peut contribuer aux lésions pulmonaires aiguës en favorisant la génération de monocytes matures producteurs de $TNF-\alpha$ [363]. De plus, au fur et à mesure du vieillissement, une population de monocytes immatures $CD14^{\text{faible}}CD16^-$ apparaît dans le sang périphérique [364].

Nous avons constaté une réduction, voire une absence, de l'expression de plusieurs éléments importants pour la maturation des monocytes, notamment *EGR1*, *CSF-1R*, *CD14*, *CD36* et *CD300E*. Cela suggère une perturbation majeure du processus de maturation des monocytes chez ces patients exprimant faiblement *TP53* dans leurs monocytes. *EGR1*, en tant que facteur de transcription, est bien connu pour son rôle dans la régulation de la différenciation monocyttaire. Son expression diminuée pourrait entraîner des monocytes moins matures et moins fonctionnels [365]. La diminution de *CSF-1R*, qui code pour le récepteur du facteur de stimulation des colonies de

monocytes (M-CSF ou CSF1), peut avoir un impact direct sur la survie, la prolifération et la maturation des monocytes [26], [366]. De plus, la baisse d'expression de CD14, suggère un défaut de la voie de maturation monocyttaire [74], [367], [368]. Les observations selon lesquelles les patients présentant une altération de *TP53* sont incapables d'atteindre un état de maturation terminale sont d'une grande importance clinique.

Grâce à l'analyse par cytométrie en flux, nous avons identifié une population CD14⁺CD300E^{dim} bloquée à un stade pré-monocyttaire chez les patients présentant une faible expression de TP53. CD300E, un récepteur de la superfamille des immunoglobulines, est souvent associé à la différenciation monocyttaire et à la maturation terminale des monocytes [87], [369]. Ces résultats mettent en évidence la nécessité de recherches complémentaires pour mieux comprendre les mécanismes précis par lesquels TP53 régule la maturation des monocytes. Des expériences de différenciation des CD34⁺ de sang de cordon en monocytes en présence de sh-SCRB (contrôle) ou de sh- dirigé contre *TP53* ont été entreprises pour répondre à cette question. Les résultats mettent clairement en évidence un retard dans la génération des monocytes matures lorsque l'expression de *TP53* est diminuée dans les CD34⁺ par rapport à la condition contrôle. Des expériences de single cell au jour 8 de la différenciation monocyttaire sont en cours dans les deux conditions afin de mieux caractériser l'implication de p53 dans l'étape finale de maturation des monocytes.

Comprendre comment p53 influe sur la maturation des monocytes ouvre de nouvelles perspectives pour la recherche de cibles thérapeutiques potentielles pour les patients atteints de CMML. Nos résultats pourraient favoriser le développement de traitements visant à rétablir la maturation normale des monocytes. Des thérapies ciblées destinées à rétablir l'expression de *TP53* pourraient également être envisagées comme une approche thérapeutique prometteuse. En 2011, une équipe a montré que la restauration de l'expression du gène *Trp53* de type sauvage, par la génération d'un allèle p53 latent, supprime la croissance des tumeurs, mais ne provoque pas la régression des tumeurs chez les souris porteuses d'une mutation missense de *Trp53* [370]. D'autres études, toujours chez la souris, ont montré que la restauration du gène *Trp53* peut supprimer la croissance des lymphomes et des sarcomes en induisant l'apoptose cellulaire [371], [372]. Ces résultats sont prometteurs pour notre étude, étant donné que nous constatons une altération de l'expression du gène *TP53* sans que ce dernier présente de mutations génétiques dans la majorité des cas. Il existe également une thérapie biologique qui utilise la Gendicine qui vise à restaurer la fonction normale du gène *TP53* dans les cellules cancéreuses en introduisant une

forme saine de ce gène via un vecteur adénoviral [373], [374]. Il a également été montré une régulation négative de *TP53* via NF-κB et vice versa. En effet, plusieurs études indiquent que les cellules exprimant NF-κB arrêtent l'activité de p53, et réciproquement, les cellules présentant une activité importante de p53, inhibent l'expression transcriptionnelle de *NF-κB* [349], [375]. Dans notre étude nous avons montré une activation importante de la voie de signalisation NF-κB chez les patients exprimant faiblement *TP53*. Il serait intéressant de réaliser des expériences de siRNA dirigé contre *NF-κB* ou des médicaments supprimant NF-κB [376] et étudier l'expression de *TP53* dans nos cellules.

Pour une validation clinique de ces résultats, il serait pertinent de les confirmer au sein d'une cohorte plus importante de patients atteints de LMMC, ce qui permettrait de caractériser de manière plus robuste leur impact sur le pronostic de ces patients. Cette démarche pourrait également contribuer à l'identification de biomarqueurs permettant de diagnostiquer la présence d'une altération de *TP53* chez les patients. Un exemple prometteur est CD300E, qui pourrait éventuellement servir de biomarqueur pour faciliter le diagnostic et reconnaître ces patients. Cette approche pourrait offrir un moyen plus simple et rapide de détecter les patients LMMC exprimant faiblement *TP53* dans leurs monocytes.

Il serait également important d'étudier l'expression de *TP53* dans les CSH de ces patients LMMC. En effet, des expériences de RNAseq, réalisées par l'équipe, sur des CD34+ issues de moelle osseuse de témoins sains (n=5) et patients LMMC (n=10), ont montré une diminution significative de l'expression de *TP53* dans les CD34+ de patients LMMC par rapport aux témoins sains. Il serait intéressant de déterminer si cette sous-expression de *TP53* dans les cellules souches a pour conséquence la génération de monocytes plus immatures.

E. Lien entre la faible expression de TP53 et la survie des patients LMMC traités à l'Hydrea

La médiane de survie après le diagnostic d'une LMMC est de deux à trois ans. Le risque de transformation en leucémie aiguë myéloïde se situe entre 15 et 30%. Cependant, il est important de noter que cette évolution est hautement variable, avec des cas où des survies de plus de dix ans ont été constatées [377]. Parmi les traitements, il est noté que les HMA sont efficaces chez environ 40-50% des patients atteints de LMMC. Même s'ils ont la capacité de restaurer l'hématopoïèse, ils ne parviennent pas à éliminer le clone muté [161]. Les patients qui initialement répondent positivement aux

HMAAs et qui rechutent après trois cycles de traitement, ont une médiane de survie de 29 mois [181] [378].

Nos résultats mettent en évidence que les patients présentant une faible expression de TP53 ont une survie réduite lorsqu'ils sont traités à l'hydroxyurée (Hydrea) mais que cela n'a pas d'impact quand les patients sont traités avec la décitabine (Dacogène). Plusieurs études ont démontré que la surexpression de TP53 muté est souvent associée à la résistance aux médicaments dans le traitement du cancer [379]. Une autre étude a montré que l'expression de type sauvage de TP53 dans les cellules aneuploïdes du cancer du côlon entraîne une résistance des cellules tumorales au traitement à l'hydroxyurée [380]. Il serait intéressant maintenant de comprendre les mécanismes moléculaires de cette différence de réponse entre l'Hydrea entre ces deux groupes de patients par des expériences *in vitro* et *in vivo*. Des essais cliniques prospectifs pourraient être envisagés pour évaluer l'efficacité de l'Hydrea par rapport au Dacogène chez différents sous-groupes de patients en fonction de leur expression de TP53. Encore une fois, CD300E est un marqueur prometteur, qui pourrait éventuellement servir de biomarqueur pour faciliter le diagnostic et reconnaître les patients avec une faible expression de TP53. Cela pourrait aider à mieux orienter le choix du traitement et déterminer quel patient doit être traité par l'Hydrea et quel patient doit être traité par du dacogène. En résumé, ces observations ouvrent la voie à des traitements plus efficaces et plus personnalisés pour ces patients.

IX. Conclusion générale

Ma thèse repose sur l'étude de deux gènes suppresseurs de tumeurs, *TET2* et *TP53*, dans les monocytes en condition physiologique et pathologique (LMMC).

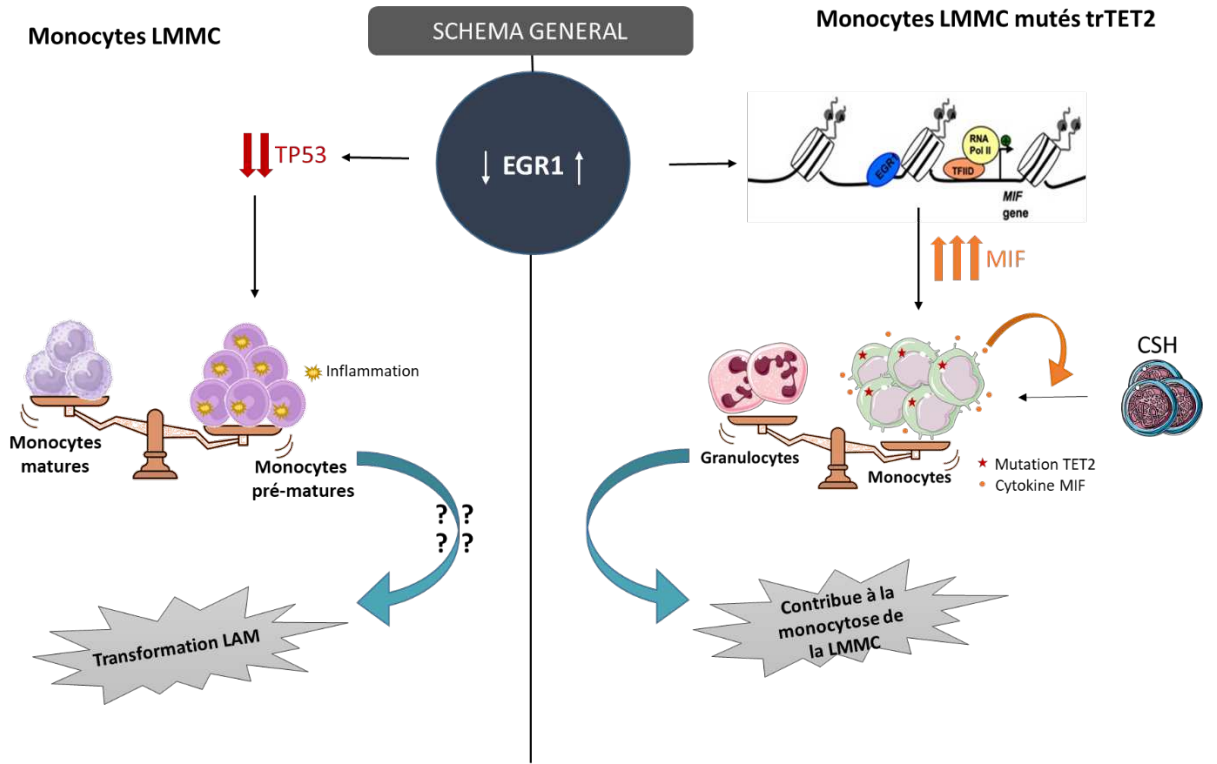
Ces deux gènes suppresseurs de tumeurs sont fréquemment sujets à des mutations dans les hémopathies malignes. Dans la LMMC, les mutations touchant *TET2* représentent 60% des cas, tandis que les mutations de *TP53* ne surviennent que dans environ 2% des cas. Un autre trait commun à ces gènes est leur capacité à influencer la transcription des gènes. *TP53* en tant que facteur de transcription et *TET2* par son rôle dans la déméthylation.

La LMMC est une maladie rare de la cellule souche hématopoïétique caractérisée par une accumulation des monocytes pendant plus de trois mois. Aucun traitement curatif n'a été identifié pour cette maladie, à l'exception de la greffe de cellules souches hématopoïétiques.

Dans la première partie, nous avons mis en évidence que l'absence d'expression de *TET2*, en condition normale et/ou dans les cas des leucémies, conduit à la surproduction de la cytokine MIF. À son tour, cette cytokine favorise la production de monocytes. Etant donné qu'il est compliqué de cibler directement *TET2* avec des médicaments, MIF pourrait représenter une cible thérapeutique plus prometteuse chez les patients LMMC avec des mutations tronquantes de *TET2*.

Dans la deuxième partie de ce travail, nous avons démontré une expression diminuée de *TP53* chez 30% des patients atteints de LMMC. Ce sous-groupe de patients est plus susceptible d'évoluer vers une LAM et a un phénotype plus inflammatoire. Ces caractéristiques en font un sous-groupe de plus mauvais pronostic. Pour la première fois, l'implication de *TP53* dans le processus de maturation terminale des monocytes primaires a été observée. *CD300E* pourrait être un biomarqueur permettant de reconnaître les patients avec une faible expression de *TP53* dans leurs monocytes. Enfin, nos résultats suggèrent qu'il est important de ne pas traiter ce sous-groupe de patients LMMC avec de l'hydroxyurée.

L'ensemble de ces travaux démontre l'importance d'explorer davantage les cellules matures au sein du clone leucémique afin de les stratifier pour favoriser la recherche de nouvelles cibles thérapeutiques et d'avoir une médecine plus personnalisée.



X. Bibliographie

- [1] A. Ivanovs, S. Rybtsov, L. Welch, R. A. Anderson, M. L. Turner, et A. Medvinsky, « Highly potent human hematopoietic stem cells first emerge in the intraembryonic aorta-gonad-mesonephros region », *J. Exp. Med.*, vol. 208, n° 12, p. 2417-2427, oct. 2011, doi: 10.1084/jem.20111688.
- [2] A. Ivanovs, S. Rybtsov, R. A. Anderson, M. L. Turner, et A. Medvinsky, « Identification of the niche and phenotype of the first human hematopoietic stem cells », *Stem Cell Rep.*, vol. 2, n° 4, p. 449-456, avr. 2014, doi: 10.1016/j.stemcr.2014.02.004.
- [3] A. Belyavsky, N. Petinati, et N. Drize, « Hematopoiesis during Ontogenesis, Adult Life, and Aging », *Int. J. Mol. Sci.*, vol. 22, n° 17, Art. n° 17, janv. 2021, doi: 10.3390/ijms22179231.
- [4] M. Ackermann, S. Liebhaber, J.-H. Klusmann, et N. Lachmann, « Lost in translation: pluripotent stem cell-derived hematopoiesis », *EMBO Mol. Med.*, vol. 7, n° 11, p. 1388-1402, nov. 2015, doi: 10.15252/emmm.201505301.
- [5] F. Notta *et al.*, « Distinct routes of lineage development reshape the human blood hierarchy across ontogeny », *Science*, vol. 351, n° 6269, p. aab2116, janv. 2016, doi: 10.1126/science.aab2116.
- [6] A. J. Becker, E. A. McCULLOCH, et J. E. Till, « Cytological Demonstration of the Clonal Nature of Spleen Colonies Derived from Transplanted Mouse Marrow Cells », *Nature*, vol. 197, n° 4866, Art. n° 4866, févr. 1963, doi: 10.1038/197452a0.
- [7] L. O. Jacobson, E. L. Simmons, E. K. Marks, et J. H. Eldredge, « Recovery from Radiation Injury », *Science*, vol. 113, n° 2940, p. 510-511, mai 1951, doi: 10.1126/science.113.2940.510.
- [8] N. S. Wolf et J. J. Trentin, « Differential proliferation of erythroid and granuloid spleen colonies following sublethal irradiation of the bone marrow donor », *J. Cell. Physiol.*, vol. 75, n° 2, p. 225-229, 1970, doi: 10.1002/jcp.1040750211.
- [9] G. J. Spangrude, S. Heimfeld, et I. L. Weissman, « Purification and Characterization of Mouse Hematopoietic Stem Cells », *Science*, vol. 241, n° 4861, p. 58-62, juill. 1988, doi: 10.1126/science.2898810.
- [10] K. Ikuta et I. L. Weissman, « Evidence that Hematopoietic Stem Cells Express Mouse c-kit but do not Depend on Steel Factor for Their Generation », *Proc. Natl. Acad. Sci. U. S. A.*, vol. 89, n° 4, p. 1502-1506, 1992.
- [11] S. Okada, H. Nakauchi, K. Nagayoshi, S. Nishikawa, Y. Miura, et T. Suda, « In vivo and in vitro stem cell function of c-kit- and Sca-1-positive murine hematopoietic cells », *Blood*, vol. 80, n° 12, p. 3044-3050, déc. 1992.
- [12] S. J. Morrison et I. L. Weissman, « The long-term repopulating subset of hematopoietic stem cells is deterministic and isolatable by phenotype », *Immunity*, vol. 1, n° 8, p. 661-673, nov. 1994, doi: 10.1016/1074-7613(94)90037-x.
- [13] M. Osawa, K. Hanada, H. Hamada, et H. Nakauchi, « Long-term lymphohematopoietic reconstitution by a single CD34-low/negative hematopoietic stem cell », *Science*, vol. 273, n° 5272, p. 242-245, juill. 1996, doi: 10.1126/science.273.5272.242.
- [14] L. Yang *et al.*, « Identification of Lin⁻Sca1⁺kit⁺CD34⁺Flt3⁻ short-term hematopoietic stem cells capable of rapidly reconstituting and rescuing myeloablated transplant recipients », *Blood*, vol. 105, n° 7, p. 2717-2723, avr. 2005, doi: 10.1182/blood-2004-06-2159.

- [15] K. Akashi, D. Traver, T. Miyamoto, et I. L. Weissman, « A clonogenic common myeloid progenitor that gives rise to all myeloid lineages », *Nature*, vol. 404, n° 6774, Art. n° 6774, mars 2000, doi: 10.1038/35004599.
- [16] M. Kondo, I. L. Weissman, et K. Akashi, « Identification of clonogenic common lymphoid progenitors in mouse bone marrow », *Cell*, vol. 91, n° 5, p. 661-672, nov. 1997, doi: 10.1016/s0092-8674(00)80453-5.
- [17] R. J. Dress *et al.*, « Plasmacytoid dendritic cells develop from Ly6D+ lymphoid progenitors distinct from the myeloid lineage », *Nat. Immunol.*, vol. 20, n° 7, p. 852-864, juill. 2019, doi: 10.1038/s41590-019-0420-3.
- [18] J. Helft, F. Anjos-Afonso, A. G. Van Der Veen, P. Chakravarty, D. Bonnet, et C. Reis E Sousa, « Dendritic Cell Lineage Potential in Human Early Hematopoietic Progenitors », *Cell Rep.*, vol. 20, n° 3, p. 529-537, juill. 2017, doi: 10.1016/j.celrep.2017.06.075.
- [19] R. Yamamoto *et al.*, « Clonal Analysis Unveils Self-Renewing Lineage-Restricted Progenitors Generated Directly from Hematopoietic Stem Cells », *Cell*, vol. 154, n° 5, p. 1112-1126, août 2013, doi: 10.1016/j.cell.2013.08.007.
- [20] R. van Furth et W. Sluiter, « Distribution of blood monocytes between a marginating and a circulating pool », *J. Exp. Med.*, vol. 163, n° 2, p. 474-479, févr. 1986, doi: 10.1084/jem.163.2.474.
- [21] F. K. Swirski *et al.*, « Identification of splenic reservoir monocytes and their deployment to inflammatory sites », *Science*, vol. 325, n° 5940, p. 612-616, juill. 2009, doi: 10.1126/science.1175202.
- [22] P. Herbomel, B. Thisse, et C. Thisse, « Ontogeny and behaviour of early macrophages in the zebrafish embryo », *Dev. Camb. Engl.*, vol. 126, n° 17, p. 3735-3745, sept. 1999, doi: 10.1242/dev.126.17.3735.
- [23] I. Hadji-Azimi, V. Coosemans, et C. Canicatti, « Atlas of adult *Xenopus laevis laevis* hematology », *Dev. Comp. Immunol.*, vol. 11, n° 4, p. 807-874, 1987, doi: 10.1016/0145-305x(87)90068-1.
- [24] A. B. Kay, « Paul Ehrlich and the Early History of Granulocytes », *Microbiol. Spectr.*, vol. 4, n° 4, p. 4.4.62, août 2016, doi: 10.1128/microbiolspec.MCHD-0032-2016.
- [25] « 1880-methodologisch-beitraege-physiologie-pathologie.pdf ». Consulté le: 6 janvier 2023. [En ligne]. Disponible sur: https://www.pei.de/SharedDocs/Downloads/DE/institut/veroeffentlichungen-von-paul-ehrlich/1877-1885/1880-methodologisch-beitraege-physiologie-pathologie.pdf?__blob=publicationFile&v=2
- [26] M. Williams, A. Mildner, et S. Yona, « Developmental and Functional Heterogeneity of Monocytes », *Immunity*, vol. 49, n° 4, p. 595-613, oct. 2018, doi: 10.1016/j.immuni.2018.10.005.
- [27] P. Szilárd, « Eine Methode zur isolierten Gewinnung der weißen Blutkörperchen », *Pflüg. Arch. Für Gesamte Physiol. Menschen Tiere*, vol. 211, n° 1, p. 597-602, déc. 1926, doi: 10.1007/BF01722203.
- [28] M. Bessis, « Une méthode permettant l'isolement des différents éléments figurés du sang », *Sang*, vol. 14, n° 262, p. 17, 1940.
- [29] W. Fleischmann, « Methoden Zur Untersuchung Des Stoffwechsels von Leukocyten Und Thrombocyten. » Abderhalden's Handbuch Der Biologischen Arbeitsmethoden 13 (4): 87. », 1937.

- [30] P. Larizza, « Beitrag zur Kenntnis der chemischen Zusammensetzung der weißen Blutzellen des menschlichen Blutes », in *Zeitschrift für die gesamte experimentelle Medizin*, vol. 101, 1937, p. 615-621.
- [31] B. L. Vallee, W. L. Hughes, et J. G. Gibson, « A method for the separation of leukocytes from whole blood by flotation on serum albumin », *Blood*, n° 1, p. 82-87, juill. 1947.
- [32] R. van Furth et Z. A. Cohn, « The origin and kinetics of mononuclear phagocytes », *J. Exp. Med.*, vol. 128, n° 3, p. 415-435, sept. 1968, doi: 10.1084/jem.128.3.415.
- [33] A. Bøyum, « Separation of Blood Leucocytes, Granulocytes and Lymphocytes », *Tissue Antigens*, vol. 4, n° 3, p. 269-274, 1974, doi: 10.1111/j.1399-0039.1974.tb00252.x.
- [34] A. Pappenheim et Ferrata, *Über die verschiedenen lymphoiden Zellformen des normaen und pathologischen Blutes.*, vol. 10:78. Fol Haematol, 1910.
- [35] R. Drissen *et al.*, « Distinct myeloid progenitor-differentiation pathways identified through single-cell RNA sequencing », *Nat. Immunol.*, vol. 17, n° 6, p. 666-676, juin 2016, doi: 10.1038/ni.3412.
- [36] J. Lee *et al.*, « Restricted dendritic cell and monocyte progenitors in human cord blood and bone marrow », *J. Exp. Med.*, vol. 212, n° 3, p. 385-399, mars 2015, doi: 10.1084/jem.20141442.
- [37] C. Varol *et al.*, « Monocytes give rise to mucosal, but not splenic, conventional dendritic cells », *J. Exp. Med.*, vol. 204, n° 1, p. 171-180, déc. 2006, doi: 10.1084/jem.20061011.
- [38] D. K. Fogg *et al.*, « A Clonogenic Bone Marrow Progenitor Specific for Macrophages and Dendritic Cells », *Science*, vol. 311, n° 5757, p. 83-87, janv. 2006, doi: 10.1126/science.1117729.
- [39] J. Hettlinger *et al.*, « Origin of monocytes and macrophages in a committed progenitor », *Nat. Immunol.*, vol. 14, n° 8, p. 821-830, août 2013, doi: 10.1038/ni.2638.
- [40] D. Sichien *et al.*, « IRF8 Transcription Factor Controls Survival and Function of Terminally Differentiated Conventional and Plasmacytoid Dendritic Cells, Respectively », *Immunity*, vol. 45, n° 3, p. 626-640, sept. 2016, doi: 10.1016/j.immuni.2016.08.013.
- [41] J. Olweus, F. Lund-Johansen, et L. Terstappen, « CD64/FcγRI is a granulo-monocytic lineage marker on CD34+ hematopoietic progenitor cells », *Blood*, vol. 85, p. 2402-13, juin 1995, doi: 10.1182/blood.V85.9.2402.bloodjournal8592402.
- [42] S. Kawamura *et al.*, « Identification of a Human Clonogenic Progenitor with Strict Monocyte Differentiation Potential: A Counterpart of Mouse cMoPs », *Immunity*, vol. 46, n° 5, p. 835-848.e4, mai 2017, doi: 10.1016/j.immuni.2017.04.019.
- [43] K. Bassler, J. Schulte-Schrepping, S. Warnat-Herresthal, A. C. Aschenbrenner, et J. L. Schultze, « The Myeloid Cell Compartment—Cell by Cell », *Annu. Rev. Immunol.*, vol. 37, n° 1, p. 269-293, avr. 2019, doi: 10.1146/annurev-immunol-042718-041728.
- [44] A. Yáñez *et al.*, « Granulocyte-Monocyte Progenitors and Monocyte-Dendritic Cell Progenitors Independently Produce Functionally Distinct Monocytes », *Immunity*, vol. 47, n° 5, p. 890-902.e4, nov. 2017, doi: 10.1016/j.immuni.2017.10.021.
- [45] S. Trzebanski et S. Jung, « Plasticity of monocyte development and monocyte fates », *Immunol. Lett.*, vol. 227, p. 66-78, nov. 2020, doi: 10.1016/j.imlet.2020.07.007.
- [46] J. C. Gasson, « Molecular physiology of granulocyte-macrophage colony-stimulating factor », *Blood*, vol. 77, n° 6, p. 1131-1145, mars 1991.
- [47] G. C. Bagby, C. A. Dinarello, P. Wallace, C. Wagner, S. Hefeneider, et E. McCall, « Interleukin 1 stimulates granulocyte macrophage colony-stimulating activity release

- by vascular endothelial cells », *J. Clin. Invest.*, vol. 78, n° 5, p. 1316-1323, nov. 1986, doi: 10.1172/JCI112717.
- [48] J. R. Lukens, M. J. Barr, D. D. Chaplin, H. Chi, et T.-D. Kanneganti, « Inflammasome-derived IL-1 β regulates the production of GM-CSF by CD4(+) T cells and $\gamma\delta$ T cells », *J. Immunol. Baltim. Md 1950*, vol. 188, n° 7, p. 3107-3115, avr. 2012, doi: 10.4049/jimmunol.1103308.
- [49] M. El-Behi *et al.*, « The encephalitogenicity of T(H)17 cells is dependent on IL-1- and IL-23-induced production of the cytokine GM-CSF », *Nat. Immunol.*, vol. 12, n° 6, p. 568-575, juin 2011, doi: 10.1038/ni.2031.
- [50] S.-N. Zhu, M. Chen, J. Jongstra-Bilen, et M. I. Cybulsky, « GM-CSF regulates intimal cell proliferation in nascent atherosclerotic lesions », *J. Exp. Med.*, vol. 206, n° 10, p. 2141-2149, sept. 2009, doi: 10.1084/jem.20090866.
- [51] M. A. Rieger, P. S. Hoppe, B. M. Smejkal, A. C. Eitelhuber, et T. Schroeder, « Hematopoietic Cytokines Can Instruct Lineage Choice », *Science*, vol. 325, n° 5937, p. 217-218, juill. 2009, doi: 10.1126/science.1171461.
- [52] D. Barreda, « Regulation of myeloid development and function by colony stimulating factors », *Dev. Comp. Immunol.*, vol. 28, n° 5, p. 509-554, mai 2004, doi: 10.1016/j.dci.2003.09.010.
- [53] D. A. Hume et K. P. A. MacDonald, « Therapeutic applications of macrophage colony-stimulating factor-1 (CSF-1) and antagonists of CSF-1 receptor (CSF-1R) signaling », *Blood*, vol. 119, n° 8, p. 1810-1820, févr. 2012, doi: 10.1182/blood-2011-09-379214.
- [54] J. A. Hamilton, « Colony-stimulating factors in inflammation and autoimmunity », *Nat. Rev. Immunol.*, vol. 8, n° 7, p. 533-544, juill. 2008, doi: 10.1038/nri2356.
- [55] X.-M. Dai *et al.*, « Targeted disruption of the mouse colony-stimulating factor 1 receptor gene results in osteopetrosis, mononuclear phagocyte deficiency, increased primitive progenitor cell frequencies, and reproductive defects », *Blood*, vol. 99, n° 1, p. 111-120, janv. 2002, doi: 10.1182/blood.v99.1.111.
- [56] K. Tobal, A. Pagliuca, B. Bhatt, N. Bailey, D. M. Layton, et G. J. Mufti, « Mutation of the human FMS gene (M-CSF receptor) in myelodysplastic syndromes and acute myeloid leukemia », *Leukemia*, vol. 4, n° 7, p. 486-489, juill. 1990.
- [57] R. Rademakers *et al.*, « Mutations in the colony stimulating factor 1 receptor (CSF1R) gene cause hereditary diffuse leukoencephalopathy with spheroids », *Nat. Genet.*, vol. 44, n° 2, p. 200-205, déc. 2011, doi: 10.1038/ng.1027.
- [58] X.-M. Dai, X.-H. Zong, V. Sylvestre, et E. R. Stanley, « Incomplete restoration of colony-stimulating factor 1 (CSF-1) function in CSF-1-deficient *Csf1op/Csf1op* mice by transgenic expression of cell surface CSF-1 », *Blood*, vol. 103, n° 3, p. 1114-1123, févr. 2004, doi: 10.1182/blood-2003-08-2739.
- [59] I. Kang et R. Bucala, « The immunobiology of MIF: function, genetics and prospects for precision medicine », *Nat. Rev. Rheumatol.*, vol. 15, n° 7, p. 427-437, juill. 2019, doi: 10.1038/s41584-019-0238-2.
- [60] T. Calandra et T. Roger, « Macrophage migration inhibitory factor: a regulator of innate immunity », *Nat. Rev. Immunol.*, vol. 3, n° 10, p. 791-800, oct. 2003, doi: 10.1038/nri1200.
- [61] T. Calandra *et al.*, « MIF as a glucocorticoid-induced modulator of cytokine production », *Nature*, vol. 377, n° 6544, p. 68-71, sept. 1995, doi: 10.1038/377068a0.
- [62] É. Giannoni, A. Schneider, T. Calandra, et T. Roger, « Le facteur d'inhibition de la migration des macrophages (MIF), un régulateur de la réponse immunitaire innée

- néonatale », *médecine/sciences*, vol. 32, n° 12, p. 1062-1064, déc. 2016, doi: 10.1051/medsci/20163212006.
- [63] M. Bozza *et al.*, « Targeted disruption of migration inhibitory factor gene reveals its critical role in sepsis », *J. Exp. Med.*, vol. 189, n° 2, p. 341-346, janv. 1999, doi: 10.1084/jem.189.2.341.
- [64] T. Calandra, J. Bernhagen, R. A. Mitchell, et R. Bucala, « The macrophage is an important and previously unrecognized source of macrophage migration inhibitory factor », *J. Exp. Med.*, vol. 179, n° 6, p. 1895-1902, juin 1994, doi: 10.1084/jem.179.6.1895.
- [65] R. P. DeKoter, J. C. Walsh, et H. Singh, « PU.1 regulates both cytokine-dependent proliferation and differentiation of granulocyte/macrophage progenitors », *EMBO J.*, vol. 17, n° 15, p. 4456-4468, août 1998, doi: 10.1093/emboj/17.15.4456.
- [66] E. W. Scott, M. C. Simon, J. Anastasi, et H. Singh, « Requirement of transcription factor PU.1 in the development of multiple hematopoietic lineages », *Science*, vol. 265, n° 5178, p. 1573-1577, sept. 1994, doi: 10.1126/science.8079170.
- [67] S. L. Nutt, D. Metcalf, A. D'Amico, M. Polli, et L. Wu, « Dynamic regulation of PU.1 expression in multipotent hematopoietic progenitors », *J. Exp. Med.*, vol. 201, n° 2, p. 221-231, janv. 2005, doi: 10.1084/jem.20041535.
- [68] D. Kurotaki *et al.*, « Transcription Factor IRF8 Governs Enhancer Landscape Dynamics in Mononuclear Phagocyte Progenitors », *Cell Rep.*, vol. 22, n° 10, p. 2628-2641, mars 2018, doi: 10.1016/j.celrep.2018.02.048.
- [69] T. Tamura, D. Kurotaki, et S. Koizumi, « Regulation of myelopoiesis by the transcription factor IRF8 », *Int. J. Hematol.*, vol. 101, n° 4, p. 342-351, avr. 2015, doi: 10.1007/s12185-015-1761-9.
- [70] M. W. Feinberg *et al.*, « The Kruppel-like factor KLF4 is a critical regulator of monocyte differentiation », *EMBO J.*, vol. 26, n° 18, p. 4138-4148, sept. 2007, doi: 10.1038/sj.emboj.7601824.
- [71] C. Nerlov, E. Querfurth, H. Kulesa, et T. Graf, « GATA-1 interacts with the myeloid PU.1 transcription factor and represses PU.1-dependent transcription », *Blood*, vol. 95, n° 8, p. 2543-2551, avr. 2000.
- [72] N. Rekhtman, F. Radparvar, T. Evans, et A. I. Skoultschi, « Direct interaction of hematopoietic transcription factors PU.1 and GATA-1: functional antagonism in erythroid cells », *Genes Dev.*, vol. 13, n° 11, p. 1398-1411, juin 1999, doi: 10.1101/gad.13.11.1398.
- [73] M. Scheller *et al.*, « Altered development and cytokine responses of myeloid progenitors in the absence of transcription factor, interferon consensus sequence binding protein », *Blood*, vol. 94, n° 11, p. 3764-3771, déc. 1999.
- [74] D. Kurotaki *et al.*, « Essential role of the IRF8-KLF4 transcription factor cascade in murine monocyte differentiation », *Blood*, vol. 121, n° 10, p. 1839-1849, mars 2013, doi: 10.1182/blood-2012-06-437863.
- [75] J. K. Alder *et al.*, « Kruppel-like factor 4 is essential for inflammatory monocyte differentiation in vivo », *J. Immunol. Baltim. Md 1950*, vol. 180, n° 8, p. 5645-5652, avr. 2008, doi: 10.4049/jimmunol.180.8.5645.
- [76] A. G. Rosmarin, Z. Yang, et K. K. Resendes, « Transcriptional regulation in myelopoiesis: Hematopoietic fate choice, myeloid differentiation, and leukemogenesis », *Exp. Hematol.*, vol. 33, n° 2, p. 131-143, févr. 2005, doi: 10.1016/j.exphem.2004.08.015.

- [77] D. E. Zhang, P. Zhang, N. D. Wang, C. J. Hetherington, G. J. Darlington, et D. G. Tenen, « Absence of granulocyte colony-stimulating factor signaling and neutrophil development in CCAAT enhancer binding protein alpha-deficient mice », *Proc. Natl. Acad. Sci. U. S. A.*, vol. 94, n° 2, p. 569-574, janv. 1997, doi: 10.1073/pnas.94.2.569.
- [78] A. Tamura *et al.*, « Accelerated apoptosis of peripheral blood monocytes in Cebpb-deficient mice », *Biochem. Biophys. Res. Commun.*, vol. 464, n° 2, p. 654-658, août 2015, doi: 10.1016/j.bbrc.2015.07.045.
- [79] T. Okuda, J. van Deursen, S. W. Hiebert, G. Grosveld, et J. R. Downing, « AML1, the target of multiple chromosomal translocations in human leukemia, is essential for normal fetal liver hematopoiesis », *Cell*, vol. 84, n° 2, p. 321-330, janv. 1996, doi: 10.1016/s0092-8674(00)80986-1.
- [80] G. Huang *et al.*, « PU.1 is a major downstream target of AML1 (RUNX1) in adult mouse hematopoiesis », *Nat. Genet.*, vol. 40, n° 1, p. 51-60, janv. 2008, doi: 10.1038/ng.2007.7.
- [81] K. Krishnaraju, B. Hoffman, et D. A. Liebermann, « Early growth response gene 1 stimulates development of hematopoietic progenitor cells along the macrophage lineage at the expense of the granulocyte and erythroid lineages », *Blood*, vol. 97, n° 5, p. 1298-1305, mars 2001, doi: 10.1182/blood.v97.5.1298.
- [82] T.-H. Pham *et al.*, « Dynamic epigenetic enhancer signatures reveal key transcription factors associated with monocytic differentiation states », *Blood*, vol. 119, n° 24, p. e161-171, juin 2012, doi: 10.1182/blood-2012-01-402453.
- [83] P. Laslo *et al.*, « Multilineage transcriptional priming and determination of alternate hematopoietic cell fates », *Cell*, vol. 126, n° 4, p. 755-766, août 2006, doi: 10.1016/j.cell.2006.06.052.
- [84] E. Barbieri *et al.*, « Targeted Enhancer Activation by a Subunit of the Integrator Complex », *Mol. Cell*, vol. 71, n° 1, p. 103-116.e7, juill. 2018, doi: 10.1016/j.molcel.2018.05.031.
- [85] V. Barbetti *et al.*, « Chromatin-associated CSF-1R binds to the promoter of proliferation-related genes in breast cancer cells », *Oncogene*, vol. 33, n° 34, p. 4359-4364, août 2014, doi: 10.1038/onc.2013.542.
- [86] S. Matarraz *et al.*, « The immunophenotype of different immature, myeloid and B-cell lineage-committed CD34+ hematopoietic cells allows discrimination between normal/reactive and myelodysplastic syndrome precursors », *Leukemia*, vol. 22, n° 6, p. 1175-1183, juin 2008, doi: 10.1038/leu.2008.49.
- [87] S. Matarraz *et al.*, « Introduction to the diagnosis and classification of monocytic-lineage leukemias by flow cytometry: MONOCYTIC-LINEAGE LEUKEMIAS BY FLOW CYTOMETRY », *Cytometry B Clin. Cytom.*, vol. 92, n° 3, p. 218-227, mai 2017, doi: 10.1002/cyto.b.21219.
- [88] H. Aguilar, D. Alvarez-Errico, A. C. García-Montero, A. Orfao, J. Sayós, et M. López-Botet, « Molecular characterization of a novel immune receptor restricted to the monocytic lineage », *J. Immunol. Baltim. Md 1950*, vol. 173, n° 11, p. 6703-6711, déc. 2004, doi: 10.4049/jimmunol.173.11.6703.
- [89] G. Meuret, J. Bammert, et G. Hoffmann, « Kinetics of Human Monocytopoiesis », *Blood*, vol. 44, n° 6, p. 801-816, déc. 1974, doi: 10.1182/blood.V44.6.801.801.
- [90] B. Passlick, D. Flieger, et H. W. Ziegler-Heitbrock, « Identification and characterization of a novel monocyte subpopulation in human peripheral blood », *Blood*, vol. 74, n° 7, p. 2527-2534, nov. 1989.

- [91] L. Ziegler-Heitbrock *et al.*, « Nomenclature of monocytes and dendritic cells in blood », *Blood*, vol. 116, n° 16, p. e74-80, oct. 2010, doi: 10.1182/blood-2010-02-258558.
- [92] A. M. Zawada *et al.*, « SuperSAGE evidence for CD14⁺⁺CD16⁺ monocytes as a third monocyte subset », *Blood*, vol. 118, n° 12, p. e50-61, sept. 2011, doi: 10.1182/blood-2011-01-326827.
- [93] S. Sprangers, T. J. de Vries, et V. Everts, « Monocyte Heterogeneity: Consequences for Monocyte-Derived Immune Cells », *J. Immunol. Res.*, vol. 2016, p. 1475435, 2016, doi: 10.1155/2016/1475435.
- [94] K. L. Wong *et al.*, « Gene expression profiling reveals the defining features of the classical, intermediate, and nonclassical human monocyte subsets », *Blood*, vol. 118, n° 5, p. e16-e31, août 2011, doi: 10.1182/blood-2010-12-326355.
- [95] T. S. Kapellos *et al.*, « Human Monocyte Subsets and Phenotypes in Major Chronic Inflammatory Diseases », *Front. Immunol.*, vol. 10, 2019, Consulté le: 6 juin 2023. [En ligne]. Disponible sur: <https://www.frontiersin.org/articles/10.3389/fimmu.2019.02035>
- [96] K.-U. Belge *et al.*, « The proinflammatory CD14⁺CD16⁺DR⁺⁺ monocytes are a major source of TNF », *J. Immunol. Baltim. Md 1950*, vol. 168, n° 7, p. 3536-3542, avr. 2002, doi: 10.4049/jimmunol.168.7.3536.
- [97] M. Frankenberger, T. Sternsdorf, H. Pechumer, A. Pforte, et H. W. Ziegler-Heitbrock, « Differential cytokine expression in human blood monocyte subpopulations: a polymerase chain reaction analysis », *Blood*, vol. 87, n° 1, p. 373-377, janv. 1996.
- [98] T. P. Hofer *et al.*, « slan-defined subsets of CD16-positive monocytes: impact of granulomatous inflammation and M-CSF receptor mutation », *Blood*, vol. 126, n° 24, p. 2601-2610, déc. 2015, doi: 10.1182/blood-2015-06-651331.
- [99] S. Yona *et al.*, « Fate mapping reveals origins and dynamics of monocytes and tissue macrophages under homeostasis », *Immunity*, vol. 38, n° 1, p. 79-91, janv. 2013, doi: 10.1016/j.immuni.2012.12.001.
- [100] A. A. Patel *et al.*, « The fate and lifespan of human monocyte subsets in steady state and systemic inflammation », *J. Exp. Med.*, vol. 214, n° 7, p. 1913-1923, juill. 2017, doi: 10.1084/jem.20170355.
- [101] F. Geissmann, S. Jung, et D. R. Littman, « Blood monocytes consist of two principal subsets with distinct migratory properties », *Immunity*, vol. 19, n° 1, p. 71-82, juill. 2003, doi: 10.1016/s1074-7613(03)00174-2.
- [102] J. Cros *et al.*, « Human CD14^{dim} monocytes patrol and sense nucleic acids and viruses via TLR7 and TLR8 receptors », *Immunity*, vol. 33, n° 3, p. 375-386, sept. 2010, doi: 10.1016/j.immuni.2010.08.012.
- [103] M. A. Ingersoll *et al.*, « Comparison of gene expression profiles between human and mouse monocyte subsets », *Blood*, vol. 115, n° 3, p. e10-19, janv. 2010, doi: 10.1182/blood-2009-07-235028.
- [104] A. V. Misharin *et al.*, « Nonclassical Ly6C⁻ Monocytes Drive the Development of Inflammatory Arthritis in Mice », *Cell Rep.*, vol. 9, n° 2, p. 591-604, oct. 2014, doi: 10.1016/j.celrep.2014.09.032.
- [105] S. A. Marsh, H. M. Arthur, et I. Spyridopoulos, « The secret life of nonclassical monocytes », *Cytometry A*, vol. 91, n° 11, p. 1055-1058, nov. 2017, doi: 10.1002/cyto.a.23280.
- [106] C. Auffray, M. H. Sieweke, et F. Geissmann, « Blood monocytes: development, heterogeneity, and relationship with dendritic cells », *Annu. Rev. Immunol.*, vol. 27, p. 669-692, 2009, doi: 10.1146/annurev.immunol.021908.132557.

- [107] A. Castagna, R. Polati, A. M. Bossi, et D. Girelli, « Monocyte/macrophage proteomics: recent findings and biomedical applications », *Expert Rev. Proteomics*, vol. 9, n° 2, p. 201-215, avr. 2012, doi: 10.1586/epr.12.11.
- [108] M. A. Tam, A. Rydström, M. Sundquist, et M. J. Wick, « Early cellular responses to *Salmonella* infection: dendritic cells, monocytes, and more », *Immunol. Rev.*, vol. 225, n° 1, p. 140-162, oct. 2008, doi: 10.1111/j.1600-065X.2008.00679.x.
- [109] F. Geissmann, M. G. Manz, S. Jung, M. H. Sieweke, M. Merad, et K. Ley, « Development of monocytes, macrophages, and dendritic cells », *Science*, vol. 327, n° 5966, p. 656-661, févr. 2010, doi: 10.1126/science.1178331.
- [110] A. C. Hearps *et al.*, « Aging is associated with chronic innate immune activation and dysregulation of monocyte phenotype and function », *Aging Cell*, vol. 11, n° 5, p. 867-875, oct. 2012, doi: 10.1111/j.1474-9726.2012.00851.x.
- [111] H. M. Sadeghi, J. F. Schnelle, J. K. Thoma, P. Nishanian, et J. L. Fahey, « Phenotypic and functional characteristics of circulating monocytes of elderly persons », *Exp. Gerontol.*, vol. 34, n° 8, p. 959-970, déc. 1999, doi: 10.1016/s0531-5565(99)00065-0.
- [112] E. F. Devèvre, M. Renovato-Martins, K. Clément, C. Sautès-Fridman, I. Cremer, et C. Poitou, « Profiling of the Three Circulating Monocyte Subpopulations in Human Obesity », *J. Immunol.*, vol. 194, n° 8, p. 3917-3923, avr. 2015, doi: 10.4049/jimmunol.1402655.
- [113] A. Shapouri-Moghaddam *et al.*, « Macrophage plasticity, polarization, and function in health and disease », *J. Cell. Physiol.*, vol. 233, n° 9, p. 6425-6440, sept. 2018, doi: 10.1002/jcp.26429.
- [114] J. B. Golden *et al.*, « Chronic Psoriatic Skin Inflammation Leads to Increased Monocyte Adhesion and Aggregation », *J. Immunol. Baltim. Md 1950*, vol. 195, n° 5, p. 2006-2018, sept. 2015, doi: 10.4049/jimmunol.1402307.
- [115] R. M. Kratofil, P. Kubes, et J. F. Deniset, « Monocyte Conversion During Inflammation and Injury », *Arterioscler. Thromb. Vasc. Biol.*, vol. 37, n° 1, p. 35-42, janv. 2017, doi: 10.1161/ATVBAHA.116.308198.
- [116] A. Anderson *et al.*, « Monocytosis Is a Biomarker of Severity in Inflammatory Bowel Disease: Analysis of a 6-Year Prospective Natural History Registry », *Inflamm. Bowel Dis.*, vol. 28, n° 1, p. 70-78, janv. 2022, doi: 10.1093/ibd/izab031.
- [117] T. McGarry *et al.*, « Rheumatoid arthritis CD14⁺ monocytes display metabolic and inflammatory dysfunction, a phenotype that precedes clinical manifestation of disease », *Clin. Transl. Immunol.*, vol. 10, n° 1, janv. 2021, doi: 10.1002/cti2.1237.
- [118] R. Medzhitov et Charles S. J. Janeway, « Innate immune recognition: mechanisms and pathways: Innate immune recognition », *Immunol. Rev.*, vol. 173, n° 1, p. 89-97, févr. 2000, doi: 10.1034/j.1600-065X.2000.917309.x.
- [119] L. A. J. O'Neill et A. G. Bowie, « The family of five: TIR-domain-containing adaptors in Toll-like receptor signalling », *Nat. Rev. Immunol.*, vol. 7, n° 5, p. 353-364, mai 2007, doi: 10.1038/nri2079.
- [120] K. L. Wong, W. H. Yeap, J. J. Y. Tai, S. M. Ong, T. M. Dang, et S. C. Wong, « The three human monocyte subsets: implications for health and disease », *Immunol. Res.*, vol. 53, n° 1-3, p. 41-57, sept. 2012, doi: 10.1007/s12026-012-8297-3.
- [121] D. Castaño, L. F. García, et M. Rojas, « Increased frequency and cell death of CD16⁺ monocytes with Mycobacterium tuberculosis infection », *Tuberc. Edinb. Scotl.*, vol. 91, n° 5, p. 348-360, sept. 2011, doi: 10.1016/j.tube.2011.04.002.

- [122] J.-Y. Zhang *et al.*, « Hyper-Activated Pro-Inflammatory CD16+ Monocytes Correlate with the Severity of Liver Injury and Fibrosis in Patients with Chronic Hepatitis B », *PLoS ONE*, vol. 6, n° 3, p. e17484, mars 2011, doi: 10.1371/journal.pone.0017484.
- [123] Y. Rodríguez-Muñoz *et al.*, « Peripheral blood monocyte subsets predict antiviral response in chronic hepatitis C », *Aliment. Pharmacol. Ther.*, vol. 34, n° 8, p. 960-971, oct. 2011, doi: 10.1111/j.1365-2036.2011.04807.x.
- [124] J. Han *et al.*, « CD14(high)CD16(+) rather than CD14(low)CD16(+) monocytes correlate with disease progression in chronic HIV-infected patients », *J. Acquir. Immune Defic. Syndr.* 1999, vol. 52, n° 5, p. 553-559, déc. 2009, doi: 10.1097/qai.0b013e3181c1d4fe.
- [125] Y. Yoshioka *et al.*, « Increased circulating CD16+ CD14dim monocytes in a patient with pulmonary alveolar proteinosis », *Respirol. Carlton Vic*, vol. 7, n° 3, p. 273-279, sept. 2002, doi: 10.1046/j.1440-1843.2002.00392.x.
- [126] N. Novak, P. Allam, E. Geiger, et T. Bieber, « Characterization of monocyte subtypes in the allergic form of atopic eczema/dermatitis syndrome », *Allergy*, vol. 57, n° 10, p. 931-935, oct. 2002, doi: 10.1034/j.1398-9995.2002.23737.x.
- [127] X. Urra *et al.*, « Monocyte Subtypes Predict Clinical Course and Prognosis in Human Stroke », *J. Cereb. Blood Flow Metab.*, vol. 29, n° 5, p. 994-1002, mai 2009, doi: 10.1038/jcbfm.2009.25.
- [128] R. N. Hanna *et al.*, « Patrolling monocytes control tumor metastasis to the lung », *Science*, vol. 350, n° 6263, p. 985-990, nov. 2015, doi: 10.1126/science.aac9407.
- [129] T. S. Griffith, S. R. Wiley, M. Z. Kubin, L. M. Sedger, C. R. Maliszewski, et N. A. Fanger, « Monocyte-mediated tumoricidal activity via the tumor necrosis factor-related cytokine, TRAIL », *J. Exp. Med.*, vol. 189, n° 8, p. 1343-1354, avr. 1999, doi: 10.1084/jem.189.8.1343.
- [130] T. Hartwig *et al.*, « The TRAIL-Induced Cancer Secretome Promotes a Tumor-Supportive Immune Microenvironment via CCR2 », *Mol. Cell*, vol. 65, n° 4, p. 730-742.e5, févr. 2017, doi: 10.1016/j.molcel.2017.01.021.
- [131] S. Lian, X. Xie, Y. Lu, et L. Jia, « Checkpoint CD47 Function On Tumor Metastasis And Immune Therapy », *OncoTargets Ther.*, vol. 12, p. 9105-9114, 2019, doi: 10.2147/OTT.S220196.
- [132] R. Du *et al.*, « HIF1alpha induces the recruitment of bone marrow-derived vascular modulatory cells to regulate tumor angiogenesis and invasion », *Cancer Cell*, vol. 13, n° 3, p. 206-220, mars 2008, doi: 10.1016/j.ccr.2008.01.034.
- [133] S. B. Coffelt *et al.*, « Angiopoietin-2 regulates gene expression in TIE2-expressing monocytes and augments their inherent proangiogenic functions », *Cancer Res.*, vol. 70, n° 13, p. 5270-5280, juill. 2010, doi: 10.1158/0008-5472.CAN-10-0012.
- [134] M. A. Venneri *et al.*, « Identification of proangiogenic TIE2-expressing monocytes (TEMs) in human peripheral blood and cancer », *Blood*, vol. 109, n° 12, p. 5276-5285, juin 2007, doi: 10.1182/blood-2006-10-053504.
- [135] X.-Q. Jiang *et al.*, « Expansion of CD14(+)CD16(+) monocytes is related to acute leukemia », *Int. J. Clin. Exp. Med.*, vol. 8, n° 8, p. 12297-12306, 2015.
- [136] X.-P. Gao *et al.*, « Pretreatment lymphocyte-to-monocyte ratio as a predictor of survival among patients with ovarian cancer: a meta-analysis », *Cancer Manag. Res.*, vol. 11, p. 1907-1920, 2019, doi: 10.2147/CMAR.S184970.

- [137] Z.-M. Li *et al.*, « Blood Lymphocyte-to-Monocyte Ratio Identifies High-Risk Patients in Diffuse Large B-Cell Lymphoma Treated with R-CHOP », *PLoS ONE*, vol. 7, n° 7, p. e41658, juill. 2012, doi: 10.1371/journal.pone.0041658.
- [138] M. Stotz *et al.*, « The preoperative lymphocyte to monocyte ratio predicts clinical outcome in patients with stage III colon cancer », *Br. J. Cancer*, vol. 110, n° 2, p. 435-440, janv. 2014, doi: 10.1038/bjc.2013.785.
- [139] A. Hamm *et al.*, « Tumour-educated circulating monocytes are powerful candidate biomarkers for diagnosis and disease follow-up of colorectal cancer », *Gut*, vol. 65, n° 6, p. 990-1000, juin 2016, doi: 10.1136/gutjnl-2014-308988.
- [140] A. D. Hurdle, O. M. Garson, et D. G. Buist, « Clinical and cytogenetic studies in chronic myelomonocytic leukaemia », *Br. J. Haematol.*, vol. 22, n° 6, p. 773-782, juin 1972.
- [141] T. Nösslinger *et al.*, « Myelodysplastic syndromes, from French-American-British to World Health Organization: comparison of classifications on 431 unselected patients from a single institution », *Blood*, vol. 98, n° 10, p. 2935-2941, nov. 2001, doi: 10.1182/blood.v98.10.2935.
- [142] A. Worsley *et al.*, « Prognostic features of chronic myelomonocytic leukaemia: a modified Bournemouth score gives the best prediction of survival », *Br. J. Haematol.*, vol. 68, n° 1, p. 17-21, janv. 1988, doi: 10.1111/j.1365-2141.1988.tb04173.x.
- [143] U. Germing, A. Kündgen, et N. Gattermann, « Risk Assessment in Chronic Myelomonocytic Leukemia (CMML) », *Leuk. Lymphoma*, vol. 45, n° 7, p. 1311-1318, juill. 2004, doi: 10.1080/1042819042000207271.
- [144] E. Such *et al.*, « Cytogenetic risk stratification in chronic myelomonocytic leukemia », *Haematologica*, vol. 96, n° 3, p. 375-383, mars 2011, doi: 10.3324/haematol.2010.030957.
- [145] J. W. Vardiman, N. L. Harris, et R. D. Brunning, « The World Health Organization (WHO) classification of the myeloid neoplasms », *Blood*, vol. 100, n° 7, p. 2292-2302, oct. 2002, doi: 10.1182/blood-2002-04-1199.
- [146] F. Onida *et al.*, « Prognostic factors and scoring systems in chronic myelomonocytic leukemia: a retrospective analysis of 213 patients », *Blood*, vol. 99, n° 3, p. 840-849, févr. 2002, doi: 10.1182/blood.V99.3.840.
- [147] M. M. Patnaik, E. A. Wassie, T. L. Lasho, C. A. Hanson, R. Ketterling, et A. Tefferi, « Blast transformation in chronic myelomonocytic leukemia: Risk factors, genetic features, survival, and treatment outcome: Blast Transformation in Chronic Myelomonocytic Leukemia », *Am. J. Hematol.*, vol. 90, n° 5, p. 411-416, mai 2015, doi: 10.1002/ajh.23962.
- [148] E. Padron *et al.*, « ETV6 and signaling gene mutations are associated with secondary transformation of myelodysplastic syndromes to chronic myelomonocytic leukemia », *Blood*, vol. 123, n° 23, p. 3675-3677, juin 2014, doi: 10.1182/blood-2014-03-562637.
- [149] K. Takahashi *et al.*, « Clinical characteristics and outcomes of therapy-related chronic myelomonocytic leukemia », *Blood*, vol. 122, n° 16, p. 2807-2811, oct. 2013, doi: 10.1182/blood-2013-03-491399.
- [150] D. A. Arber *et al.*, « The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia », *Blood*, vol. 127, n° 20, p. 2391-2405, mai 2016, doi: 10.1182/blood-2016-03-643544.
- [151] J. D. Khoury *et al.*, « The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Myeloid and Histiocytic/Dendritic Neoplasms », *Leukemia*, vol. 36, n° 7, p. 1703-1719, juill. 2022, doi: 10.1038/s41375-022-01613-1.

- [152] D. E. Rollison *et al.*, « Epidemiology of myelodysplastic syndromes and chronic myeloproliferative disorders in the United States, 2001-2004, using data from the NAACCR and SEER programs », *Blood*, vol. 112, n° 1, p. 45-52, juill. 2008, doi: 10.1182/blood-2008-01-134858.
- [153] P. J. Williamson, A. R. Kruger, P. J. Reynolds, T. J. Hamblin, et D. G. Oscier, « Establishing the incidence of myelodysplastic syndrome », *Br. J. Haematol.*, vol. 87, n° 4, p. 743-745, août 1994, doi: 10.1111/j.1365-2141.1994.tb06733.x.
- [154] E. Such *et al.*, « Development and validation of a prognostic scoring system for patients with chronic myelomonocytic leukemia », *Blood*, vol. 121, n° 15, p. 3005-3015, avr. 2013, doi: 10.1182/blood-2012-08-452938.
- [155] M. M. Patnaik *et al.*, « Spliceosome mutations involving *SRSF2*, *SF3B1*, and *U2AF35* in chronic myelomonocytic leukemia: Prevalence, clinical correlates, and prognostic relevance », *Am. J. Hematol.*, vol. 88, n° 3, p. 201-206, mars 2013, doi: 10.1002/ajh.23373.
- [156] P. A. Pophali et M. M. Patnaik, « The Role of New Tyrosine Kinase Inhibitors in Chronic Myeloid Leukemia », *Cancer J. Sudbury Mass*, vol. 22, n° 1, p. 40-50, 2016, doi: 10.1097/PPO.000000000000165.
- [157] M. M. Patnaik, T. L. Lasho, C. M. Finke, A. Pardanani, et A. Tefferi, « Targeted next generation sequencing of PDGFRB rearranged myeloid neoplasms with monocytosis », *Am. J. Hematol.*, vol. 91, n° 3, p. E12-14, mars 2016, doi: 10.1002/ajh.24267.
- [158] A. Pardanani *et al.*, « FIP1L1-PDGFR α in eosinophilic disorders: prevalence in routine clinical practice, long-term experience with imatinib therapy, and a critical review of the literature », *Leuk. Res.*, vol. 30, n° 8, p. 965-970, août 2006, doi: 10.1016/j.leukres.2005.11.011.
- [159] D. A. Arber et A. Orazi, « Update on the pathologic diagnosis of chronic myelomonocytic leukemia », *Mod. Pathol. Off. J. U. S. Can. Acad. Pathol. Inc*, vol. 32, n° 6, p. 732-740, juin 2019, doi: 10.1038/s41379-019-0215-y.
- [160] E. A. Wassie *et al.*, « Molecular and prognostic correlates of cytogenetic abnormalities in chronic myelomonocytic leukemia: a Mayo Clinic-French Consortium Study: Cytogenetic abnormalities in chronic myelomonocytic leukemia », *Am. J. Hematol.*, vol. 89, n° 12, p. 1111-1115, déc. 2014, doi: 10.1002/ajh.23846.
- [161] J. Merlevede *et al.*, « Mutation allele burden remains unchanged in chronic myelomonocytic leukaemia responding to hypomethylating agents », *Nat. Commun.*, vol. 7, n° 1, p. 10767, févr. 2016, doi: 10.1038/ncomms10767.
- [162] M. M. Patnaik et A. Tefferi, « Chronic myelomonocytic leukemia: 2022 update on diagnosis, risk stratification, and management », *Am. J. Hematol.*, vol. 97, n° 3, p. 352-372, mars 2022, doi: 10.1002/ajh.26455.
- [163] V. Grossmann *et al.*, « Molecular profiling of chronic myelomonocytic leukemia reveals diverse mutations in >80% of patients with TET2 and EZH2 being of high prognostic relevance », *Leukemia*, vol. 25, n° 5, p. 877-879, mai 2011, doi: 10.1038/leu.2011.10.
- [164] M. M. Patnaik *et al.*, « Number and type of TET2 mutations in chronic myelomonocytic leukemia and their clinical relevance », *Blood Cancer J.*, vol. 6, n° 9, p. e472, sept. 2016, doi: 10.1038/bcj.2016.82.
- [165] M. Meggendorfer *et al.*, « SRSF2 mutations in 275 cases with chronic myelomonocytic leukemia (CMML) », *Blood*, vol. 120, n° 15, p. 3080-3088, oct. 2012, doi: 10.1182/blood-2012-01-404863.

- [166] M. M. Patnaik et A. Tefferi, « Cytogenetic and molecular abnormalities in chronic myelomonocytic leukemia », *Blood Cancer J.*, vol. 6, n° 2, p. e393, févr. 2016, doi: 10.1038/bcj.2016.5.
- [167] A. Kohlmann *et al.*, « Next-generation sequencing technology reveals a characteristic pattern of molecular mutations in 72.8% of chronic myelomonocytic leukemia by detecting frequent alterations in TET2, CBL, RAS, and RUNX1 », *J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol.*, vol. 28, n° 24, p. 3858-3865, août 2010, doi: 10.1200/JCO.2009.27.1361.
- [168] R. Itzykson et E. Solary, « An evolutionary perspective on chronic myelomonocytic leukemia », *Leukemia*, vol. 27, n° 7, p. 1441-1450, juill. 2013, doi: 10.1038/leu.2013.100.
- [169] R. Itzykson *et al.*, « Prognostic score including gene mutations in chronic myelomonocytic leukemia », *J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol.*, vol. 31, n° 19, p. 2428-2436, juill. 2013, doi: 10.1200/JCO.2012.47.3314.
- [170] L. B. Alexandrov *et al.*, « Clock-like mutational processes in human somatic cells », *Nat. Genet.*, vol. 47, n° 12, p. 1402-1407, déc. 2015, doi: 10.1038/ng.3441.
- [171] R. Itzykson *et al.*, « Clonal architecture of chronic myelomonocytic leukemias », *Blood*, vol. 121, n° 12, p. 2186-2198, mars 2013, doi: 10.1182/blood-2012-06-440347.
- [172] K. Yoshida *et al.*, « Frequent pathway mutations of splicing machinery in myelodysplasia », *Nature*, vol. 478, n° 7367, p. 64-69, sept. 2011, doi: 10.1038/nature10496.
- [173] R. Itzykson *et al.*, « Two Distinct Mechanisms Contribute to Granulomonocytic Hyperplasia in Chronic Myelomonocytic Leukemias (CMML) », *Blood*, vol. 120, n° 21, p. 309-309, nov. 2012, doi: 10.1182/blood.V120.21.309.309.
- [174] E. Padron *et al.*, « GM-CSF-dependent pSTAT5 sensitivity is a feature with therapeutic potential in chronic myelomonocytic leukemia », *Blood*, vol. 121, n° 25, p. 5068-5077, juin 2013, doi: 10.1182/blood-2012-10-460170.
- [175] N. Kotecha *et al.*, « Single-cell profiling identifies aberrant STAT5 activation in myeloid malignancies with specific clinical and biologic correlates », *Cancer Cell*, vol. 14, n° 4, p. 335-343, oct. 2008, doi: 10.1016/j.ccr.2008.08.014.
- [176] K. Geissler *et al.*, « High Spontaneous In Vitro Myeloid Colony Formation in Chronic Myelomonocytic Leukemia is Associated with Mutations in Rasopathy Genes, Myeloproliferation and Inferior Prognosis », *Blood*, vol. 128, n° 22, p. 5503-5503, déc. 2016, doi: 10.1182/blood.V128.22.5503.5503.
- [177] D. Selimoglu-Buet *et al.*, « Characteristic repartition of monocyte subsets as a diagnostic signature of chronic myelomonocytic leukemia », *Blood*, vol. 125, n° 23, p. 3618-3626, juin 2015, doi: 10.1182/blood-2015-01-620781.
- [178] D. Selimoglu-Buet *et al.*, « A miR-150/TET3 pathway regulates the generation of mouse and human non-classical monocyte subset », *Nat. Commun.*, vol. 9, n° 1, p. 5455, déc. 2018, doi: 10.1038/s41467-018-07801-x.
- [179] E. Solary, O. Wagner-Ballon, et D. Selimoglu-Buet, « Incorporating flow cytometry and next-generation sequencing in the diagnosis of CMML. Are we ready for prime? », *Best Pract. Res. Clin. Haematol.*, vol. 33, n° 2, p. 101134, juin 2020, doi: 10.1016/j.beha.2019.101134.
- [180] S. Tarfi *et al.*, « Disappearance of slan-positive non-classical monocytes for diagnosis of chronic myelomonocytic leukemia with an associated inflammatory state »,

- Haematologica*, vol. 105, n° 4, p. e147-e152, avr. 2020, doi: 10.3324/haematol.2019.219782.
- [181] J. Kwon, « Diagnosis and treatment of chronic myelomonocytic leukemia », *BLOOD Res.*, vol. 56, n° S1, p. S5-S16, avr. 2021, doi: 10.5045/br.2021.2020321.
- [182] A. M. Hunter, L. Zhang, et E. Padron, « Current Management and Recent Advances in the Treatment of Chronic Myelomonocytic Leukemia », *Curr. Treat. Options Oncol.*, vol. 19, n° 12, p. 67, oct. 2018, doi: 10.1007/s11864-018-0581-6.
- [183] E. Wattel *et al.*, « A randomized trial of hydroxyurea versus VP16 in adult chronic myelomonocytic leukemia. Groupe Français des Myélodysplasies and European CMML Group », *Blood*, vol. 88, n° 7, p. 2480-2487, oct. 1996.
- [184] A. Unnikrishnan *et al.*, « Integrative Genomics Identifies the Molecular Basis of Resistance to Azacitidine Therapy in Myelodysplastic Syndromes », *Cell Rep.*, vol. 20, n° 3, p. 572-585, juill. 2017, doi: 10.1016/j.celrep.2017.06.067.
- [185] M. A. Sekeres *et al.*, « Randomized Phase II Study of Azacitidine Alone or in Combination With Lenalidomide or With Vorinostat in Higher-Risk Myelodysplastic Syndromes and Chronic Myelomonocytic Leukemia: North American Intergroup Study SWOG S1117 », *J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol.*, vol. 35, n° 24, p. 2745-2753, août 2017, doi: 10.1200/JCO.2015.66.2510.
- [186] M. Kenealy *et al.*, « Azacitidine with or without lenalidomide in higher risk myelodysplastic syndrome & low blast acute myeloid leukemia », *Haematologica*, vol. 104, n° 4, p. 700-709, avr. 2019, doi: 10.3324/haematol.2018.201152.
- [187] A. Bazinet *et al.*, « Azacitidine plus venetoclax in patients with high-risk myelodysplastic syndromes or chronic myelomonocytic leukaemia: phase 1 results of a single-centre, dose-escalation, dose-expansion, phase 1-2 study », *Lancet Haematol.*, vol. 9, n° 10, p. e756-e765, oct. 2022, doi: 10.1016/S2352-3026(22)00216-2.
- [188] H. Eissa *et al.*, « Allogeneic hematopoietic cell transplantation for chronic myelomonocytic leukemia: relapse-free survival is determined by karyotype and comorbidities », *Biol. Blood Marrow Transplant. J. Am. Soc. Blood Marrow Transplant.*, vol. 17, n° 6, p. 908-915, juin 2011, doi: 10.1016/j.bbmt.2010.09.018.
- [189] H. D. Liu *et al.*, « Allogeneic Hematopoietic Cell Transplantation for Adult Chronic Myelomonocytic Leukemia », *Biol. Blood Marrow Transplant. J. Am. Soc. Blood Marrow Transplant.*, vol. 23, n° 5, p. 767-775, mai 2017, doi: 10.1016/j.bbmt.2017.01.078.
- [190] K. Moran-Crusio *et al.*, « Tet2 loss leads to increased hematopoietic stem cell self-renewal and myeloid transformation », *Cancer Cell*, vol. 20, n° 1, p. 11-24, juill. 2011, doi: 10.1016/j.ccr.2011.06.001.
- [191] Z. Li *et al.*, « Deletion of Tet2 in mice leads to dysregulated hematopoietic stem cells and subsequent development of myeloid malignancies », *Blood*, vol. 118, n° 17, p. 4509-4518, oct. 2011, doi: 10.1182/blood-2010-12-325241.
- [192] C. Parikh, R. Subrahmanyam, et R. Ren, « Oncogenic NRAS, KRAS, and HRAS exhibit different leukemogenic potentials in mice », *Cancer Res.*, vol. 67, n° 15, p. 7139-7146, août 2007, doi: 10.1158/0008-5472.CAN-07-0778.
- [193] V. Caraffini *et al.*, « Loss of RAF kinase inhibitor protein is involved in myelomonocytic differentiation and aggravates RAS-driven myeloid leukemogenesis », *Haematologica*, vol. 105, n° 2, p. 375-386, 2020, doi: 10.3324/haematol.2018.209650.
- [194] B. H. Lee *et al.*, « FLT3 mutations confer enhanced proliferation and survival properties to multipotent progenitors in a murine model of chronic myelomonocytic

- leukemia », *Cancer Cell*, vol. 12, n° 4, p. 367-380, oct. 2007, doi: 10.1016/j.ccr.2007.08.031.
- [195] C. B. Goodwin *et al.*, « PI3K p110 δ uniquely promotes gain-of-function Shp2-induced GM-CSF hypersensitivity in a model of JMML », *Blood*, vol. 123, n° 18, p. 2838-2842, mai 2014, doi: 10.1182/blood-2013-10-535104.
- [196] K. Lv *et al.*, « CBL family E3 ubiquitin ligases control JAK2 ubiquitination and stability in hematopoietic stem cells and myeloid malignancies », *Genes Dev.*, vol. 31, n° 10, p. 1007-1023, mai 2017, doi: 10.1101/gad.297135.117.
- [197] P. Zhang *et al.*, « Chromatin regulator Asx1 loss and Nf1 haploinsufficiency cooperate to accelerate myeloid malignancy », *J. Clin. Invest.*, vol. 128, n° 12, p. 5383-5398, déc. 2018, doi: 10.1172/JCI121366.
- [198] Y. Wei *et al.*, « KDM6B overexpression activates innate immune signaling and impairs hematopoiesis in mice », *Blood Adv.*, vol. 2, n° 19, p. 2491-2504, oct. 2018, doi: 10.1182/bloodadvances.2018024166.
- [199] M.-Y. Wu, K. W. Eldin, et A. L. Beaudet, « Identification of chromatin remodeling genes *Arid4a* and *Arid4b* as leukemia suppressor genes », *J. Natl. Cancer Inst.*, vol. 100, n° 17, p. 1247-1259, sept. 2008, doi: 10.1093/jnci/djn253.
- [200] A. Yoshimi *et al.*, « Robust patient-derived xenografts of MDS/MPN overlap syndromes capture the unique characteristics of CMML and JMML », *Blood*, vol. 130, n° 4, p. 397-407, juill. 2017, doi: 10.1182/blood-2017-01-763219.
- [201] A. Beke *et al.*, « Multilayer intraclonal heterogeneity in chronic myelomonocytic leukemia », *Haematologica*, vol. 105, n° 1, p. 112-123, janv. 2020, doi: 10.3324/haematol.2018.208488.
- [202] F. Lyko, « The DNA methyltransferase family: a versatile toolkit for epigenetic regulation », *Nat. Rev. Genet.*, vol. 19, n° 2, p. 81-92, févr. 2018, doi: 10.1038/nrg.2017.80.
- [203] J. Du, L. M. Johnson, S. E. Jacobsen, et D. J. Patel, « DNA methylation pathways and their crosstalk with histone methylation », *Nat. Rev. Mol. Cell Biol.*, vol. 16, n° 9, p. 519-532, sept. 2015, doi: 10.1038/nrm4043.
- [204] S. Ito *et al.*, « Tet proteins can convert 5-methylcytosine to 5-formylcytosine and 5-carboxylcytosine », *Science*, vol. 333, n° 6047, p. 1300-1303, sept. 2011, doi: 10.1126/science.1210597.
- [205] M. Ko *et al.*, « Impaired hydroxylation of 5-methylcytosine in myeloid cancers with mutant TET2 », *Nature*, vol. 468, n° 7325, p. 839-843, déc. 2010, doi: 10.1038/nature09586.
- [206] E. R. Mardis *et al.*, « Recurring mutations found by sequencing an acute myeloid leukemia genome », *N. Engl. J. Med.*, vol. 361, n° 11, p. 1058-1066, sept. 2009, doi: 10.1056/NEJMoa0903840.
- [207] T. Haferlach *et al.*, « Landscape of genetic lesions in 944 patients with myelodysplastic syndromes », *Leukemia*, vol. 28, n° 2, p. 241-247, févr. 2014, doi: 10.1038/leu.2013.336.
- [208] E. Papaemmanuil *et al.*, « Clinical and biological implications of driver mutations in myelodysplastic syndromes », *Blood*, vol. 122, n° 22, p. 3616-3627; quiz 3699, nov. 2013, doi: 10.1182/blood-2013-08-518886.
- [209] L. Cimmino *et al.*, « TET1 is a tumor suppressor of hematopoietic malignancy », *Nat. Immunol.*, vol. 16, n° 6, p. 653-662, juin 2015, doi: 10.1038/ni.3148.

- [210] A. J. Pulikkottil *et al.*, « TET3 promotes AML growth and epigenetically regulates glucose metabolism and leukemic stem cell associated pathways », *Leukemia*, vol. 36, n° 2, p. 416-425, févr. 2022, doi: 10.1038/s41375-021-01390-3.
- [211] D. Schübeler, « Function and information content of DNA methylation », *Nature*, vol. 517, n° 7534, p. 321-326, janv. 2015, doi: 10.1038/nature14192.
- [212] E. Solary, O. A. Bernard, A. Tefferi, F. Fuks, et W. Vainchenker, « The Ten-Eleven Translocation-2 (TET2) gene in hematopoiesis and hematopoietic diseases », *Leukemia*, vol. 28, n° 3, p. 485-496, mars 2014, doi: 10.1038/leu.2013.337.
- [213] K. Shide *et al.*, « TET2 is essential for survival and hematopoietic stem cell homeostasis », *Leukemia*, vol. 26, n° 10, p. 2216-2223, oct. 2012, doi: 10.1038/leu.2012.94.
- [214] E. Pronier *et al.*, « Inhibition of TET2-mediated conversion of 5-methylcytosine to 5-hydroxymethylcytosine disturbs erythroid and granulomonocytic differentiation of human hematopoietic progenitors », *Blood*, vol. 118, n° 9, p. 2551-2555, sept. 2011, doi: 10.1182/blood-2010-12-324707.
- [215] F. Delhommeau *et al.*, « Mutation in *TET2* in Myeloid Cancers », *N. Engl. J. Med.*, vol. 360, n° 22, p. 2289-2301, mai 2009, doi: 10.1056/NEJMoa0810069.
- [216] F. Pan, O. Weeks, F.-C. Yang, et M. Xu, « The TET2 interactors and their links to hematological malignancies: TET2 Interactors to TET2 Loss-Mediated Hematological Malignancies », *IUBMB Life*, vol. 67, n° 6, p. 438-445, juin 2015, doi: 10.1002/iub.1389.
- [217] X. Jin *et al.*, « Oncogenic N-Ras and Tet2 haploinsufficiency collaborate to dysregulate hematopoietic stem and progenitor cells », *Blood Adv.*, vol. 2, n° 11, p. 1259-1271, juin 2018, doi: 10.1182/bloodadvances.2018017400.
- [218] S. J. Song *et al.*, « The oncogenic microRNA miR-22 targets the TET2 tumor suppressor to promote hematopoietic stem cell self-renewal and transformation », *Cell Stem Cell*, vol. 13, n° 1, p. 87-101, juill. 2013, doi: 10.1016/j.stem.2013.06.003.
- [219] O. Kosmider *et al.*, « TET2 mutation is an independent favorable prognostic factor in myelodysplastic syndromes (MDSs) », *Blood*, vol. 114, n° 15, p. 3285-3291, oct. 2009, doi: 10.1182/blood-2009-04-215814.
- [220] S. Weissmann *et al.*, « Landscape of TET2 mutations in acute myeloid leukemia », *Leukemia*, vol. 26, n° 5, p. 934-942, mai 2012, doi: 10.1038/leu.2011.326.
- [221] S. Jaiswal *et al.*, « Age-related clonal hematopoiesis associated with adverse outcomes », *N. Engl. J. Med.*, vol. 371, n° 26, p. 2488-2498, déc. 2014, doi: 10.1056/NEJMoa1408617.
- [222] C. M. Hirsch *et al.*, « Consequences of mutant TET2 on clonality and subclonal hierarchy », *Leukemia*, vol. 32, n° 8, p. 1751-1761, août 2018, doi: 10.1038/s41375-018-0150-9.
- [223] S. M. C. Langemeijer *et al.*, « Acquired mutations in TET2 are common in myelodysplastic syndromes », *Nat. Genet.*, vol. 41, n° 7, p. 838-842, juill. 2009, doi: 10.1038/ng.391.
- [224] O. Abdel-Wahab *et al.*, « Genetic characterization of TET1, TET2, and TET3 alterations in myeloid malignancies », *Blood*, vol. 114, n° 1, p. 144-147, juill. 2009, doi: 10.1182/blood-2009-03-210039.
- [225] R. Bejar *et al.*, « TET2 mutations predict response to hypomethylating agents in myelodysplastic syndrome patients », *Blood*, vol. 124, n° 17, p. 2705-2712, oct. 2014, doi: 10.1182/blood-2014-06-582809.

- [226] G. Coltro *et al.*, « Clinical, molecular, and prognostic correlates of number, type, and functional localization of TET2 mutations in chronic myelomonocytic leukemia (CMML)—a study of 1084 patients », *Leukemia*, vol. 34, n° 5, p. 1407-1421, mai 2020, doi: 10.1038/s41375-019-0690-7.
- [227] H. Awada *et al.*, « Invariant phenotype and molecular association of biallelic TET2 mutant myeloid neoplasia », *Blood Adv.*, vol. 3, n° 3, p. 339-349, févr. 2019, doi: 10.1182/bloodadvances.2018024216.
- [228] C. Chang, D. T. Simmons, M. A. Martin, et P. T. Mora, « Identification and partial characterization of new antigens from simian virus 40-transformed mouse cells », *J. Virol.*, vol. 31, n° 2, p. 463-471, août 1979, doi: 10.1128/JVI.31.2.463-471.1979.
- [229] D. P. Lane et L. V. Crawford, « T antigen is bound to a host protein in SV40-transformed cells », *Nature*, vol. 278, n° 5701, p. 261-263, mars 1979, doi: 10.1038/278261a0.
- [230] D. I. H. Linzer et A. J. Levine, « Characterization of a 54K Dalton cellular SV40 tumor antigen present in SV40-transformed cells and uninfected embryonal carcinoma cells », *Cell*, vol. 17, n° 1, p. 43-52, mai 1979, doi: 10.1016/0092-8674(79)90293-9.
- [231] A. J. Levine et M. Oren, « The first 30 years of p53: growing ever more complex », *Nat. Rev. Cancer*, vol. 9, n° 10, p. 749-758, oct. 2009, doi: 10.1038/nrc2723.
- [232] C. A. Finlay, P. W. Hinds, et A. J. Levine, « The p53 proto-oncogene can act as a suppressor of transformation », *Cell*, vol. 57, n° 7, p. 1083-1093, juin 1989, doi: 10.1016/0092-8674(89)90045-7.
- [233] P.-L. Chen, Y. Chen, R. Bookstein, et W.-H. Lee, « Genetic Mechanisms of Tumor Suppression by the Human p53 Gene », *Science*, vol. 250, n° 4987, p. 1576-1580, déc. 1990, doi: 10.1126/science.2274789.
- [234] J.-C. Bourdon, « p53 and its isoforms in cancer », *Br. J. Cancer*, vol. 97, n° 3, p. 277-282, août 2007, doi: 10.1038/sj.bjc.6603886.
- [235] O. W. McBride, D. Merry, et D. Givol, « The gene for human p53 cellular tumor antigen is located on chromosome 17 short arm (17p13) », *Proc. Natl. Acad. Sci. U. S. A.*, vol. 83, n° 1, p. 130-134, janv. 1986, doi: 10.1073/pnas.83.1.130.
- [236] H. Lee *et al.*, « Local structural elements in the mostly unstructured transcriptional activation domain of human p53 », *J. Biol. Chem.*, vol. 275, n° 38, p. 29426-29432, sept. 2000, doi: 10.1074/jbc.M003107200.
- [237] W. D. Funk, D. T. Pak, R. H. Karas, W. E. Wright, et J. W. Shay, « A transcriptionally active DNA-binding site for human p53 protein complexes », *Mol. Cell. Biol.*, vol. 12, n° 6, p. 2866-2871, juin 1992, doi: 10.1128/mcb.12.6.2866-2871.1992.
- [238] N. Baptiste, P. Friedlander, X. Chen, et C. Prives, « The proline-rich domain of p53 is required for cooperation with anti-neoplastic agents to promote apoptosis of tumor cells », *Oncogene*, vol. 21, n° 1, p. 9-21, janv. 2002, doi: 10.1038/sj.onc.1205015.
- [239] J. H. Bayle, B. Elenbaas, et A. J. Levine, « The carboxyl-terminal domain of the p53 protein regulates sequence-specific DNA binding through its nonspecific nucleic acid-binding activity », *Proc. Natl. Acad. Sci. U. S. A.*, vol. 92, n° 12, p. 5729-5733, juin 1995, doi: 10.1073/pnas.92.12.5729.
- [240] S. Y. Shieh, M. Ikeda, Y. Taya, et C. Prives, « DNA damage-induced phosphorylation of p53 alleviates inhibition by MDM2 », *Cell*, vol. 91, n° 3, p. 325-334, oct. 1997, doi: 10.1016/s0092-8674(00)80416-x.
- [241] S. Chuikov *et al.*, « Regulation of p53 activity through lysine methylation », *Nature*, vol. 432, n° 7015, p. 353-360, nov. 2004, doi: 10.1038/nature03117.

- [242] J. K. Kurash *et al.*, « Methylation of p53 by Set7/9 mediates p53 acetylation and activity in vivo », *Mol. Cell*, vol. 29, n° 3, p. 392-400, févr. 2008, doi: 10.1016/j.molcel.2007.12.025.
- [243] L. Liu *et al.*, « p53 sites acetylated in vitro by PCAF and p300 are acetylated in vivo in response to DNA damage », *Mol. Cell. Biol.*, vol. 19, n° 2, p. 1202-1209, févr. 1999, doi: 10.1128/MCB.19.2.1202.
- [244] K. Sakaguchi *et al.*, « DNA damage activates p53 through a phosphorylation-acetylation cascade », *Genes Dev.*, vol. 12, n° 18, p. 2831-2841, sept. 1998, doi: 10.1101/gad.12.18.2831.
- [245] K. Linke, P. D. Mace, C. A. Smith, D. L. Vaux, J. Silke, et C. L. Day, « Structure of the MDM2/MDMX RING domain heterodimer reveals dimerization is required for their ubiquitylation in trans », *Cell Death Differ.*, vol. 15, n° 5, p. 841-848, mai 2008, doi: 10.1038/sj.cdd.4402309.
- [246] D. A. Freedman, L. Wu, et A. J. Levine, « Functions of the MDM2 oncoprotein », *Cell. Mol. Life Sci. CMLS*, vol. 55, n° 1, p. 96-107, janv. 1999, doi: 10.1007/s000180050273.
- [247] A. M. Bode et Z. Dong, « Post-translational modification of p53 in tumorigenesis », *Nat. Rev. Cancer*, vol. 4, n° 10, p. 793-805, oct. 2004, doi: 10.1038/nrc1455.
- [248] D. R. Green et G. Kroemer, « Cytoplasmic functions of the tumour suppressor p53 », *Nature*, vol. 458, n° 7242, p. 1127-1130, avr. 2009, doi: 10.1038/nature07986.
- [249] G. Shaulsky, N. Goldfinger, A. Ben-Ze'ev, et V. Rotter, « Nuclear accumulation of p53 protein is mediated by several nuclear localization signals and plays a role in tumorigenesis », *Mol. Cell. Biol.*, vol. 10, n° 12, p. 6565-6577, déc. 1990, doi: 10.1128/mcb.10.12.6565-6577.1990.
- [250] M. F. Lavin et N. Gueven, « The complexity of p53 stabilization and activation », *Cell Death Differ.*, vol. 13, n° 6, p. 941-950, juin 2006, doi: 10.1038/sj.cdd.4401925.
- [251] M. L. Agarwal, A. Agarwal, W. R. Taylor, et G. R. Stark, « p53 controls both the G2/M and the G1 cell cycle checkpoints and mediates reversible growth arrest in human fibroblasts », *Proc. Natl. Acad. Sci. U. S. A.*, vol. 92, n° 18, p. 8493-8497, août 1995, doi: 10.1073/pnas.92.18.8493.
- [252] J. Martín-Caballero, J. M. Flores, P. García-Palencia, et M. Serrano, « Tumor susceptibility of p21(Waf1/Cip1)-deficient mice », *Cancer Res.*, vol. 61, n° 16, p. 6234-6238, août 2001.
- [253] C. Deng, P. Zhang, J. W. Harper, S. J. Elledge, et P. Leder, « Mice lacking p21CIP1/WAF1 undergo normal development, but are defective in G1 checkpoint control », *Cell*, vol. 82, n° 4, p. 675-684, août 1995, doi: 10.1016/0092-8674(95)90039-x.
- [254] Y. Shen et E. White, « p53-dependent apoptosis pathways », *Adv. Cancer Res.*, vol. 82, p. 55-84, 2001, doi: 10.1016/s0065-230x(01)82002-9.
- [255] H. Sardhara, « Apoptosis : Mechanism and Morphologic Changes ». Consulté le: 3 août 2023. [En ligne]. Disponible sur: <https://www.biotechfront.com/2021/01/mechanism-and-morphologic-changes.html>
- [256] G. T. Bommer *et al.*, « p53-mediated activation of miRNA34 candidate tumor-suppressor genes », *Curr. Biol. CB*, vol. 17, n° 15, p. 1298-1307, août 2007, doi: 10.1016/j.cub.2007.06.068.
- [257] B. J. Aubrey, G. L. Kelly, A. Janic, M. J. Herold, et A. Strasser, « How does p53 induce apoptosis and how does this relate to p53-mediated tumour suppression? », *Cell Death Differ.*, vol. 25, n° 1, p. 104-113, janv. 2018, doi: 10.1038/cdd.2017.169.

- [258] J. E. Chipuk et D. R. Green, « Dissecting p53-dependent apoptosis », *Cell Death Differ.*, vol. 13, n° 6, p. 994-1002, juin 2006, doi: 10.1038/sj.cdd.4401908.
- [259] M. Mihara *et al.*, « p53 has a direct apoptogenic role at the mitochondria », *Mol. Cell*, vol. 11, n° 3, p. 577-590, mars 2003, doi: 10.1016/s1097-2765(03)00050-9.
- [260] M. Mijit, V. Caracciolo, A. Melillo, F. Amicarelli, et A. Giordano, « Role of p53 in the Regulation of Cellular Senescence », *Biomolecules*, vol. 10, n° 3, p. 420, mars 2020, doi: 10.3390/biom10030420.
- [261] Y. Abuetafah *et al.*, « DNA damage response revisited: the p53 family and its regulators provide endless cancer therapy opportunities », *Exp. Mol. Med.*, vol. 54, n° 10, p. 1658-1669, oct. 2022, doi: 10.1038/s12276-022-00863-4.
- [262] R. M. Kortlever, P. J. Higgins, et R. Bernards, « Plasminogen activator inhibitor-1 is a critical downstream target of p53 in the induction of replicative senescence », *Nat. Cell Biol.*, vol. 8, n° 8, p. 877-884, août 2006, doi: 10.1038/ncb1448.
- [263] A. L. Hughes, L. Gollapudi, T. L. Sladek, et K. E. Neet, « Mediation of nerve growth factor-driven cell cycle arrest in PC12 cells by p53. Simultaneous differentiation and proliferation subsequent to p53 functional inactivation », *J. Biol. Chem.*, vol. 275, n° 48, p. 37829-37837, déc. 2000, doi: 10.1074/jbc.M003146200.
- [264] J. Zhang, W. Yan, et X. Chen, « p53 is required for nerve growth factor-mediated differentiation of PC12 cells via regulation of TrkA levels », *Cell Death Differ.*, vol. 13, n° 12, p. 2118-2128, déc. 2006, doi: 10.1038/sj.cdd.4401972.
- [265] H. Zheng *et al.*, « p53 and Pten control neural and glioma stem/progenitor cell renewal and differentiation », *Nature*, vol. 455, n° 7216, p. 1129-1133, oct. 2008, doi: 10.1038/nature07443.
- [266] X. Wang *et al.*, « p53 functions as a negative regulator of osteoblastogenesis, osteoblast-dependent osteoclastogenesis, and bone remodeling », *J. Cell Biol.*, vol. 172, n° 1, p. 115-125, janv. 2006, doi: 10.1083/jcb.200507106.
- [267] C. J. Lengner *et al.*, « Osteoblast differentiation and skeletal development are regulated by Mdm2-p53 signaling », *J. Cell Biol.*, vol. 172, n° 6, p. 909-921, mars 2006, doi: 10.1083/jcb.200508130.
- [268] Y. Tamir et E. Bengal, « p53 protein is activated during muscle differentiation and participates with MyoD in the transcription of muscle creatine kinase gene », *Oncogene*, vol. 17, n° 3, p. 347-356, juill. 1998, doi: 10.1038/sj.onc.1201929.
- [269] C. Kandoth *et al.*, « Mutational landscape and significance across 12 major cancer types », *Nature*, vol. 502, n° 7471, p. 333-339, oct. 2013, doi: 10.1038/nature12634.
- [270] W. A. Freed-Pastor et C. Prives, « Mutant p53: one name, many proteins », *Genes Dev.*, vol. 26, n° 12, p. 1268-1286, juin 2012, doi: 10.1101/gad.190678.112.
- [271] M. Olivier, M. Hollstein, et P. Hainaut, « TP53 mutations in human cancers: origins, consequences, and clinical use », *Cold Spring Harb. Perspect. Biol.*, vol. 2, n° 1, p. a001008, janv. 2010, doi: 10.1101/cshperspect.a001008.
- [272] J.-W. Pan *et al.*, « The molecular landscape of Asian breast cancers reveals clinically relevant population-specific differences », *Nat. Commun.*, vol. 11, n° 1, p. 6433, déc. 2020, doi: 10.1038/s41467-020-20173-5.
- [273] P. A. J. Muller et K. H. Vousden, « Mutant p53 in Cancer: New Functions and Therapeutic Opportunities », *Cancer Cell*, vol. 25, n° 3, p. 304-317, mars 2014, doi: 10.1016/j.ccr.2014.01.021.
- [274] P. A. J. Muller et K. H. Vousden, « p53 mutations in cancer », *Nat. Cell Biol.*, vol. 15, n° 1, p. 2-8, janv. 2013, doi: 10.1038/ncb2641.

- [275] R. Brosh et V. Rotter, « When mutants gain new powers: news from the mutant p53 field », *Nat. Rev. Cancer*, vol. 9, n° 10, p. 701-713, oct. 2009, doi: 10.1038/nrc2693.
- [276] D. Wolf, N. Harris, et V. Rotter, « Reconstitution of p53 expression in a nonproducer Ab-MuLV-transformed cell line by transfection of a functional p53 gene », *Cell*, vol. 38, n° 1, p. 119-126, août 1984, doi: 10.1016/0092-8674(84)90532-4.
- [277] G. Shaulsky, N. Goldfinger, et V. Rotter, « Alterations in tumor development in vivo mediated by expression of wild type or mutant p53 proteins », *Cancer Res.*, vol. 51, n° 19, p. 5232-5237, oct. 1991.
- [278] G. Blandino, A. J. Levine, et M. Oren, « Mutant p53 gain of function: differential effects of different p53 mutants on resistance of cultured cells to chemotherapy », *Oncogene*, vol. 18, n° 2, p. 477-485, janv. 1999, doi: 10.1038/sj.onc.1202314.
- [279] C. Lu et W. S. El-Deiry, « Targeting p53 for enhanced radio- and chemo-sensitivity », *Apoptosis Int. J. Program. Cell Death*, vol. 14, n° 4, p. 597-606, avr. 2009, doi: 10.1007/s10495-009-0330-1.
- [280] K. V. Chin, K. Ueda, I. Pastan, et M. M. Gottesman, « Modulation of activity of the promoter of the human MDR1 gene by Ras and p53 », *Science*, vol. 255, n° 5043, p. 459-462, janv. 1992, doi: 10.1126/science.1346476.
- [281] J. A. Bush et G. Li, « Cancer chemoresistance: the relationship between p53 and multidrug transporters », *Int. J. Cancer*, vol. 98, n° 3, p. 323-330, mars 2002, doi: 10.1002/ijc.10226.
- [282] C. Gaiddon, M. Lokshin, J. Ahn, T. Zhang, et C. Prives, « A subset of tumor-derived mutant forms of p53 down-regulate p63 and p73 through a direct interaction with the p53 core domain », *Mol. Cell. Biol.*, vol. 21, n° 5, p. 1874-1887, mars 2001, doi: 10.1128/MCB.21.5.1874-1887.2001.
- [283] Y. Li et C. Prives, « Are interactions with p63 and p73 involved in mutant p53 gain of oncogenic function? », *Oncogene*, vol. 26, n° 15, p. 2220-2225, avr. 2007, doi: 10.1038/sj.onc.1210311.
- [284] Y. Tomita *et al.*, « WT p53, but not tumor-derived mutants, bind to Bcl2 via the DNA binding domain and induce mitochondrial permeabilization », *J. Biol. Chem.*, vol. 281, n° 13, p. 8600-8606, mars 2006, doi: 10.1074/jbc.M507611200.
- [285] O. C. Rodriguez *et al.*, « Dietary downregulation of mutant p53 levels via glucose restriction: mechanisms and implications for tumor therapy », *Cell Cycle Georget. Tex.*, vol. 11, n° 23, p. 4436-4446, déc. 2012, doi: 10.4161/cc.22778.
- [286] E. Morselli *et al.*, « p53 inhibits autophagy by interacting with the human ortholog of yeast Atg17, RB1CC1/FIP200 », *Cell Cycle*, vol. 10, n° 16, p. 2763-2769, août 2011, doi: 10.4161/cc.10.16.16868.
- [287] G. Shaulsky, N. Goldfinger, A. Peled, et V. Rotter, « Involvement of wild-type p53 in pre-B-cell differentiation in vitro », *Proc. Natl. Acad. Sci. U. S. A.*, vol. 88, n° 20, p. 8982-8986, oct. 1991, doi: 10.1073/pnas.88.20.8982.
- [288] S. Peller *et al.*, « The onset of p53-dependent apoptosis plays a role in terminal differentiation of human normoblasts », *Oncogene*, vol. 22, n° 30, p. 4648-4655, juill. 2003, doi: 10.1038/sj.onc.1206541.
- [289] S. Le Goff *et al.*, « p53 activation during ribosome biogenesis regulates normal erythroid differentiation », *Blood*, vol. 137, n° 1, p. 89-102, janv. 2021, doi: 10.1182/blood.2019003439.
- [290] S. Soddu *et al.*, « Wild-type p53 gene expression induces granulocytic differentiation of HL-60 cells », *Blood*, vol. 83, n° 8, p. 2230-2237, avr. 1994.

- [291] D. Banerjee *et al.*, « Transfection of wild-type but not mutant p53 induces early monocytic differentiation in HL60 cells and increases their sensitivity to stress », *Cell Growth Differ. Mol. Biol. J. Am. Assoc. Cancer Res.*, vol. 6, n° 11, p. 1405-1413, nov. 1995.
- [292] D. Ronen, D. Schwartz, Y. Teitz, N. Goldfinger, et V. Rotter, « Induction of HL-60 cells to undergo apoptosis is determined by high levels of wild-type p53 protein whereas differentiation of the cells is mediated by lower p53 levels », *Cell Growth Differ. Mol. Biol. J. Am. Assoc. Cancer Res.*, vol. 7, n° 1, p. 21-30, janv. 1996.
- [293] Y. Liu *et al.*, « p53 Regulates Hematopoietic Stem Cell Quiescence », *Cell Stem Cell*, vol. 4, n° 1, p. 37-48, janv. 2009, doi: 10.1016/j.stem.2008.11.006.
- [294] M. TeKippe, D. E. Harrison, et J. Chen, « Expansion of hematopoietic stem cell phenotype and activity in Trp53-null mice », *Exp. Hematol.*, vol. 31, n° 6, p. 521-527, juin 2003, doi: 10.1016/s0301-472x(03)00072-9.
- [295] C. J. Kemp, T. Wheldon, et A. Balmain, « p53-deficient mice are extremely susceptible to radiation-induced tumorigenesis », *Nat. Genet.*, vol. 8, n° 1, p. 66-69, sept. 1994, doi: 10.1038/ng0994-66.
- [296] Z. Zhao *et al.*, « p53 loss promotes acute myeloid leukemia by enabling aberrant self-renewal », *Genes Dev.*, vol. 24, n° 13, p. 1389-1402, juill. 2010, doi: 10.1101/gad.1940710.
- [297] A. A. Sablina, A. V. Budanov, G. V. Ilyinskaya, L. S. Agapova, J. E. Kravchenko, et P. M. Chumakov, « The antioxidant function of the p53 tumor suppressor », *Nat. Med.*, vol. 11, n° 12, p. 1306-1313, déc. 2005, doi: 10.1038/nm1320.
- [298] H. Jung *et al.*, « TXNIP Maintains the Hematopoietic Cell Pool by Switching the Function of p53 under Oxidative Stress », *Cell Metab.*, vol. 18, n° 1, p. 75-85, juill. 2013, doi: 10.1016/j.cmet.2013.06.002.
- [299] S. V. Boregowda, V. Krishnappa, J. Strivelli, C. L. Haga, C. N. Booker, et D. G. Phinney, « Basal p53 expression is indispensable for mesenchymal stem cell integrity », *Cell Death Differ.*, vol. 25, n° 4, p. 679-692, mars 2018, doi: 10.1038/s41418-017-0004-4.
- [300] I. Ringshausen, C. C. O'Shea, A. J. Finch, L. B. Swigart, et G. I. Evan, « Mdm2 is critically and continuously required to suppress lethal p53 activity in vivo », *Cancer Cell*, vol. 10, n° 6, p. 501-514, déc. 2006, doi: 10.1016/j.ccr.2006.10.010.
- [301] M. B. Kastan *et al.*, « Levels of p53 Protein Increase with Maturation in Human Hematopoietic Cells¹ », *Cancer Res.*, vol. 51, n° 16, p. 4279-4286, août 1991.
- [302] L. Busque, M. Buscarlet, L. Mollica, et R. L. Levine, « Concise Review: Age-Related Clonal Hematopoiesis: Stem Cells Tempting the Devil », *Stem Cells Dayt. Ohio*, vol. 36, n° 9, p. 1287-1294, sept. 2018, doi: 10.1002/stem.2845.
- [303] M. Xie *et al.*, « Age-related mutations associated with clonal hematopoietic expansion and malignancies », *Nat. Med.*, vol. 20, n° 12, p. 1472-1478, déc. 2014, doi: 10.1038/nm.3733.
- [304] G. Genovese *et al.*, « Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence », *N. Engl. J. Med.*, vol. 371, n° 26, p. 2477-2487, déc. 2014, doi: 10.1056/NEJMoa1409405.
- [305] F. Zink *et al.*, « Clonal hematopoiesis, with and without candidate driver mutations, is common in the elderly », *Blood*, vol. 130, n° 6, p. 742-752, août 2017, doi: 10.1182/blood-2017-02-769869.

- [306] C. C. Coombs *et al.*, « Therapy-Related Clonal Hematopoiesis in Patients with Non-hematologic Cancers Is Common and Associated with Adverse Clinical Outcomes », *Cell Stem Cell*, vol. 21, n° 3, p. 374-382.e4, sept. 2017, doi: 10.1016/j.stem.2017.07.010.
- [307] S. Chen *et al.*, « Mutant p53 drives clonal hematopoiesis through modulating epigenetic pathway », *Nat. Commun.*, vol. 10, n° 1, p. 5649, déc. 2019, doi: 10.1038/s41467-019-13542-2.
- [308] S. Peller et V. Rotter, « TP53 in hematological cancer: low incidence of mutations with significant clinical relevance », *Hum. Mutat.*, vol. 21, n° 3, p. 277-284, mars 2003, doi: 10.1002/humu.10190.
- [309] The European Reference Network GENTURIS, T. Frebourg, S. Bajalica Lagercrantz, C. Oliveira, R. Magenheim, et D. G. Evans, « Guidelines for the Li–Fraumeni and heritable TP53-related cancer syndromes », *Eur. J. Hum. Genet.*, vol. 28, n° 10, p. 1379-1386, oct. 2020, doi: 10.1038/s41431-020-0638-4.
- [310] S. Srivastava, Z. Q. Zou, K. Pirollo, W. Blattner, et E. H. Chang, « Germ-line transmission of a mutated p53 gene in a cancer-prone family with Li-Fraumeni syndrome », *Nature*, vol. 348, n° 6303, p. 747-749, déc. 1990, doi: 10.1038/348747a0.
- [311] V. Grossmann *et al.*, « A novel hierarchical prognostic model of AML solely based on molecular mutations », *Blood*, vol. 120, n° 15, p. 2963-2972, oct. 2012, doi: 10.1182/blood-2012-03-419622.
- [312] F. G. Rücker *et al.*, « TP53 alterations in acute myeloid leukemia with complex karyotype correlate with specific copy number alterations, monosomal karyotype, and dismal outcome », *Blood*, vol. 119, n° 9, p. 2114-2121, mars 2012, doi: 10.1182/blood-2011-08-375758.
- [313] Z. Y. Xu-Monette *et al.*, « Dysfunction of the TP53 tumor suppressor gene in lymphoid malignancies », *Blood*, vol. 119, n° 16, p. 3668-3683, avr. 2012, doi: 10.1182/blood-2011-11-366062.
- [314] H. Seifert *et al.*, « The prognostic impact of 17p (p53) deletion in 2272 adults with acute myeloid leukemia », *Leukemia*, vol. 23, n° 4, p. 656-663, avr. 2009, doi: 10.1038/leu.2008.375.
- [315] H. Döhner *et al.*, « Genomic Aberrations and Survival in Chronic Lymphocytic Leukemia », *N. Engl. J. Med.*, vol. 343, n° 26, p. 1910-1916, déc. 2000, doi: 10.1056/NEJM200012283432602.
- [316] A. Stengel *et al.*, « TP53 mutations occur in 15.7% of ALL and are associated with MYC-rearrangement, low hypodiploidy, and a poor prognosis », *Blood*, vol. 124, n° 2, p. 251-258, juill. 2014, doi: 10.1182/blood-2014-02-558833.
- [317] S. Xiong *et al.*, « Spontaneous tumorigenesis in mice overexpressing the p53-negative regulator Mdm4 », *Cancer Res.*, vol. 70, n° 18, p. 7148-7154, sept. 2010, doi: 10.1158/0008-5472.CAN-10-1457.
- [318] S. N. Jones, A. R. Hancock, H. Vogel, L. A. Donehower, et A. Bradley, « Overexpression of Mdm2 in mice reveals a p53-independent role for Mdm2 in tumorigenesis », *Proc. Natl. Acad. Sci. U. S. A.*, vol. 95, n° 26, p. 15608-15612, déc. 1998, doi: 10.1073/pnas.95.26.15608.
- [319] M. Gurney *et al.*, « Somatic TP53 single nucleotide variants, indels and copy number alterations in chronic myelomonocytic leukemia (CMML) », *Leukemia*, juill. 2023, doi: 10.1038/s41375-023-01964-3.

- [320] Q. Zhang *et al.*, « Tet2 is required to resolve inflammation by recruiting Hdac2 to specifically repress IL-6 », *Nature*, vol. 525, n° 7569, p. 389-393, sept. 2015, doi: 10.1038/nature15252.
- [321] L. Lv *et al.*, « Vpr Targets TET2 for Degradation by CRL4VprBP E3 Ligase to Sustain IL-6 Expression and Enhance HIV-1 Replication », *Mol. Cell*, vol. 70, n° 5, p. 961-970.e5, juin 2018, doi: 10.1016/j.molcel.2018.05.007.
- [322] B. Cong, Q. Zhang, et X. Cao, « The function and regulation of TET2 in innate immunity and inflammation », *Protein Cell*, vol. 12, n° 3, p. 165-173, mars 2021, doi: 10.1007/s13238-020-00796-6.
- [323] A. H. Cull, B. Snetsinger, R. Buckstein, R. A. Wells, et M. J. Rauh, « Tet2 restrains inflammatory gene expression in macrophages », *Exp. Hematol.*, vol. 55, p. 56-70.e13, nov. 2017, doi: 10.1016/j.exphem.2017.08.001.
- [324] J. Lugin *et al.*, « Histone deacetylase inhibitors repress macrophage migration inhibitory factor (MIF) expression by targeting MIF gene transcription through a local chromatin deacetylation », *Biochim. Biophys. Acta*, vol. 1793, n° 11, p. 1749-1758, nov. 2009, doi: 10.1016/j.bbamcr.2009.09.007.
- [325] T. Kasama, K. Ohtsuka, M. Sato, R. Takahashi, K. Wakabayashi, et K. Kobayashi, « Macrophage migration inhibitory factor: a multifunctional cytokine in rheumatic diseases », *Arthritis*, vol. 2010, p. 106202, 2010, doi: 10.1155/2010/106202.
- [326] Y. Al-Abed et S. VanPatten, « MIF as a disease target: ISO-1 as a proof-of-concept therapeutic », *Future Med. Chem.*, vol. 3, n° 1, p. 45-63, janv. 2011, doi: 10.4155/fmc.10.281.
- [327] B. E. Lippitz, « Cytokine patterns in patients with cancer: a systematic review », *Lancet Oncol.*, vol. 14, n° 6, p. e218-e228, mai 2013, doi: 10.1016/S1470-2045(12)70582-X.
- [328] J. Fukuzawa *et al.*, « Contribution of Macrophage Migration Inhibitory Factor to Extracellular Signal-regulated Kinase Activation by Oxidative Stress in Cardiomyocytes », *J. Biol. Chem.*, vol. 277, n° 28, p. 24889-24895, juill. 2002, doi: 10.1074/jbc.M112054200.
- [329] L. Leng *et al.*, « MIF Signal Transduction Initiated by Binding to CD74 », *J. Exp. Med.*, vol. 197, n° 11, p. 1467-1476, juin 2003, doi: 10.1084/jem.20030286.
- [330] D. Mahalingam *et al.*, « Phase I study of imalumab (BAX69), a fully human recombinant antioxidized macrophage migration inhibitory factor antibody in advanced solid tumours », *Br. J. Clin. Pharmacol.*, vol. 86, n° 9, p. 1836-1848, sept. 2020, doi: 10.1111/bcp.14289.
- [331] D. Heinrichs *et al.*, « Macrophage migration inhibitory factor (MIF) exerts antifibrotic effects in experimental liver fibrosis via CD74 », *Proc. Natl. Acad. Sci.*, vol. 108, n° 42, p. 17444-17449, oct. 2011, doi: 10.1073/pnas.1107023108.
- [332] K. L. Meyer-Siegler, K. A. Iczkowski, L. Leng, R. Bucala, et P. L. Vera, « Inhibition of Macrophage Migration Inhibitory Factor or Its Receptor (CD74) Attenuates Growth and Invasion of DU-145 Prostate Cancer Cells », *J. Immunol.*, vol. 177, n° 12, p. 8730-8739, déc. 2006, doi: 10.4049/jimmunol.177.12.8730.
- [333] T. Calandra *et al.*, « Protection from septic shock by neutralization of macrophage migration inhibitory factor », *Nat. Med.*, vol. 6, n° 2, p. 164-170, févr. 2000, doi: 10.1038/72262.

- [334] M. Merk *et al.*, « The *D*-dopachrome tautomerase (*DDT*) gene product is a cytokine and functional homolog of macrophage migration inhibitory factor (MIF) », *Proc. Natl. Acad. Sci.*, vol. 108, n° 34, août 2011, doi: 10.1073/pnas.1102941108.
- [335] J. Pohl, U. B. Hendgen-Cotta, P. Stock, P. Luedike, et T. Rassaf, « Elevated MIF-2 levels predict mortality in critically ill patients », *J. Crit. Care*, vol. 40, p. 52-57, août 2017, doi: 10.1016/j.jcrc.2017.03.012.
- [336] D. J. Wallace, M. H. Weisman, W. A. Wegener, H. Horne, et D. M. Goldenberg, « THU0288 IMMUN-115 (Humanized Anti-CD74 Antibody) for Subcutaneous (SC) Administration: A Phase Ib Study in Patients with Systemic Lupus Erythematosus (SLE) », *Ann. Rheum. Dis.*, vol. 75, n° Suppl 2, p. 291.1-291, juin 2016, doi: 10.1136/annrheumdis-2016-eular.3310.
- [337] P. V. Tilstam *et al.*, « MIF but not MIF-2 recruits inflammatory macrophages in an experimental polymicrobial sepsis model », *J. Clin. Invest.*, vol. 131, n° 23, p. e127171, déc. 2021, doi: 10.1172/JCI127171.
- [338] M. Merk, R. A. Mitchell, S. Endres, et R. Bucala, « D-dopachrome tautomerase (D-DT or MIF-2): doubling the MIF cytokine family », *Cytokine*, vol. 59, n° 1, p. 10-17, juill. 2012, doi: 10.1016/j.cyto.2012.03.014.
- [339] E. R. Kasthuber et S. W. Lowe, « Putting p53 in Context », *Cell*, vol. 170, n° 6, p. 1062-1078, sept. 2017, doi: 10.1016/j.cell.2017.08.028.
- [340] F. Toledo et G. M. Wahl, « Regulating the p53 pathway: in vitro hypotheses, in vivo veritas », *Nat. Rev. Cancer*, vol. 6, n° 12, p. 909-923, déc. 2006, doi: 10.1038/nrc2012.
- [341] N. Kon, Y. Kobayashi, M. Li, C. L. Brooks, T. Ludwig, et W. Gu, « Inactivation of HAUSP in vivo modulates p53 function », *Oncogene*, vol. 29, n° 9, p. 1270-1279, mars 2010, doi: 10.1038/onc.2009.427.
- [342] S. Bang, S. Kaur, et M. Kurokawa, « Regulation of the p53 Family Proteins by the Ubiquitin Proteasomal Pathway », *Int. J. Mol. Sci.*, vol. 21, n° 1, p. 261, déc. 2019, doi: 10.3390/ijms21010261.
- [343] X. Zhang, E. Devany, M. R. Murphy, G. Glazman, M. Persaud, et F. E. Kleiman, « PARN deadenylase is involved in miRNA-dependent degradation of TP53 mRNA in mammalian cells », *Nucleic Acids Res.*, vol. 43, n° 22, p. 10925-10938, déc. 2015, doi: 10.1093/nar/gkv959.
- [344] J. Chen, K. Guo, et M. B. Kastan, « Interactions of nucleolin and ribosomal protein L26 (RPL26) in translational control of human p53 mRNA », *J. Biol. Chem.*, vol. 287, n° 20, p. 16467-16476, mai 2012, doi: 10.1074/jbc.M112.349274.
- [345] P. Nair, S. Muthukumar, S. F. Sells, S.-S. Han, V. P. Sukhatme, et V. M. Rangnekar, « Early Growth Response-1-dependent Apoptosis Is Mediated by p53 », *J. Biol. Chem.*, vol. 272, n° 32, p. 20131-20138, août 1997, doi: 10.1074/jbc.272.32.20131.
- [346] A. Stoddart *et al.*, « Haploinsufficiency of del(5q) genes, *Egr1* and *Apc*, cooperate with *Tp53* loss to induce acute myeloid leukemia in mice », *Blood*, vol. 123, n° 7, p. 1069-1078, févr. 2014, doi: 10.1182/blood-2013-07-517953.
- [347] R. P. Huang *et al.*, « Decreased *Egr-1* expression in human, mouse and rat mammary cells and tissues correlates with tumor formation », *Int. J. Cancer*, vol. 72, n° 1, p. 102-109, juill. 1997, doi: 10.1002/(sici)1097-0215(19970703)72:1<102::aid-ijc15>3.0.co;2-l.
- [348] T. Cooks, C. C. Harris, et M. Oren, « Caught in the cross fire: p53 in inflammation », *Carcinogenesis*, vol. 35, n° 8, p. 1680-1690, août 2014, doi: 10.1093/carcin/bgu134.

- [349] A. V. Gudkov, K. V. Gurova, et E. A. Komarova, « Inflammation and p53: A Tale of Two Stresses », *Genes Cancer*, vol. 2, n° 4, p. 503-516, avr. 2011, doi: 10.1177/1947601911409747.
- [350] M. Karin, « Nuclear factor- κ B in cancer development and progression », *Nature*, vol. 441, n° 7092, p. 431-436, mai 2006, doi: 10.1038/nature04870.
- [351] A. Mantovani, P. Allavena, A. Sica, et F. Balkwill, « Cancer-related inflammation », *Nature*, vol. 454, n° 7203, p. 436-444, juill. 2008, doi: 10.1038/nature07205.
- [352] G. Trinchieri, « Cancer and Inflammation: An Old Intuition with Rapidly Evolving New Concepts », *Annu. Rev. Immunol.*, vol. 30, n° 1, p. 677-706, avr. 2012, doi: 10.1146/annurev-immunol-020711-075008.
- [353] A. V. Gudkov et E. A. Komarova, « p53 and the Carcinogenicity of Chronic Inflammation », *Cold Spring Harb. Perspect. Med.*, vol. 6, n° 11, p. a026161, nov. 2016, doi: 10.1101/cshperspect.a026161.
- [354] L. A. Donehower *et al.*, « Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours », *Nature*, vol. 356, n° 6366, p. 215-221, mars 1992, doi: 10.1038/356215a0.
- [355] J. H. Madenspacher *et al.*, « p53 Integrates host defense and cell fate during bacterial pneumonia », *J. Exp. Med.*, vol. 210, n° 5, p. 891-904, mai 2013, doi: 10.1084/jem.20121674.
- [356] L. Marruecos *et al.*, « Single loss of a Trp53 allele triggers an increased oxidative, DNA damage and cytokine inflammatory responses through deregulation of I κ B α expression », *Cell Death Dis.*, vol. 12, n° 4, p. 359, avr. 2021, doi: 10.1038/s41419-021-03638-3.
- [357] J. Lugin, N. Rosenblatt-Velin, R. Parapanov, et L. Liaudet, « The role of oxidative stress during inflammatory processes », *Biol. Chem.*, vol. 395, n° 2, p. 203-230, févr. 2014, doi: 10.1515/hsz-2013-0241.
- [358] Y. Yang, A. V. Bazhin, J. Werner, et S. Karakhanova, « Reactive oxygen species in the immune system », *Int. Rev. Immunol.*, vol. 32, n° 3, p. 249-270, juin 2013, doi: 10.3109/08830185.2012.755176.
- [359] C. Y. Ok *et al.*, « TP53 mutation characteristics in therapy-related myelodysplastic syndromes and acute myeloid leukemia is similar to de novo diseases », *J. Hematol. Oncol. J Hematol Oncol*, vol. 8, n° 1, p. 45, déc. 2015, doi: 10.1186/s13045-015-0139-z.
- [360] P. Fenaux *et al.*, « P53 gene mutations in acute myeloid leukemia with 17p monosomy », *Blood*, vol. 78, n° 7, p. 1652-1657, oct. 1991, doi: 10.1182/blood.V78.7.1652.1652.
- [361] P. Fenaux *et al.*, « Mutations of the P53 gene in acute myeloid leukaemia », *Br. J. Haematol.*, vol. 80, n° 2, p. 178-183, févr. 1992, doi: 10.1111/j.1365-2141.1992.tb08897.x.
- [362] for the International Working Group for MDS Molecular Prognostic Committee *et al.*, « TP53 mutation status divides myelodysplastic syndromes with complex karyotypes into distinct prognostic subgroups », *Leukemia*, vol. 33, n° 7, p. 1747-1758, juill. 2019, doi: 10.1038/s41375-018-0351-2.
- [363] Z. Xing *et al.*, « Immature monocytes contribute to cardiopulmonary bypass-induced acute lung injury by generating inflammatory descendants », *Thorax*, vol. 72, n° 3, p. 245-255, mars 2017, doi: 10.1136/thoraxjnl-2015-208023.

- [364] Y. Cao *et al.*, « Phenotypic and functional alterations of monocyte subsets with aging », *Immun. Ageing*, vol. 19, n° 1, p. 63, déc. 2022, doi: 10.1186/s12979-022-00321-9.
- [365] M. Trizzino *et al.*, « EGR1 is a gatekeeper of inflammatory enhancers in human macrophages », *Sci. Adv.*, vol. 7, n° 3, p. eaaz8836, janv. 2021, doi: 10.1126/sciadv.aaz8836.
- [366] R. Rojo *et al.*, « Deletion of a Csf1r enhancer selectively impacts CSF1R expression and development of tissue macrophage populations », *Nat. Commun.*, vol. 10, n° 1, p. 3215, juill. 2019, doi: 10.1038/s41467-019-11053-8.
- [367] Y. P. Zhu, G. D. Thomas, et C. C. Hedrick, « 2014 Jeffrey M. Hoeg Award Lecture: Transcriptional Control of Monocyte Development », *Arterioscler. Thromb. Vasc. Biol.*, vol. 36, n° 9, p. 1722-1733, sept. 2016, doi: 10.1161/ATVBAHA.116.304054.
- [368] H. W. Ziegler-Heitbrock, « Definition of human blood monocytes », *J. Leukoc. Biol.*, vol. 67, n° 5, p. 603-606, mai 2000, doi: 10.1002/jlb.67.5.603.
- [369] F. V. Mello *et al.*, « Maturation-associated gene expression profiles along normal human bone marrow monopoiesis », *Br. J. Haematol.*, vol. 176, n° 3, p. 464-474, févr. 2017, doi: 10.1111/bjh.14467.
- [370] Y. Wang *et al.*, « Restoring expression of wild-type p53 suppresses tumor growth but does not cause tumor regression in mice with a p53 missense mutation », *J. Clin. Invest.*, vol. 121, n° 3, p. 893-904, mars 2011, doi: 10.1172/JCI44504.
- [371] C. P. Martins, L. Brown-Swigart, et G. I. Evan, « Modeling the Therapeutic Efficacy of p53 Restoration in Tumors », *Cell*, vol. 127, n° 7, p. 1323-1334, déc. 2006, doi: 10.1016/j.cell.2006.12.007.
- [372] M. A. Christophorou *et al.*, « Temporal dissection of p53 function in vitro and in vivo », *Nat. Genet.*, vol. 37, n° 7, p. 718-726, juill. 2005, doi: 10.1038/ng1572.
- [373] S. Pearson, H. Jia, et K. Kandachi, « China approves first gene therapy », *Nat. Biotechnol.*, vol. 22, n° 1, p. 3-4, janv. 2004, doi: 10.1038/nbt0104-3.
- [374] W.-W. Zhang *et al.*, « The First Approved Gene Therapy Product for Cancer Ad- p53 (Gendicine): 12 Years in the Clinic », *Hum. Gene Ther.*, vol. 29, n° 2, p. 160-179, févr. 2018, doi: 10.1089/hum.2017.218.
- [375] T. L. Lee *et al.*, « A novel nuclear factor-kappaB gene signature is differentially expressed in head and neck squamous cell carcinomas in association with TP53 status », *Clin. Cancer Res. Off. J. Am. Assoc. Cancer Res.*, vol. 13, n° 19, p. 5680-5691, oct. 2007, doi: 10.1158/1078-0432.CCR-07-0670.
- [376] K. V. Gurova *et al.*, « Small molecules that reactivate p53 in renal cell carcinoma reveal a NF-kappaB-dependent mechanism of p53 suppression in tumors », *Proc. Natl. Acad. Sci. U. S. A.*, vol. 102, n° 48, p. 17448-17453, nov. 2005, doi: 10.1073/pnas.0508888102.
- [377] M. M. Patnaik, « How I diagnose and treat chronic myelomonocytic leukemia », *Haematologica*, vol. 107, n° 7, p. 1503-1517, juill. 2022, doi: 10.3324/haematol.2021.279500.
- [378] R. Itzykson *et al.*, « Diagnosis and Treatment of Chronic Myelomonocytic Leukemias in Adults: Recommendations From the European Hematology Association and the European LeukemiaNet », *HemaSphere*, vol. 2, n° 6, p. e150, déc. 2018, doi: 10.1097/HS9.000000000000150.

- [379] K. Hientz, A. Mohr, D. Bhakta-Guha, et T. Efferth, « The role of p53 in cancer drug resistance and targeted chemotherapy », *Oncotarget*, vol. 8, n° 5, p. 8921-8946, janv. 2017, doi: 10.18632/oncotarget.13475.
- [380] X. Fang *et al.*, « p53 mediates hydroxyurea resistance in aneuploid cells of colon cancer », *Exp. Cell Res.*, vol. 376, n° 1, p. 39-48, mars 2019, doi: 10.1016/j.yexcr.2019.01.013.

XI. Annexes

Annexe n°1

Bencheikh L, Diop MK, Rivière J, Imanci A, Pierron G, Souquere S, Naimo A, Morabito M, Dussiot M, De Leeuw F, Lobry C, Solary E, Droin N. **Dynamic gene regulation by nuclear colony-stimulating factor 1 receptor in human monocytes and macrophages**. Nat Commun. 2019 Apr 26;10(1):1935. doi: 10.1038/s41467-019-09970-9. PMID: 31028249; PMCID: PMC6486619.

Contribution personnelle : Dans cette étude j'ai effectué des cross-link et réalisée des expériences de ChIP-seq sur des monocytes de patients atteints de LMMC (Figure.9).

Annexe n°2

Bencheikh L, Imanci A, Plocque A, Solary É, Droin N. **Rôle dynamique de CSF1R nucléaire dans la lignée monocyttaire [Dynamic role of nuclear CSF1R in the monocytic lineage]**. Med Sci (Paris). 2019 Dec;35(12):1225-1227. French. doi: 10.1051/medsci/2019236. Epub 2020 Jan 6. PMID: 31903947.

Contribution personnelle : Participation à la rédaction.

Annexe n°3

Arkoun B, Robert E, Boudia F, Mazzi S, Dufour V, Siret A, Mammasse Y, Aid Z, Vieira M, Imanci A, Aglave M, Cambot M, Petermann R, Souquere S, Rameau P, Catelain C, Diot R, Tachdjian G, Hermine O, Droin N, Debili N, Plo I, Malinge S, Soler E, Raslova H, Mercher T, Vainchenker W. **Stepwise GATA1 and SMC3 mutations alter megakaryocyte differentiation in a Down syndrome leukemia model**. J Clin Invest. 2022 Jul 15;132(14):e156290. doi: 10.1172/JCI156290. PMID: 35587378; PMCID: PMC9282925.

Contribution personnelle : Dans cet article, j'ai réalisé des expériences de Cut&Tag sur des mégacaryocytes, de l'incubation avec l'anticorps primaires à la purification et à la réalisation des librairies pour le séquençage (Fig.5 et Fig.7). J'ai également participé à l'écriture du matériel et méthode de cette partie.

Annexe n°4

Botten GA, Zhang Y, Dudnyk K, Kim YJ, Liu X, Sanders JT, Imanci A, Droin N, Cao H, Kaphle P, Dickerson KE, Kumar KR, Chen M, Chen W, Solary E, Ly P, Zhou J, Xu J. **Structural variation cooperates with permissive chromatin to control enhancer hijacking-mediated oncogenic transcription**. Blood. 2023 Jul 27;142(4):336-351. doi: 10.1182/blood.2022017555. PMID: 36947815; PMCID: PMC10447518.




Contribution personnelle : Dans ce papier j'ai participé à la purification des CD14+ des prélèvements de sang de certains patients LMMC dont l'ADN génomique a été extrait pour des analyses génomique au Etats-Unis.

ARTICLE

<https://doi.org/10.1038/s41467-019-09970-9>

OPEN

Dynamic gene regulation by nuclear colony-stimulating factor 1 receptor in human monocytes and macrophages

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Despite their location at the cell surface, several receptor tyrosine kinases (RTK) are also found in the nucleus, as either intracellular domains or full length proteins. However, their potential nuclear functions remain poorly understood. Here we find that a fraction of full length Colony Stimulating Factor-1 Receptor (CSF-1R), an RTK involved in monocyte/macrophage generation, migrates to the nucleus upon CSF-1 stimulation in human primary monocytes. Chromatin-immunoprecipitation identifies the preferential recruitment of CSF-1R to intergenic regions, where it co-localizes with H3K4me1 and interacts with the transcription factor EGR1. When monocytes are differentiated into macrophages with CSF-1, CSF-1R is redirected to transcription starting sites, colocalizes with H3K4me3, and interacts with ELK and YY1 transcription factors. CSF-1R expression and chromatin recruitment is modulated by small molecule CSF-1R inhibitors and altered in monocytes from chronic myelomonocytic leukemia patients. Unraveling this dynamic non-canonical CSF-1R function suggests new avenues to explore the poorly understood functions of this receptor and its ligands.

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Receptor tyrosine kinases (RTK) constitute the largest family of catalytic receptors with 58 members¹. Upon stimulation, RTKs undergo homo-oligomers or hetero-oligomers formation required for cytoplasmic catalytic domain auto-phosphorylation. In turn, multiple signaling pathways are activated and relay information from the cell membrane to intracellular compartments to trigger critical cellular processes¹. Beside this canonical mode of signaling, several RTKs are also present in the cell nucleus as either intracellular domains (ICD), or full length proteins^{2,3}. ICD are generated by proteolytic cleavage or alternative mRNA splicing³. Full length RTKs usually translocate from the cell surface to the nucleus through Golgi apparatus and endoplasmic reticulum⁴ before nuclear addressing through importin β -mediated pathways⁵. In the nucleus, RTKs phosphorylate proteins^{6,7} and regulate gene transcription⁶ to modulate DNA damage response⁷, cell proliferation⁶, survival⁸, and migration⁹. Therapeutic strategies targeting RTKs were developed, mostly to treat cancers, and RTKs nuclear accumulation could modulate their efficacy¹⁰.

Colony-Stimulating Factor 1 Receptor (CSF-1R), also called M-CSFR (Macrophage colony-stimulating factor), is encoded by CFMS (Cellular Feline McDonough Sarcoma) proto oncogene and is a class III RTK expressed by phagocytic mononuclear cells¹¹. CSF-1R is involved in several human diseases, its constitutive inactivating mutation induces a leuko-encephalopathy while its stimulation supports tumor progression and chronic inflammatory diseases. CSF-1R-targeting strategies are currently tested clinically in treating tumor infiltrated with macrophages¹².

Upon binding of either Colony-Stimulating Factor 1 (CSF-1) or interleukin (IL)-34¹³, its two known ligands, CSF-1R undergoes oligomerization, relieving catalytic domain inhibition¹⁴ and activating signal transduction^{15,16}, which is subsequently attenuated by CSF-1R ubiquitinylation, internalization, and degradation¹⁷. The nuclear localization of CSF-1R remains a controversial issue. CSF-1R proteolytic cleavage generating an ICD that migrates in the nucleus was initially reported¹⁸. Holoreceptor nuclear localization was subsequently described in human epithelial cancer cell lines¹⁹ and murine macrophages²⁰, where CSF-1R could be phosphorylated²⁰ and binds the promoter of selected genes¹⁹. Little is known, however about nuclear CSF-1R functional importance in physiological and pathological conditions.

Here, we demonstrate that a fraction of CSF-1R is in the nucleus of human monocytes, where it is recruited to DNA, co-localizes with H3K4me1 histone marks and interacts with EGRI. Upon CSF-1 exposure, which induces monocyte differentiation into macrophages, CSF-1R localization on chromatin changes within a few hours, it colocalizes with H3K4me3, and promotes gene expression through interaction with transcription factors YY1 and ELK. This function is affected by small molecule CSF-1R inhibitors and altered in dysplastic monocytes collected from chronic myelomonocytic leukemia (CMML) patients. These results emphasize a dynamic role of nuclear CSF-1R in monocyte differentiation into macrophages.

Results

A fraction of CSF-1R is located in human monocyte nucleus.

We sorted human monocytes from healthy donor peripheral blood and detected CSF-1R in both the cytoplasm and the nucleus by confocal microscopy (Fig. 1a, b), which was further confirmed by orthogonal views (Fig. 1c). The signal specificity was confirmed by the use of a blocking peptide mimicking the epitope recognized by CSF-1R antibody, and by monocyte transfection with CFMS siRNA (Fig. 1d). CSF-1R was also detected in monocyte nucleus by imaging flow cytometry

(Supplementary Fig. 1a, b). Monocyte fractionation into nuclear versus cytoplasmic and membrane fractions followed by immunoblotting confirmed CSF-1R nuclear detection as a full-length protein with partially (130 kDa) and fully glycosylated (170 kDa) forms (Fig. 1e). Again, signal specificity was demonstrated by two CFMS targeting siRNAs, which totally abolished the signal in the nucleus and the cytoplasmic and membrane fractions (Supplementary Fig. 1c). Finally, CSF-1R localization was observed by electron microscopy in heterochromatin and euchromatin (Fig. 1f), which was validated by a distinct antibody targeting CSF-1R N-terminal fragment (Supplementary Fig. 1d). Monocyte fractionation without denaturation, followed by immunoblotting, detected a transient CSF-1R dimerization in the membrane and cytoplasmic fraction after 10 min of CSF-1 treatment, which was not detected in nuclear extracts, even after prolonged immunoblot exposure, suggesting the nuclear expression of monomeric holoreceptor (Supplementary Fig. 1e). All together, these results demonstrate the presence of a fraction of full-length CSF-1R in human monocyte nucleus.

Nuclear CSF-1R holoreceptor location is driven by its ligand.

Peripheral blood monocytes were exposed to AF488-labeled recombinant CSF-1 for 15 min before fixation and staining with anti-CSF-1R antibody. As expected, CSF-1 and CSF-1R co-localized mainly at the plasma membrane and in the cytoplasm. CSF-1 was also detected in monocyte nucleus where it co-localizes with CSF-1R (Fig. 2a and movie as Supplementary Movie 1). Of note, CSF-1R staining after CSF-1 treatment (Fig. 2a) was more punctual compared with resting monocytes (Fig. 1a). This nuclear localization of CSF-1 and CSF-1R could not be related to nuclear localization signals (NLS) as CSF-1R primary sequence is devoid of this sequence and the putative NLS (amino acids 521 to 524) in CSF-1 sequence¹⁹ is deleted from the recombinant CSF-1 used in this experiment. CSF-1 nuclear accumulation could be prevented by monocyte pre-treatment for 3 h with small molecule CSF-1R inhibitors, either BLZ945 or GW2580 (Fig. 2b). Confocal imaging further showed that monocyte exposure to CSF-1R inhibitors for 3 h partially depleted nuclear CSF-1R (Fig. 2c), which could be prevented by leptomycin B, an inhibitor of CRM1-mediated nuclear export (Fig. 2d). All together, these results suggest a role for CSF-1 in CSF-1R nuclear localization in human monocytes.

CSF-1R is recruited on chromatin in human monocyte nucleus.

Chromatin immunoprecipitation followed by deep sequencing (ChIP-Seq) was performed with an anti-CSF-1R antibody in monocytes sorted from three healthy donors. Broad peak calling using MACS2 algorithm identified a mean number of 36,884 peaks, of which 4980 (13%) were common to the three samples (Fig. 3a and Supplementary Data 1). Intersection with Irreproducible Discovery Rate (IDR) analysis selected a set of 2303 common peaks (Supplementary Data 2). Cis-Regulatory Annotation System (CEAS) indicated that CSF-1R was preferentially recruited to intergenic (63.5%) regions (Fig. 3b). To gain insight into CSF-1R function at the chromatin level, we performed genome-wide analysis of H3K4me1 and H3K4me3 marks by ChIP-seq. For example, CSF-1R was recruited upstream of *KLF6*, *PU.1* (Fig. 3c, d), *CSF2RB* and *CEBPD* genes (Supplementary Fig. 2a) and to the last intron of *PU.1* gene (Fig. 3d). CSF-1R colocalization with histone mark H3K4me1 suggests regulating/enhancer regions^{21,22}. ChIP-seq results were validated by ChIP-qPCR in independent healthy donor monocytes with two anti-CSF-1R antibodies that recognize its N-terminal and C-terminal parts, respectively (Supplementary Fig. 2b). Motif analysis of ChIP-seq data using HOMER, focused on peaks shared by the three donors, indicated that CSF-1R could be recruited on several

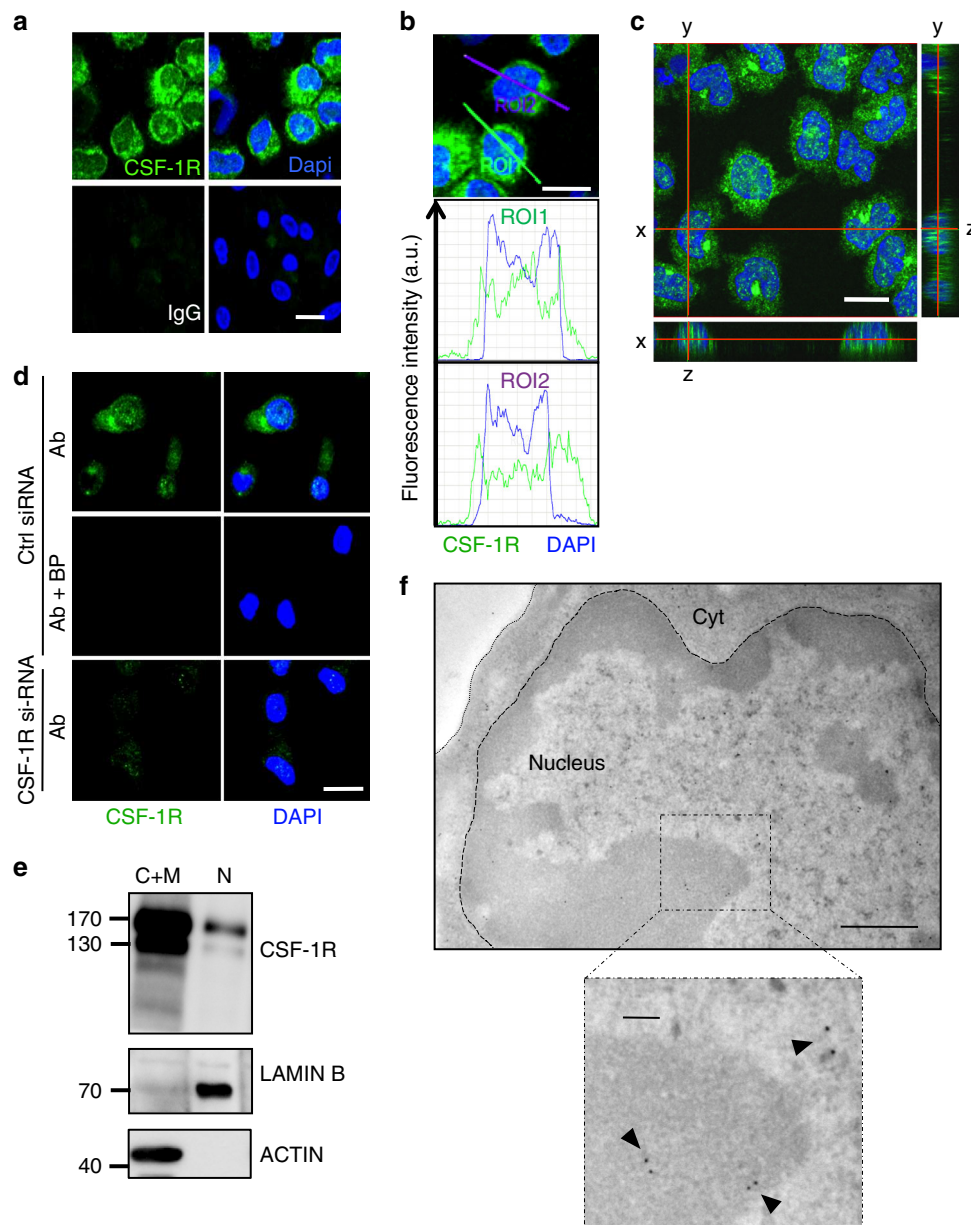


Fig. 1 A fraction of CSF-1R is located in the nucleus of human monocytes. **a** Sorted peripheral blood human monocytes were stained with an anti-CSF-1R antibody (Cter sc-692) or a control IgG (green) and Dapi (blue), followed by confocal imaging analysis ($n = 3$, scale: $10 \mu\text{m}$). **b** Quantification of the signal generated by CSF-1R labeling (green) and Dapi (blue) according to indicated axes (A.U.: arbitrary units, ROI: region of interest, scale: $10 \mu\text{m}$). **c** Monocytes were stained with an anti-CSF-1R antibody (Cter sc-692) and Dapi (blue), followed by confocal imaging analysis (stack of 50 pictures of $0.2 \mu\text{m}$) to reconstitute an orthogonal view (scale: $10 \mu\text{m}$). **d** The specificity of CSF-1R labeling with sc-692 antibody (Ab in green) was explored in monocytes transfected 24 h before with a CSF-1R specific or a control si-RNA and treated or not with sc-692 blocking peptide (BP, $n = 2$, scale: $10 \mu\text{m}$, Dapi in blue). **e** Monocytes were fractionated into cytoplasmic plus membrane (C+M) and nuclear (N) fractions and analyzed by immunoblotting with antibodies that recognize CSF-1R (Cell signaling #3152), Lamin B (N fraction) and actin (C+M fraction) ($n = 3$). **f** Immunogold analysis of CSF-1R expression in monocytes using the anti-Cter sc-692 antibody and electron microscopy ($n = 2$, scale: 500 nm ; insert: 100 nm)

transcription factor binding sites, the most significant being EGR2 and EGR1 motifs (Fig. 3e). The ten biological pathways with highest enrichment identified by gene ontology (GO) analysis were related to monocyte and macrophage functions (Supplementary Table 1), suggesting a role for nuclear CSF-1R in supporting monocyte trophic functions.

CSF-1R interacts with EGR1 to downregulate gene expression. Since CSF-1R is recruited on EGR1 motifs and EGR1 is a transcription factor involved in monocytopoiesi²³, we performed co-

immunoprecipitation experiments in whole monocyte extracts, which demonstrated an interaction between the two proteins (Fig. 4a). We reasoned that CSF-1R and EGR1 peaks might colocalize in genome-wide analysis. ChIP-seq experiments were performed on two healthy donor monocyte samples with an anti-EGR1 antibody. Broad peak calling using MACS2 algorithm identified 3542 common peaks (Supplementary Data 3). A large fraction of EGR1 peaks (56.9%) co-localized with CSF-1R peaks and were mainly localized to intergenic regions (Fig. 4b, Supplementary Fig. 3a and Supplementary Data 4). Ranking heatmap centered on CSF-1R common peaks further showed their

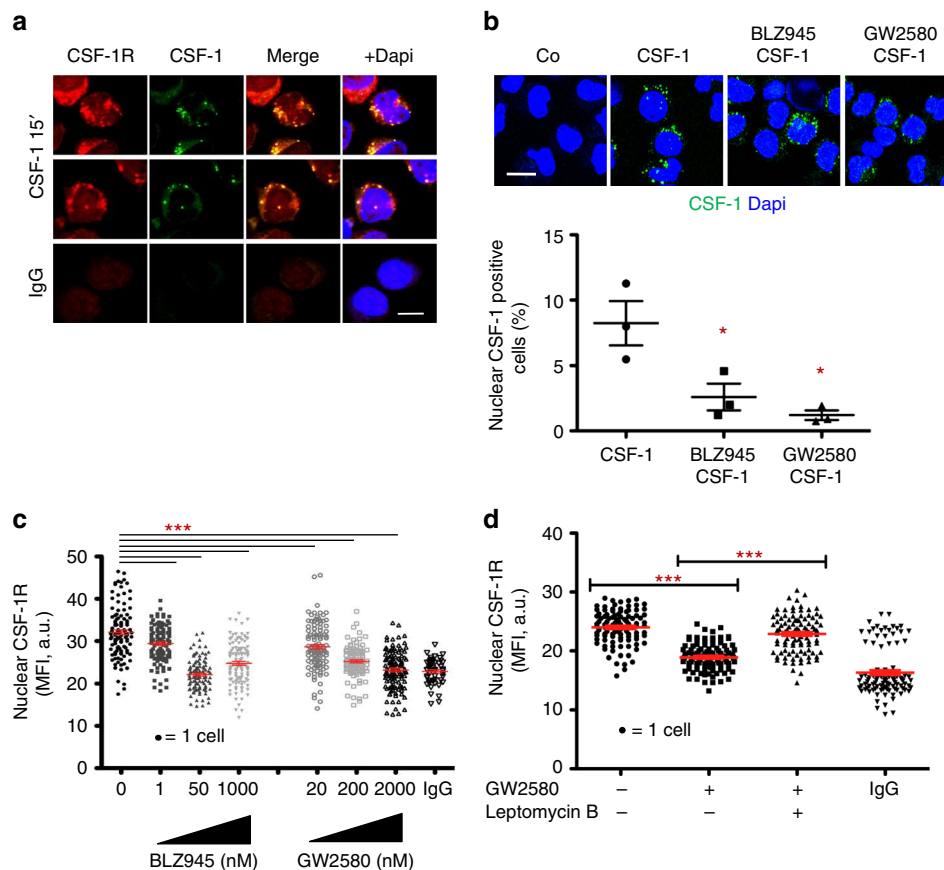


Fig. 2 Nuclear localization of CSF-1R holoreceptor is driven by its ligand. **a** Peripheral blood monocytes were fixed and stained for CSF-1R or control IgG (red) and Dapi (blue) after 15 min exposure to AF488-labeled CSF-1 (1 µg/mL in green) followed by confocal imaging analysis ($n = 3$, scale: 7 µm). **b** Monocytes were pre-treated for 3 h with CSF-1R small molecule inhibitors (BLZ945 50 nM; GW2580 200 nM or control DMSO), exposed 15 min to AF488-labeled CSF-1 (except Co, 1 µg/mL, in green), fixed and stained for Dapi (blue) before analysis by confocal imaging; Lower panel: quantification of nuclear CSF-1 positive cells (mean \pm SEM of 3 independent experiments, scale: 10 µm, Mann–Whitney test: $*P < 0.05$). **c** Monocytes were treated or not with indicated concentrations of BLZ945 and GW2580 before imaging flow cytometry analysis of CSF-1R nuclear localization (MFI, mean fluorescence intensity, mean \pm SEM, Unpaired t test: $***P < 0.001$, $n = 3$). **d** Monocytes were treated or not for 3 h with 200 nM GW2580, in the absence or presence of 20 nM Leptomycin B, fixed and stained for CSF-1R or control IgG before imaging flow cytometry analysis of CSF-1R nuclear localization (mean \pm SEM, Paired t test: $***P < 0.001$, $n = 2$)

colocalisation with EGR1 and H3K4me1 while being distant from H3K4me3 peaks (Fig. 4c). We also observed that 78.2% of common CSF-1R and EGR1 peaks colocalized with H3K4me1 mark (Supplementary Data 5), which is exemplified on specific genes, namely *PU.1*, *KLF6* (Fig. 4d), *C3*, *CEBPD* and *CMKLR1* (Supplementary Fig. 3b). Motif analysis obviously identified EGR2 and EGR1 motifs as EGR1 recruitment sequences (Supplementary Fig. 3c). Collectively, these results argue for EGR1 and CSF-1R co-recruitment on chromatin with H3K4me1 mark in monocyte nucleus.

To explore the role of EGR1 in recruiting CSF-1R at the chromatin level, we deleted *EGR1* gene in THP1 monocytic cell line using Crispr/Cas9 technology. Preliminary experiments detected 2124 CSF-1R peaks common to primary human monocytes and THP1 cells. We obtained three clones with *EGR1* homozygous deletion, which was validated by Sanger sequencing (Supplementary Fig. 4a) and RT-qPCR (Supplementary Fig. 4b). We performed CSF-1R ChIP-seq analysis in these clones and their wildtype counterpart. Importantly, the quantity of DNA captured by the anti-CSF-1R antibody in *EGR1*-deleted clones was very low as compared with wild type cells (Supplementary Fig. 4c). Deep sequencing of these libraries, whose results were pooled for analysis, indicated that *EGR1*

deletion abrogated CSF-1R localization at EGR1 sites, for example on *PU.1*, *CEBPD* (Fig. 4e), *CALML5*, *TLR10*, *TOM1*, and *ROR2* genes (Supplementary Fig. 4d).

We then transfected monocytes with *CFMS*-targeting or scrambled siRNA to establish whether CSF-1R could be involved in gene regulation. CSF-1R downregulation induced a significant increase in the expression of several genes on which CSF1R was detected by ChIP-seq experiments, including *CEBPD*, *SRC*, *FGR*, and *KLF6* (Fig. 5a). Since this experiment could not uncouple membrane and nuclear CSF-1R functions, we also cloned four identified EGR1 motif sequences upstream of a *LUCIFERASE* reporter gene and transfected monocytes with this construct and *CFMS*-specific or control siRNA. An increased *LUCIFERASE* expression was observed after CSF-1R downregulation, suggesting a repressive role of CSF-1R on EGR1 sites (Fig. 5b). Since ChIP-seq experiments identified CSF-1R and EGR1 on *PU.1* promoter (Fig. 4d), we also cloned *PU.1* promoter with (*PU.1* promoter 1) or without (*PU.1* promoter 2) CSF-1R recruitment site upstream of *LUCIFERASE* gene. In monocytes transfected with the first plasmid, we observed an increased *LUCIFERASE* expression after CSF-1R downregulation (Fig. 5c), together with an increased expression of *PU.1* (Fig. 5d). These effects were not observed with the second plasmid encoding a promoter deleted of CSF-1R

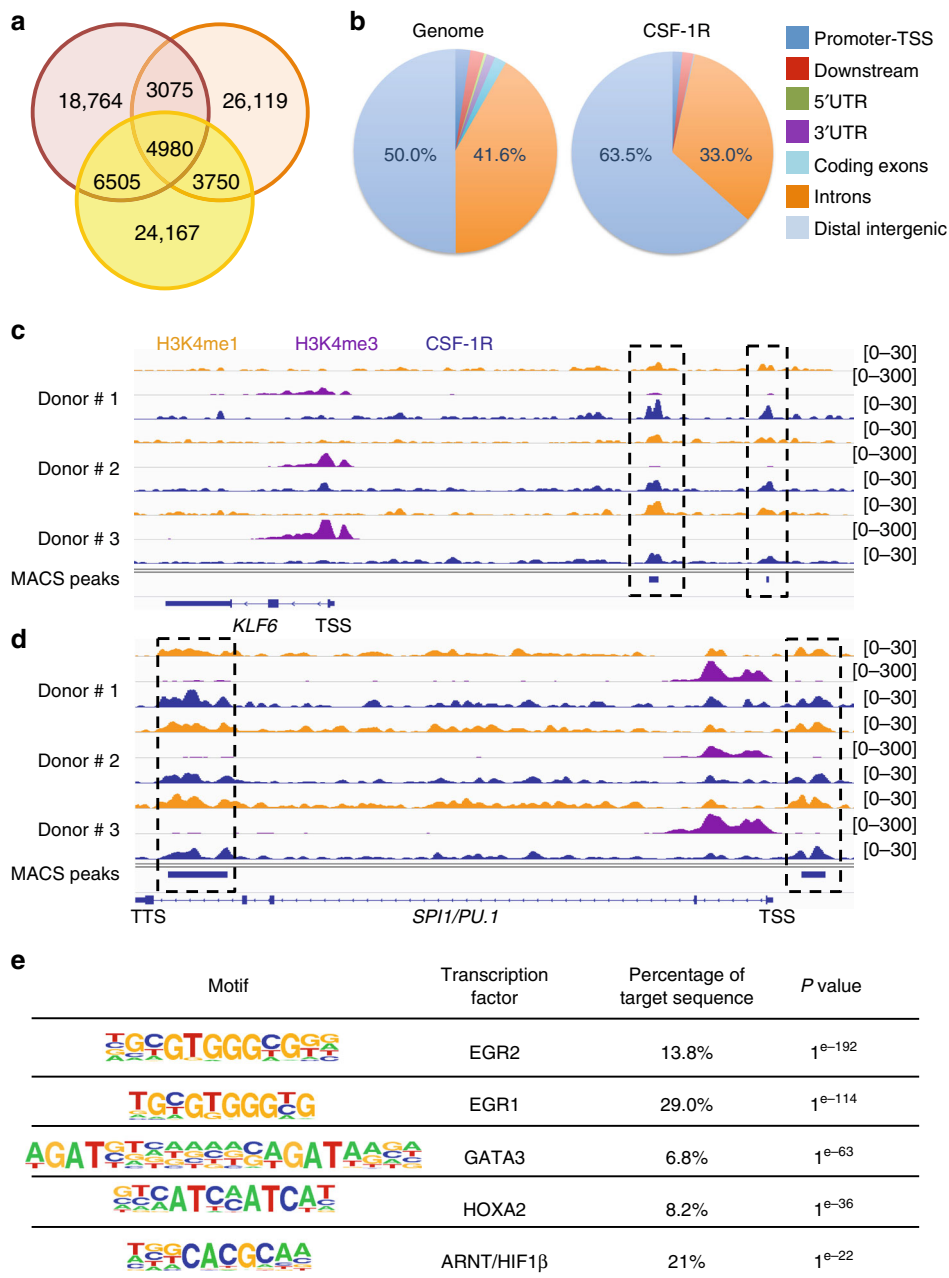


Fig. 3 CSF-1R is recruited to the chromatin in human monocyte nucleus. **a** Venn diagram of peak calling (input normalization) from ChIP-seq experiment with sc-46662 anti-CSF-1R antibody performed on peripheral blood monocytes collected from 3 healthy donors. **b** Repartition of the ChIP-seq peaks common to the 3 donors on the genome (right panel) as compared with hg19 reference genome annotation (left panel). **c, d** Peak calling for CSF-1R (blue), H3K4me1 (orange) and H3K4me3 (purple). ChIP-seq on *KLF6* (**c**) and *SPI1/PU.1* (**d**) genes in the three healthy donor monocytes (TSS: transcription starting site, TTS: transcription termination site). **e** Motif analysis of ChIP-seq peaks common to the 3 healthy donor monocyte samples

recruitment site (Fig. 5c, d). Collectively, these data indicate that CSF-1R negatively regulates *PU.1* gene expression in human monocytes.

Differential CSF-1R chromatin localization in macrophages.

Primary monocytes exposed to CSF-1 during 72 h differentiate into macrophages. Immunoblot analysis of nuclear extracts collected during this process detected a transient decrease in nuclear CSF-1R expression, 6 to 24 h after CSF-1 stimulation. After 72 h, the lower molecular weight CSF-1R was increased in macrophages (Fig. 6a). CSF-1R nuclear staining was quantified over the time after CSF-1 treatment by confocal imaging, showing again a

decrease of CSF-1R nuclear localization after 24 and 72 h compared with resting monocytes (Fig. 6b). Confocal imaging also detected CSF-1R in the nucleus of a fraction of these macrophages (Fig. 6c, d), which was confirmed by orthogonal views (Fig. 6e) and further validated by immunogold staining and electron microscopy (Fig. 6f). Interestingly, nuclear CSF-1R was mainly located on the verge of heterochromatin, which suggested its association with actively transcribed regions²⁴.

CSF-1R ChIP-Seq was performed in macrophages generated by exposure of monocytes from 3 healthy donors to CSF-1 during 72 h (same donors used in Fig. 3). Peak calling identified a mean number of 29,060 peaks, of which 19.3% to the three samples (Supplementary Fig. 5a). Common peaks (Supplementary Data 6)

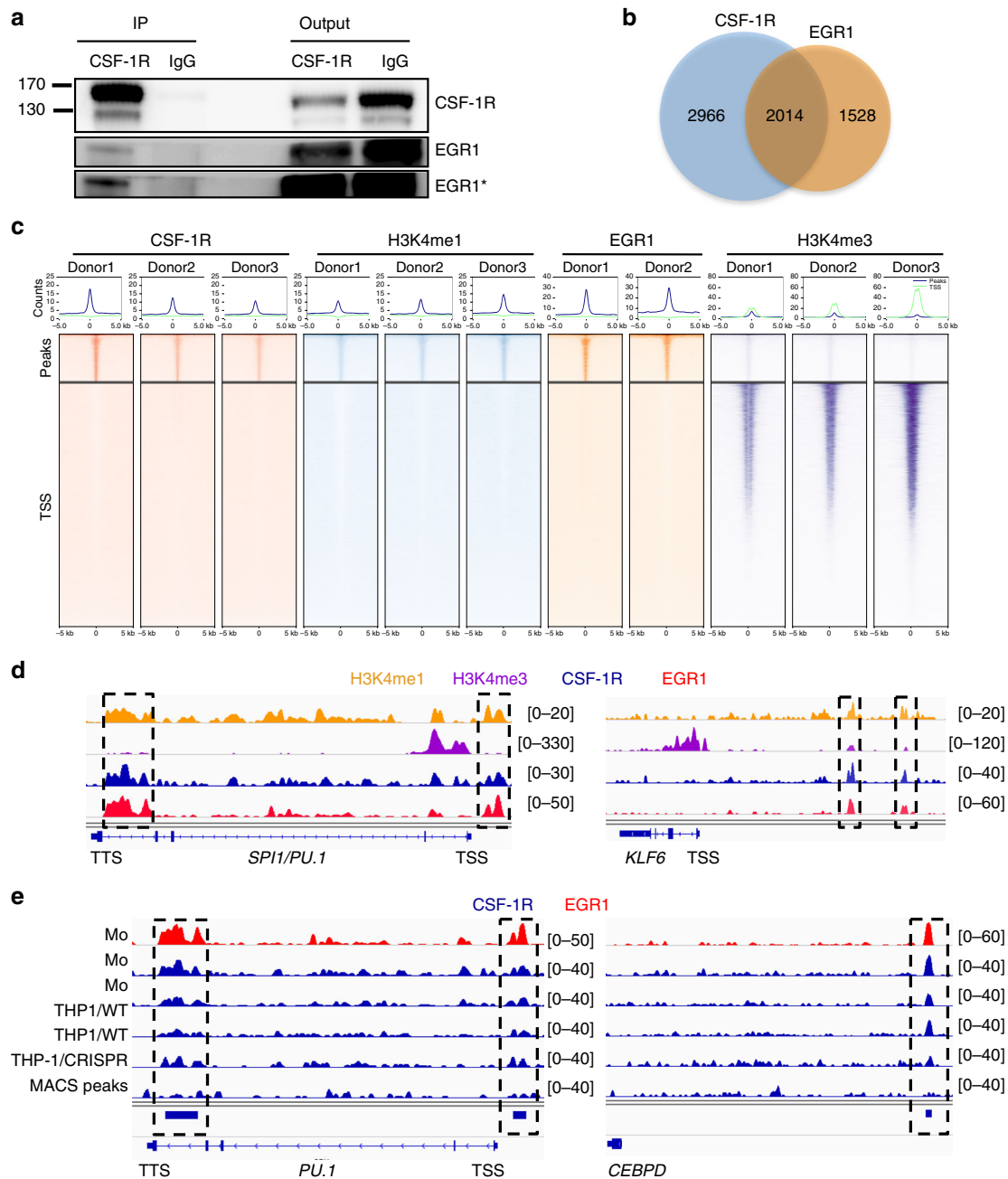


Fig. 4 CSF-1R interacts with EGR1 to modulate gene expression. **a** Co-immunoprecipitation experiment using an anti-CSF-1R (sc-365719) or a control IgG in monocytes followed by immunoblotting with anti-CSF-1R and anti-EGR1 antibodies ($n = 3$). Asterisk (*) indicates a longer exposure of the blot. **b** Venn diagram of peak calling (input normalization) common to three donors from ChIP-seq experiment with sc-46662 anti-CSF-1R and sc-110X anti-EGR1 antibodies. **c** Ranking heatmap centered on CSF-1R peaks (top) and on gene transcription starting sites (TSS) of CSF-1R, H3K4me1, EGR1 and H3K4me3 peaks in monocytes. **d** Peak calling for H3K4me1 (orange), H3K4me3 (purple), EGR1 (red), CSF-1R (blue). ChIP-seq on *PU.1* and *KLF6* genes in monocytes (TSS: transcription starting site, TTS: transcription termination site). **e** Peak calling on *PU.1* and *CEBPD* genes for EGR1 in monocytes and for CSF-1R in monocytes (Mo) from two donors (in blue, same donors as in Fig. 3), wild-type THP-1 clones (THP1/WT), THP-1 clones deleted for EGR1 (pool of 3 clones, THP1/CRISPR)

indicated that CSF-1R was less recruited to intergenic regions (26.4%) as compared with untreated monocytes (63.5%, Fig. 3b) while a significant fraction of CSF-1R was detected on promoter-TSS (transcription starting site) (11.9%), exonic (9.1%) and 5' UTR (9.9%) regions (Supplementary Fig. 5b). CSF-1R promoter-TSS recruitment was validated by ChIP-qPCR for *CFMS*, *MYB*, *EZH2*, *FOS*, *YY1* and *IL6R* genes in macrophages from independent healthy donors, *RAC2* being used as a negative control (Supplementary Fig. 5c).

Comparison of peak localization in monocytes and macrophages identified 1192 common peaks (representing 23.9% and

21.2% of monocyte and macrophage peaks, respectively, Supplementary Data 7). ChIP-Seq experiments with histone marks H3K4me1 and H3K4me3 performed on the same samples showed a higher fraction of CSF-1R associated with H3K4me1 in monocytes and H3K4me3 in macrophages (Fig. 7a and Supplementary Data 8–11, respectively). Ranking heatmaps centered on TSS indicated that CSF-1R is relocalized around the TSS where it colocalizes with H3K4me3 in macrophages (Fig. 7b), as exemplified for *MAFB*, *JUN*, and *MYC* genes (Supplementary Fig. 5d). CSF-1R location also changed from the promoter region of *PU.1* gene in monocytes to *PU.1* first intron in

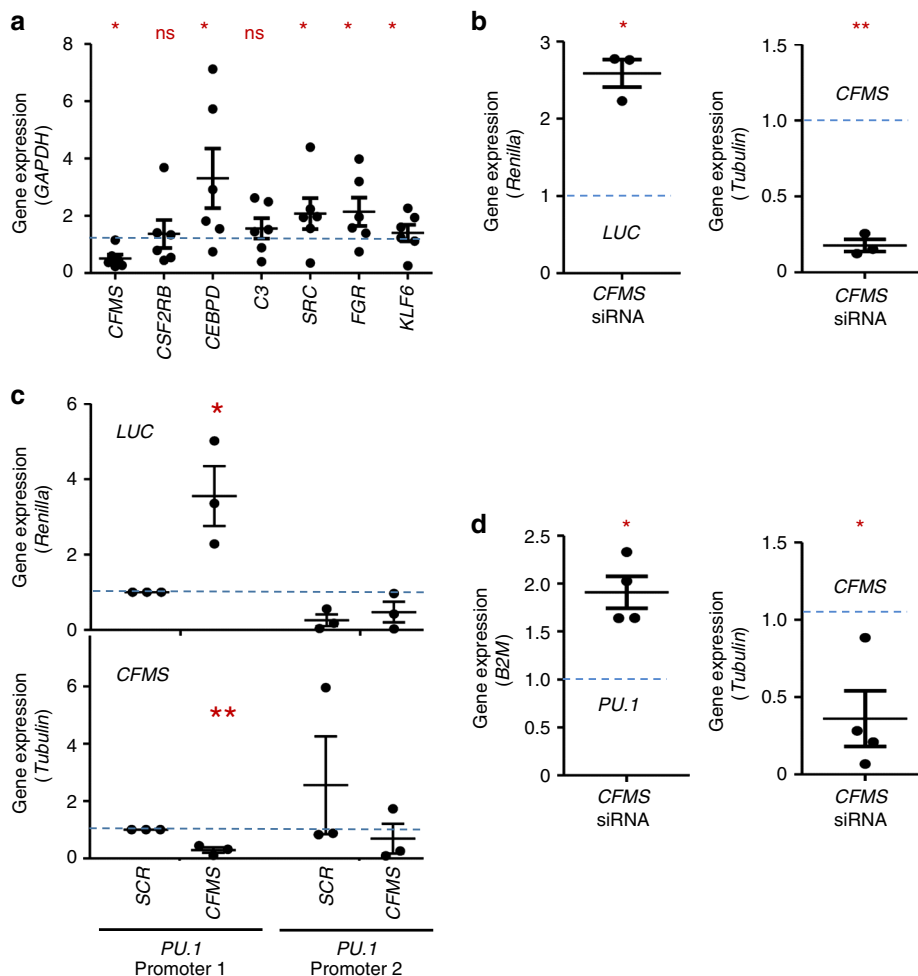


Fig. 5 CSF-1R negatively regulates *PU.1* gene expression in human monocytes. **a** The expression of indicated genes was measured by RT-qPCR in monocytes transfected 72 h before with a scramble (SCR) or a *CFMS* specific siRNA (mean \pm SEM of 6 independent experiments, results in *CFMS*-siRNA transfected cells normalized to SCR-siRNA = blue hatched line; Mann-Whitney test: * P < 0.05; ns, non significant). **b** Expression of *LUCIFERASE* (*LUC*, normalized to *Renilla*) and *CFMS* (normalized to *TUBULIN*) genes in monocytes, 48 h after transfection with a scramble (SCR) or a *CFMS* specific siRNA and PGL4.17-EGR1 motif plasmid (mean \pm SEM of 3 independent experiments, results in *CFMS*-siRNA transfected cells normalized to SCR-siRNA = blue hatched line; Paired t test: * P < 0.05, ** P < 0.01). **c** Expression of *LUCIFERASE* (normalized to *Renilla*) and *CFMS* (normalized to *TUBULIN*) in monocytes 48 h after transfection with SCR or *CFMS* specific siRNA and PGL4.17-*PU.1* promoter 1 (complete) or PGL4.17-*PU.1* promoter 2 (deleted of CSF-1R recruitment site) plasmids (mean \pm SEM of 3 independent experiments, normalization to control siRNA with *PU.1* promoter 1 = blue hatched line, Paired t test: * P < 0.05, ** P < 0.01). **d** Expression of *PU.1* and *CFMS* genes in monocytes 72 h after transfection with SCR or *CFMS* specific siRNA (mean \pm SEM of 4 independent experiments, normalization to control siRNA, Paired t test: * P < 0.05)

macrophages where it colocalizes with H3K4me3 mark (Supplementary Fig. 5e). GO analysis of CSF-1R-interacting genes in macrophages showed a dramatic change as compared with monocytes as biological pathways with highest enrichment were related mainly to epigenetics and transcription regulation (Supplementary Table 2). Enforcing this dynamic process, motif analysis revealed CSF-1R recruitment on different transcription factor binding sites such as E2F4, ELK4, YY1 (yin yang 1), and ELK1, not seen in monocytes (Fig. 7c). All together, CSF-1-induced monocyte differentiation into macrophages modifies CSF-1R recruitment on chromatin. ELK proteins bind to serum response elements to modulate immune response^{25,26}, while YY1 is involved in macrophage response to lipopolysaccharides²⁷. Co-immunoprecipitation (Fig. 7d) and immunofluorescence (Supplementary Fig. 5f) experiments in macrophages argued for CSF-1R interaction with ELK1 and YY1 proteins.

siRNA mediated downregulation of CSF-1R in CSF-1 treated monocytes induced a significant decrease in *YY1*, *ASXL1*, *CBL*, and *CJUN* expression (Supplementary Fig. 6a), supporting the

idea that CSF-1R may directly or indirectly participate to the transcription of these genes in macrophages. We then selected, two genes whose expression upon CSF-1R downregulation was compared in monocytes and macrophages. The first one is *KLF6* whose expression increases in monocytes when CSF-1R is downregulated (Fig. 5a) while being not affected by CSF-1R downregulation in macrophages (Supplementary Fig. 6b). The other one is *CSF2RB*, which is not significantly modified by *CFMS* siRNA in monocytes (Fig. 5a) while its expression is dramatically decreased by CSF-1R downregulation in macrophages (Supplementary Fig. 6b).

CSF-1 modulates fastly CSF-1R recruitment on chromatin.

Having observed that monocyte exposure to CSF-1 for 72 h induced CSF-1R relocation on gene TSS, we explored the kinetics of this event by treating monocytes with CSF-1 for only 6 h. ChIP-seq experiments (Supplementary Data 12) identified an increase in CSF-1R recruitment on exonic, promoter-TSS, TTS

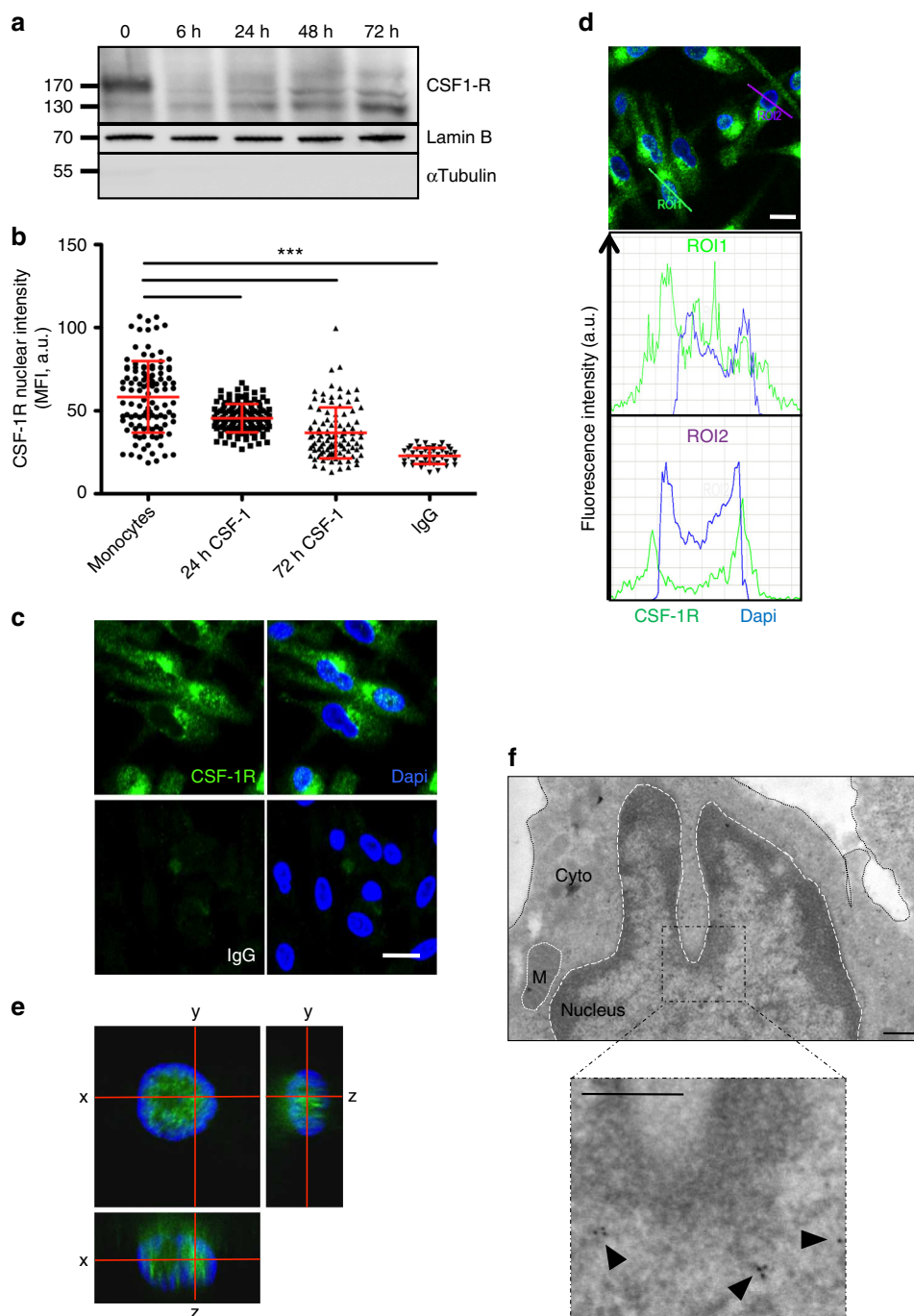


Fig. 6 Differential CSF-1R chromatin localization in CSF-1-induced macrophages. **a** Nuclear proteins were extracted from monocytes stimulated with 100 ng/mL CSF-1 during the indicated times, and analyzed by immunoblotting for CSF-1R, Lamin B (nucleus marker) and α -tubulin (cytosol marker) ($n = 3$). **b** Monocytes were stimulated or not with 100 ng/mL CSF-1 during the indicated times, stained with an anti-CSF-1R antibody (Cter sc-692) or a control IgG and Dapi, followed by confocal imaging analysis of CSF-1R nuclear localization (MFI, mean fluorescence intensity, A.U. arbitrary units, mean \pm SD, Paired t test: *** $P < 0.001$, $n = 1$). **c** Monocytes were treated 3 days with 100 ng/mL CSF-1, fixed and stained for CSF-1R or control IgG (green) and Dapi (blue) followed by confocal imaging ($n = 3$, scale: 10 μ m). **d** Quantification of the signal generated by CSF-1R labeling (green) and Dapi labeling (blue) according to indicated axes (A.U.: arbitrary units, ROI: region of interest, scale: 10 μ m). **e** Macrophages were stained with an anti-CSF-1R antibody (C-ter sc-692) and Dapi (blue), followed by confocal imaging analysis (stack of 50 pictures of 0.2 μ m) to reconstitute an orthogonal view (scale: 10 μ m). **f** Monocytes were differentiated 3 days with 100 ng/mL CSF-1, fixed and stained for CSF-1R (sc-692) followed by electron microscopy ($n = 1$, scale: 500 nm)

(transcription termination site) and 5'UTR regions compared with unstimulated monocytes (Fig. 8a, b). The fraction of CSF-1R peaks colocalizing with H3K4me3 after 6 h of CSF-1 exposure was 90% (Supplementary Data 13). Only 28% of CSF-1R peaks remained similar to unstimulated cells (Fig. 8c and Supplementary Data 14). CSF-1R was detected on a fraction of chromatin

sites identified in macrophages, e.g., *PU1* TSS, while some CSF-1R localizations as *CSF2RB* TSS were only transiently detected in cells treated for 6 h with CSF-1 (Fig. 8c). Pathway analysis indicated that CSF-1R localizes mainly to genes involved in CSF-1 signaling, phagocytosis, and migration (Supplementary Table 3). Most of these pathways differ from those identified in resting

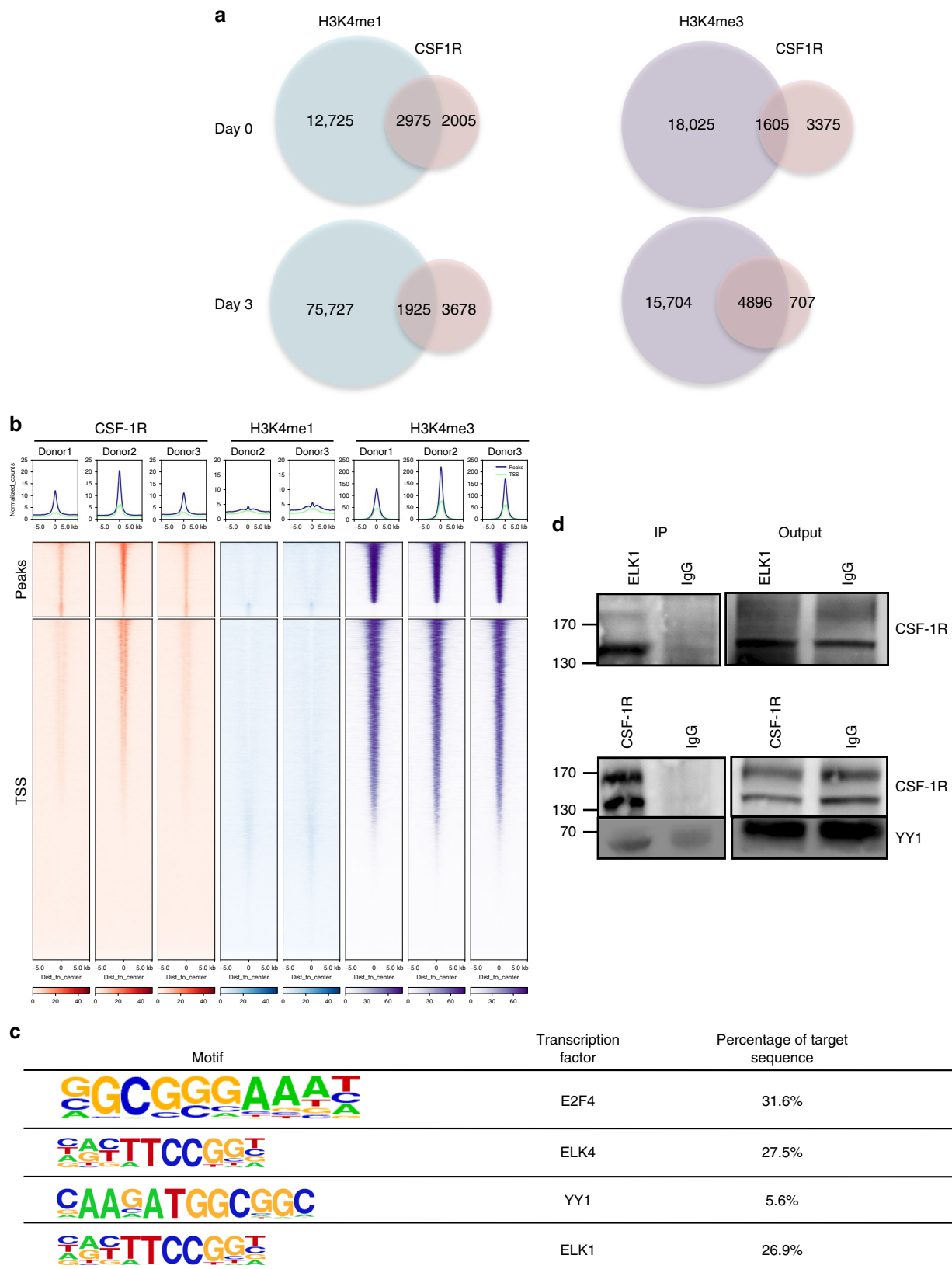


Fig. 7 Differential CSF-1R chromatin localization in CSF-1-induced macrophages. **a** Venn diagram of ChIP-seq peak number in untreated monocytes (Day 0) and monocytes treated with 100 ng/mL CSF-1 for 3 days (Day 3) showing peaks common to the 3 donors. Left: CSF-1R and H3K4me1 peaks; right: CSF-1R and H3K4me3 peaks. **b** Ranking heatmap centered on CSF-1R peaks (top) and on gene transcription starting sites (TSS) of CSF-1R, H3K4me1 and H3K4me3 peaks in CSF-1-differentiated macrophages (3 days 100 ng/mL). **c** Motif analysis of ChIP-seq experiment on 3 day CSF-1 differentiated monocytes (100 ng/mL). **d** Co-immunoprecipitation of CSF1R and ELK1 (upper panel) or YY1 (lower panel). Immunoprecipitation (IP) was performed with an anti-ELK1 (sc-365876) or anti-CSF-1R (sc-365719) and a control IgG in monocytes treated for 3 days with 100 ng/mL CSF-1, followed by immunoblotting with an anti-CSF-1R ($n = 2$ each) or an anti-YY1 ($n = 3$) antibodies

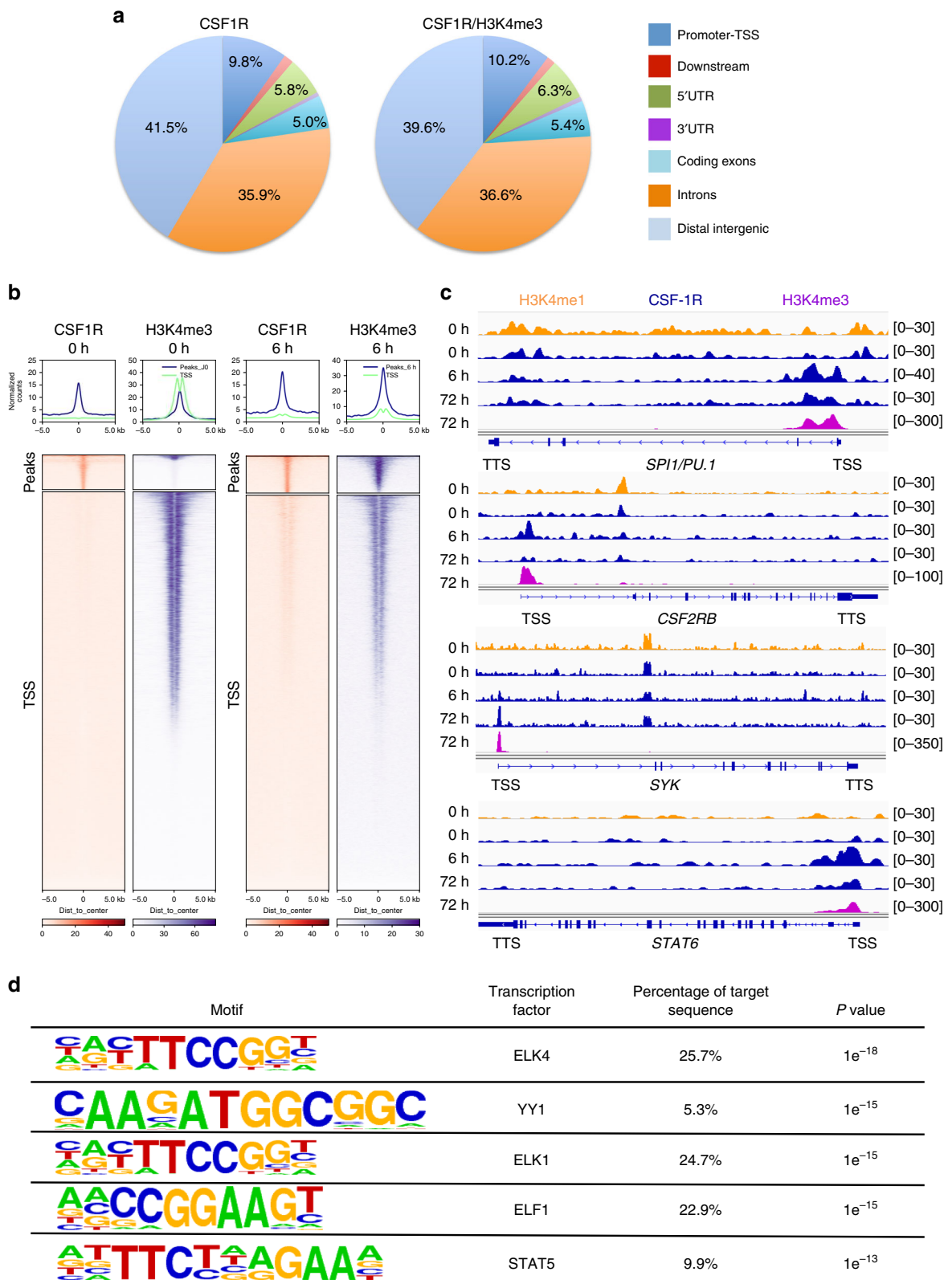


Fig. 8 CSF-1 modulates CSF-1R recruitment on chromatin within a few hours. **a** Repartition of CSF-1R ChIP-seq peaks and the intersection of CSF-1R and H3K4me3 peaks on the genome of monocytes exposed for 6 h to 100 ng/mL CSF-1. Reference genome is shown on Fig. 3b. **b** Heatmap centered on CSF-1R peaks (top) and on gene transcription starting sites (TSS) of CSF-1R and H3K4me3 peaks in unstimulated monocytes (0 h) and monocytes exposed to CSF-1 for 6 h (6 h). **c** ChIP-Seq experiments showing CSF-1R, H3K4me1 and H3K4me3 peak localization on *PU.1*, *CSF2RB*, *SYK* and *STAT6* genes in monocytes, either untreated (0 h) or treated with CSF1 for 6 h (6 h) or 3 days (72 h) (TSS: transcription starting site, TTS: transcription termination site). **d** Motif analysis of the CSF-1R ChIP-seq peaks from monocytes stimulated with 100 ng/mL CSF-1 for 6 h

monocytes, as well as in macrophages. Motif analysis indicated CSF-1R localization on ELK and YY1 motifs, as in macrophages, and ELF1 and STAT5 motifs, which were only transiently detected after 6 h of CSF-1 stimulation (Fig. 8d). These results demonstrate a dynamic CSF-1R/chromatin interaction through monocyte to macrophage differentiation upon CSF-1 exposure.

Abnormal CSF-1R expression in CMML monocytes. CMML is a myelodysplastic/myeloproliferative syndrome characterized by an abnormal expansion of circulating classical monocytes^{28,29}. We detected a heterogeneous decrease in *CFMS* mRNA and CSF-1R protein expression in patient monocytes compared with healthy donor monocytes (Fig. 9a, b). In many patient samples, the decreased expression of CSF-1R precluded CSF-1R ChIP-seq experiments. In those in which we succeeded in carrying out CSF-1R immunoprecipitation, we generated a bed file of 2276 reproducible peaks (intersection of 4980 peaks obtained with the monoclonal N-terminal CSF-1R antibody with 23,480 peaks obtained with the polyclonal, C-terminal CSF-1R antibody). Some enrichments on chromatin sites were conserved in healthy donors and patient monocytes, e.g., on intergenic area on chromosome 1 and *CEBPA* and *BCL2* genes (Fig. 9c) whereas others were lost in some patient cells, e.g., in *BPIFB1* gene, downstream of *AP1M1* gene and in intergenic areas on chromosome 11 and 22 (Fig. 9d). These results indicated a heterogeneous disruption of CSF-1R interaction with chromatin in CMML patient monocytes.

Discussion

The present study demonstrates the presence of a fraction of CSF-1R in human primary monocyte nucleus and its recruitment to chromatin where it colocalizes with H3K4me1 marks and interacts with EGR1 to modulate gene expression. Upon CSF-1-induced monocyte differentiation into macrophage, nuclear CSF-1R is relocated on chromatin within a few hours to differentially influence gene expression through interaction with YY1 and ELK transcription factors. Small molecule inhibitors of CSF-1R interfere with these nuclear functions that are otherwise disorganized in CMML dysplastic monocytes.

Several RTKs have been shown to modulate gene transcription by interacting with transcription factors^{30,31}, carrying these proteins to the nucleus³², promoting their recruitment to DNA³³, or phosphorylating nuclear proteins^{34,35}. CSF-1R itself was shown to be recruited to specific genes in breast cancer cells¹⁹. We now demonstrate a physiological and dynamic role of CSF-1R in regulating gene expression along the differentiation of human monocytes. These cells, which circulate in the blood stream, are considered as an intermediate developmental stage between bone marrow precursors and some tissue macrophage populations. Nuclear CSF-1R may be a molecular component of monocyte differentiation into macrophages through modulating the expression of key regulatory genes such as *CEBPD* and *KLF6*³⁶.

CSF-1R staining was diffuse in resting monocytes. The punctual staining detected in response to CSF-1 treatment may indicate internalization in endocytic vesicles following receptor activation at the membrane level, and the formation of protein complexes involving CSF-1R along specific chromatin sites in the nucleus. We noticed a decrease in nuclear CSF1R upon treatment with CSF-1R small molecule inhibitors, suggesting that interaction of the receptor with its ligand may drive its nuclear accumulation. The slight but reproducible increase in nuclear CSF1R observed at highest BLZ945 concentration could indicate a toxic effect of the compound.

A distinct interaction of CSF-1R with chromatin is observed among cell types at the level of individual genes, e.g., CSF-1R is recruited to distinct sequences on *CMYC* and *CJUN* genes in

macrophages and breast cancer cells, respectively¹⁹. As CSF-1R lacks DNA binding domain, it may be recruited to these DNA sequences through interaction with other proteins, including transcription factors. These interactions may change with the functional program followed by these cells, e.g., CSF-1R interaction with EGR1 detected in monocytes is lost in macrophages in which the transcription factor expression is downregulated³⁶. Changes in epigenetic landscape along monocyte differentiation may be part of the molecular mechanisms that modulate CSF-1R interaction with nuclear chromatin²². An endotoxin challenge would probably modify the pattern of CSF-1R/chromatin interaction in either monocytes or macrophages.

CSF-1R relocation to active genes with an H3K4me3 mark was detected as soon as 6 h after CSF-1 stimulation without being totally similar to that observed after 72 h of CSF-1 exposure, suggesting a dynamic pattern of CSF-1R recruitment to chromatin. Monocytes are highly plastic cells whose differentiation is diverted towards osteoclasts when RANKL is combined with CSF-1³⁷, while a combination of CSF-2 and IL-4 promotes myeloid dendritic cell formation³⁸. Each of these processes may generate a specific pattern of nuclear CSF-1R interaction with chromatin. Finally, CSF-1R expression level and chromatin recruitment are altered in CMML patient monocytes, which may be related to splicing and epigenetic aberrations that characterize this disease^{29,39} and further supports the dysplastic nature of these cells.

Nuclear CSF-1R could also play an important role in CSF-1 capacity to instruct hematopoietic stem cell commitment towards monocyte lineage through promoting *PU.1/SPI1* gene transcription, as depicted in mice⁴⁰. The differential recruitment of CSF1R on *PU.1/SPI1* sequence in monocytes and macrophages suggests a subtle regulation of this gene in response to CSF-1. More generally, CSF-1R nuclear functions could be shared with other RTK subfamily members such as PDGF-R (Platelet-derived growth factor receptor), which was detected in cell nucleus, as well as KIT (Mast/Stem cell growth factor receptor) and FLT3 (Fms-like tyrosine kinase 3), whose nuclear localization remains to be explored. Finally, therapeutic strategies targeting these receptors are currently developed, e.g., CSF-1R-targeting strategies are used to deplete tumor-associated macrophages¹². Further investigations will indicate how nuclear location of CSF1R could modulate the response to these drugs^{10,30}.

Methods

Antibodies and reagents. For immunofluorescence, imaging cytometry and electron microscopy experiments, we used anti-CSF-1R antibodies (sc-692, sc-365719), anti ELK-1 antibody (sc-365876) and corresponding isotype controls (normal rabbit IgG sc-2027, normal mouse IgG sc-2025) from Santa Cruz Biotechnology (Clintonsville, Nanterre, France). Secondary antibodies (Alexa Fluor 488 goat anti-Rabbit) were purchased at ThermoFisher Scientific (Waltham, USA) and at TEBU-Bio (Le Perray-en-Yvelines, France) (goat anti-rabbit and anti-mouse with gold particles). For immunoblot experiments, we used Cell Signaling Technology (Clintonsville, Nanterre, France) antibodies: anti-CSF-1R (#3152), anti-YY1 (#2185), anti-HDAC2 (#2540), anti- α TUBULIN (#3873); Santa Cruz Biotechnology antibodies: anti-GAPDH (sc-32233), anti-EGR1 (sc-110), anti-LAMIN B (sc-6217); anti-ACTIN (A5441) from Sigma-Aldrich (Saint-Quentin Fallavier, France) and anti-H3K4me3 from Active Motif (39159) (La Hulpe, Belgium). Secondary antibodies were purchased at ThermoFisher Scientific (anti-rabbit, anti-mouse and anti-goat HRP-conjugated). For immunoprecipitation experiments, we used anti-CSF-1R (sc-365719), anti-ELK1 (sc-365876) or control Mouse IgG (sc-2025) from Santa Cruz Biotechnology. For ChIP-qPCR and ChIP-seq experiments we used two anti-CSF-1R antibodies (sc-692 and sc-46662), EGR1 antibody (sc-110X), corresponding isotype controls (normal rabbit IgG sc-2027, normal mouse IgG sc-2025) from Santa Cruz Biotechnology, anti-H3K4me3 and H3K4me1 from Active Motif (39159 and 39297, respectively). Chemical inhibitors of CSF-1R were purchased at Interchim (BLZ945, ThermoFisher Scientific) and Sigma-Aldrich (GW2580).

Monocyte sorting and culture. Peripheral blood mononucleated cells (PBMC) were obtained from healthy donor buffy coats (Etablissement Français du sang, Rungis, France) or newly diagnosed CMML patients whose samples were collected

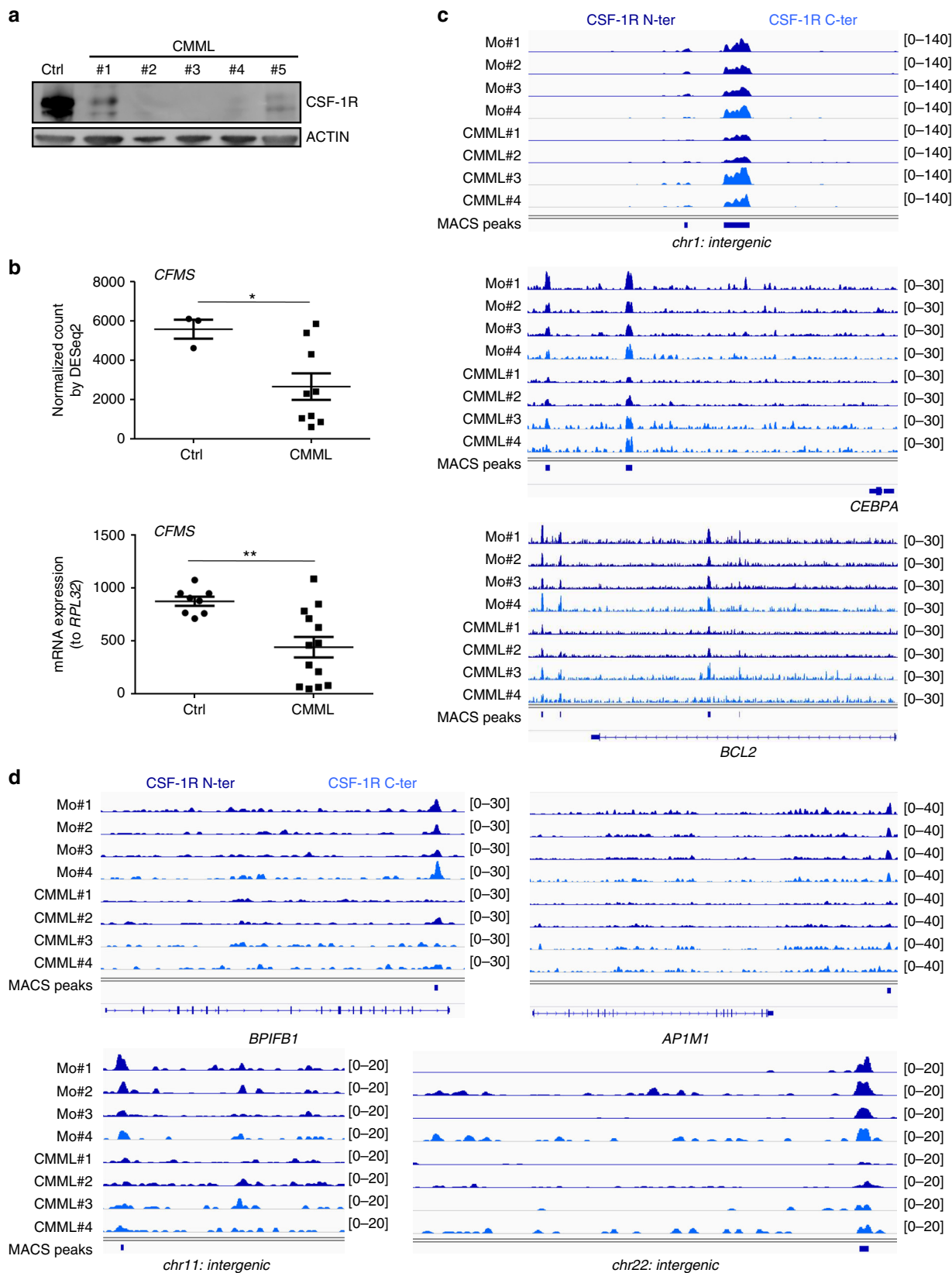


Fig. 9 CSF-1R is abnormally expressed and recruited on chromatin in CMML monocytes. **a** Total proteins were extracted from isolated control or CMML monocytes and analyzed by immunoblotting with antibodies that recognize CSF-1R and actin. **b** *CFMS* expression was assessed by RNA-sequencing and qPCR in monocytes of 9 and 13 CMML patients, respectively, compared with 3 and 8 control buffy coats (Ctrl), respectively (mean \pm SEM, Mann-Whitney test: * $P < 0.05$, ** $P < 0.01$). **c** ChIP-Seq experiments showing CSF-1R peak localization on intergenic area on chromosome 1 (chr1: intergenic), upstream of *CEBPA* and on *BCL2* genes that are conserved in control and CMML monocytes. **d** ChIP-Seq experiments showing CSF-1R peak localization on *BPIFB1*, downstream of *AP1M1* and on intergenic area on chromosome 11 and 22 (chr11: intergenic and chr22: intergenic) that can be lost in CMML compared with healthy donor monocytes (CSF-1R N-ter: sc-46662 monoclonal antibody in dark blue, CSF-1R C-ter: sc-692 polyclonal antibody in light blue)

with informed consent following the authorization provided by the ethical committee Ile-de-France 1 (DC-2014-2091), separated on Pancoll (Pan-Biotech, Dutscher, Brumath, France). Peripheral blood CD14⁺ monocytes were sorted with magnetic beads and the AutoMacs system (Miltenyi Biotech, Paris, France)⁴¹. Monocytes used for nucleofection experiments were sorted with Monocyte isolation kit II (Miltenyi Biotech). After sorting, monocytes were cultured overnight at 10⁶/mL in RPMI 1640 Glutamax medium (ThermoFisher Scientific) supplemented with 10% heat inactivated fetal bovine serum (FBS, Lonza, Amboise, France), 1% penicillin/streptomycin, and 2 mM L-Glutamine (ThermoFisher Scientific). Cells were then seeded at 0.5 × 10⁶/mL and differentiated into macrophages in the same medium containing recombinant human CSF-1 (100 ng/mL Peprotech, Neuilly-Sur-Seine, France) during 1 to 3 days. Cells were detached using PBS-EDTA and centrifuged at 300×g into dry pellets for further RNA or protein extraction.

Cell lines. 293T and THP-1 cell lines were purchased at ATCC (LGC Standards, Molsheim, France). THP-1 were cultured in the same medium used for primary cells and 293T in Dulbecco's Modified Eagle's Medium (ThermoFisher Scientific) supplemented with 10% heat inactivated fetal bovine serum, 1% penicillin/streptomycin, and 2 mM L-Glutamine.

Confocal microscopy. 150 × 10³ monocytes were cytopspined 5 min at 65×g then fixed 10 min with 4% paraformaldehyde (PFA). For adherent macrophages cultured in chamber slides (Nunc Lab-Tek II, ThermoFisher Scientific), the medium was removed and the cells directly fixed with PFA on the slide. After 3 washes in PBS, cells were incubated with anti-CSF-1R antibody (2 µg/mL) or isotype control (2 µg/mL rabbit IgG) and with blocking peptide if necessary (sc-692P at 2 µg/mL, Santa Cruz Biotechnology) diluted in 0.05% saponin during 1 h at room temperature (RT), followed by wash. The secondary antibody was added (10 µg/mL) with 0.05% saponin during 30 min followed by wash. Slides were mounted using Vectashield mounting medium with Dapi (Vectorlabs, Clinisciences). Specimens were examined on Leica confocal microscopes (TCS SPE and SP8, Leica Microsystems, Nanterre, France). The nuclear CSF-1R fluorescence was quantified using ImageJ software. The nucleus areas were selected in each cell using Dapi staining before measuring the mean intensity of CSF-1R fluorescence inside each nucleus with ROI manager. Live cell imaging movie and orthogonal view were created with ImageJ software.

CSF-1 chemical modification. Human recombinant CSF-1 (Peprotech) was coupled with Alexa Fluor 488 using the Alexa fluor 488 Microscale Protein Labeling Kit (ThermoFisher Scientific) according to the supplier instructions.

Live cell imaging. Adherent cells cultured in chamber slides were serum-deprived, then stained with 100 ng/mL Hoechst (BD Biosciences, Le Pont de Claix, France) during 30 min, stimulated 15 min with 1 µg/mL fluorescent CSF-1 before wash and living cell observation with a spinning disk microscope (Andor Technology, Belfast, UK) during 10 min (1-min delay between each image).

Electron microscopy. Cells were centrifuged and fixed as a pellet with 1.6% glutaraldehyde (Sigma-Aldrich) in 0.1 M phosphate buffer, rinsed and equilibrated in 30% methanol, deposited in a Leica EM AFS2/FSP automatic reagent handling apparatus (Leica Microsystems) and embedded in Lowicryl K4M under UV for 40 h at -20 °C and under UV for 40 h at +20 °C. For immunogold electron microscopy (I-EM), ultra-thin sections were incubated for 1 h at room temperature with the anti-rabbit CSF-1R antibody diluted 1/10 in PBT (1× PBS + 5% BSA + 0.25% triton X-100) and for 30 min with a secondary anti-rabbit antibody coupled to 10-nm gold particles (BBInternational, Cardiff, UK) diluted 1/25 in PBS. Thin-sections were briefly contrasted with uranyl acetate and analyzed with a Tecnai Spirit (FEI, Hillsboro, OR). Digital images were taken with a SIS MegaviewIII charge-coupled device camera (Olympus, Tokyo, Japan).

Imaging cytometry. Two to 10 × 10⁵ cells were fixed with 4% PFA for 10 min at 4 °C, washed with 0.05% saponin and incubated 1 h at 4 °C with primary CSF-1R antibody or control isotype diluted in 0.05% saponin. After washing, cells were incubated with a secondary antibody (goat anti-rabbit AF488, 20 µg/mL) diluted in 0.05% saponin for 1 h at 4 °C, washed, and resuspended in 2% FBS-PBS before adding 200 ng/mL Dapi (BD Biosciences). Samples were analyzed on ImageStream X MK2. Focused cells were selected using Gradient RMS function and analysis was performed on singlet CSF-1R⁺/Dapi⁺ cells on IDEAS software.

Cell fractionation and protein extraction. For extracting the cytoplasmic/membrane and the nuclear fractions, cells were incubated 15 min on ice in a buffer containing 10 mM HEPES, 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA, 1 mM DTT, 0.5 mM PMSF (Buffer 1). NP-40 (0.625%) was added and the cells briefly vortexed. Samples were then centrifuged 1 min at 16,000×g at 4 °C. The supernatant (constituted of cytoplasmic plus membrane fraction) was collected and the pellet rinsed with Buffer 1 3 times (centrifugation 1 min at 16,000×g and supernatant discarded). The rinsed pellets (consisting of the nuclear fraction) were resuspended in 2× Laemmli sample buffer (Bio-Rad, Marnes-la-Coquette, France)

supplemented with 0.1 M DTT and 1× Protease inhibitor cocktail (Roche) during 15 min with agitation at 4 °C. 4× Laemmli sample buffer (Bio-Rad) supplemented with 0.1 M DTT was added to the cytoplasmic fraction and all the samples were then boiled 10 min at 95 °C. To explore the location of CSF-1R dimers, the same protocol was used without DTT in both buffers. After compartment separation, the samples were sonicated 3 times and 4× Laemmli added to cytoplasmic fraction. Samples were then used without boiling. Extraction of total proteins from CMML peripheral blood sorted monocytes was performed with RNA/DNA/Protein Purification Plus Kit (NorgenBiotek, Interchim, Montluçon, France) according to the supplier instructions. 4× Laemmli, 0.1 M DTT and 1× Protease inhibitor cocktail were added and the samples were boiled 10 min at 95 °C.

Immunoprecipitation. Cells were lysed 20 min on ice in a buffer containing 50 mM Tris pH 7.4, 150 mM NaCl, 1% NP-40, 10% Glycerol, 1 mM Na₃VO₄ and 1× Protease inhibitor cocktail (100 µL for 10 × 10⁶ cells). Samples were then centrifuged 15 min at 4 °C at 18,000×g and the supernatant containing the proteins was collected. Ten µg of CSF-1R or ELK1 antibody or negative control IgG were added for 10 × 10⁶ cells and the samples incubated one night at 4 °C with agitation. One hundred µL of Protein A/G PLUS-Agarose (Santa Cruz biotechnology) were incubated with the samples during 2 h at 4 °C with agitation. The complexes were then precipitated with 1 min centrifugation at 2000×g and the supernatant, consisting of «output» was collected. The remaining beads were washed 5 times with 500 µL of lysis buffer without NP-40. 2× Laemmli with 0.1 M DTT and Protease inhibitor cocktail 1× was added on the beads and outputs. The samples were then boiled 10 min at 95 °C.

Immunoblotting. Proteins were separated on polyacrylamide gel and transferred to nitrocellulose membrane (ThermoFisher Scientific). Membranes were blocked with 5% bovine serum albumin in PBS, with 0.1% Tween-20 (Sigma-Aldrich) for 40 min at RT, incubated overnight at 4 °C with the primary antibodies (dilution 1/1000e), washed in PBS-0.1% Tween-20, incubated further with HRP-conjugated secondary antibody (400 ng/mL) for 1 h at RT and washed again before analysis using Immobilon Western Chemiluminescent HRP Substrate system (Millipore, Molsheim, France). The chemiluminescent emission was registered by imageQuant LAS 4000 camera (GE Healthcare Life Science, Vélizy, France). Uncropped scans of key immunoblots are shown in Supplementary Fig. 7.

Transient transfection and siRNA knockdown. Small interfering RNA and plasmids were introduced into monocytes by nucleofection (Lonza). Briefly, 5 × 10⁶ monocytes resuspended in 100 µL nucleofector solution with 1 nmol siRNA (CSF1-R (1): 5'-GGCUCAACCUCAAAGUCAUGGUGGA-3'; CSF1-R (2): 5'-GCAUCCGGCUGAAAGUGCAGAAAGU-3'; CSF1-R (3): 5'-GCUCAGCCAGCAGCGUUGAUGUUAA-3') and 150 ng total plasmids were electroporated. Cells were incubated 24 h with 5 ml of pre-warmed complete medium and 10 ng/mL IL-3 (Peprotech) were subsequently added. 48–72 h after transfection, cells were collected using EDTA for gene expression analysis. We used Stealth siRNA duplex (ThermoFisher Scientific) targeting *CFMS* and Stealth RNAi[™] siRNA Negative Control. Small interfering RNAs were introduced into macrophages by transfection with lipofectamin 2000 (ThermoFisher Scientific). Monocytes were cultured for 2 days with CSF-1 (100 ng/mL), medium was then replaced by the same medium without antibiotics, and cells were transfected with 0.1 nmol siRNA for 0.3 × 10⁶ cells according to the supplier instructions. Fourty-eight hours after transfection, cells were collected using EDTA for gene expression analysis.

Gene expression analysis. Total RNA was isolated from primary monocytes or macrophages with RNeasy mini Kit (Qiagen). Reverse transcription was carried out using random hexamers (ThermoFisher Scientific) and reverse transcriptase (Super Script II, ThermoFisher Scientific). Quantitative real-time PCR was performed using SYBR-Green (ThermoFisher Scientific) in an applied biosystem 7500 thermocycler using the standard SYBR-Green detection protocol as outlined by the manufacturers (Applied Biosystems, ThermoFisher Scientific). Briefly, 100–200 ng of total cDNA, 50 nM (each) primers, and 1× SYBR-Green mixture were used in a total volume of 20 µL. Specific primer sequences are provided in Supplementary Table 4.

ChIP-qPCR and ChIP-seq experiments. Cells were cross-linked with addition of 1% formaldehyde directly to the culture medium for 10 min at RT with agitation. Fixation was stopped by addition of 125 mM glycine during 5 min at RT with agitation and samples were then washed 2 times in ice-cold PBS before addition of SDS lysis buffer (Millipore, 10 µL per 1 × 10⁶ cells) supplemented with 1% protease inhibitor cocktail (Active Motif). Samples were vortexed and incubated 15 min on ice before 10 min sonication at 40 W (Covaris S220, Woodingdean, UK). The chromatin immunoprecipitation was carried out using ChIP-it express kit according to manufacturer's instruction (Active Motif). Enriched DNA from ChIP and Input DNA fragments were end-repaired, extended with an 'A' base on the 3' end, ligated with indexed paired-end adapters (NEXTflex, Bioo Scientific, Proteigene, Saint Marcel, France) using the Bravo Platform (Agilent, Les Ulis, France), size-selected after 4 cycles of PCR with AMPure XP beads (Beckman Coulter, Villepinte, France) and amplified by PCR for 10 cycles more. Fifty-cycle single-end

sequencing was performed using Illumina HiSeq 2000 (Illumina, San Diego, USA) or fifty-cycle paired-end sequencing was done using Illumina NovaSeq 6000.

ChIP-qPCRs were performed in the same way as RT-qPCRs with 2 μ L of ChIP or IgG samples instead of cDNA. Specific forward and reverse primers are in Supplementary Table 5.

ChIP-seq analysis. Reads were aligned into human genome hg19 with BWA aln (v0.7.5a). Peak calling for H3K4me3 and EGR1 was performed using MACS 2.1 using default options for narrow peaks. For CSF-1R and H3K4me1 ChIP-seq analysis, MACS2.1 was used using the following parameters: `–nomodel–broad–extsize 200`. Annotation and motif analyses have been done with HOMER (v4.9.1) using a window of 600 bp around peak summit. Bigwig files normalized for sequencing depths have been generated with deeptools v3.1.2. Integrative Genomics Viewer (IGV 2.4.14) was used for representation. Heatmaps of short read coverage across the genomic regions of interest (TSS, peaks) were done using deeptools v3.1.2.

Plasmids. For luciferase assays, we cloned EGR1 known motif identified by ChIP-seq analysis for CSF-1R recruitment in a Luciferase reporter system (pGL4.17 vector, Promega, Charbonnières-les-Bains, France). Four EGR1 motif identified sequences (F primer: 5'-CTAGCTGCGTGGGTGTGCG TGGGTGTGCGTGGG TGTGCGTGGGTGA-3'; R: 5'-AGCTTCACCCACGCACCCACGCACACC CACGCACA CCCACGCAG-3') were ordered at Eurofins (Les Ulis, France), denatured 5 min at 95 °C and hybridized by slow temperature drop before enzymatic cloning. CSF1-R binding PU.1 promoter sequences (PU.1 promoter 1 (complete) F primer: 5'-ATTAGTAGCTCTACTCATCCCTCCAC-3'; R: 5'-TAA GCTTCCAGCCGGGCTCCGAG-3', PU.1 promoter 2 (without CSF-1R recruitment sequence) F: 5'-ATTAGTAGCGGTAGATGGGTGGATAG-3'; R: 5'-ATTT AGATCTCCAGCCGGGCTCCGA-3') were first amplified with PCR from healthy monocyte genomic DNA, purified, sub-cloned in TOPO 2.1 vector (ThermoFisher Scientific) before enzymatic cloning in pGL4.17 vector. Luciferase plasmids were co-transfected with Renilla control reporter vector used for transfection efficiency normalization as described previously⁴².

CRISPR Knockout. Two EGR1 guide RNA sequences and Cas9 were encoded in V2 CRISPR-GFP or Cherry plasmid (one for each guide). Guide sequences were designed in the first exon of EGR1 with CRISPR software (<http://crispor.tefor.net/>) and cloned in one of the two lentiviral vectors: EGR1 guide 1 F: 5'-AAACGG CCGGTTACATGCGGGGC-3'; R: 5'-CACCGCCCGCATGTAACCCGGC C-3', EGR1 guide 2 F: 5'-AAACTCGGCGTAGGCCACTGCTTAC-3'; R: 5'-CAAC GTAAGCAGTGGCCTACGCCGA-3'. For lentiviral production, 293 T were co-transfected with plasmid of interest along with pCMV and pMD2.G plasmids using jetPrime reagent (Polyplus transfection, Ozyme) according to the manufacturer instructions. Retroviral particles were collected 48 h after transfection and concentrated by ultracentrifugation. THP-1 cells were co-transduced with a MOI of 2.5 with both guides and single-cell sorted based on their positive GFP and Cherry expression (BD Influx). EGR1 knockout was assessed by PCR and Sanger sequencing with the following primers: F: 5'-ATAGAGCGGATCCGGGGAGT C-3'; R: 5'-GAAACCCGGCTCTCATTCTAAGATC-3'.

Statistical analysis. All statistical analyses (Paired and unpaired *t* tests and Mann–Whitney test for Ctrl versus CMML *CEMS* expression) were performed using prism software.

Gene ontology (GO) analysis. Gene ontology annotation was performed using geneontology.org website. We selected the most accurate pathways based on higher fold change.

Patients. Peripheral blood samples were collected on EDTA from 28 patients with a CMML diagnosis according to the World Health Organization 2016 criteria⁴³. All the procedures were approved by the institutional board of Gustave Roussy and the ethical committee Ile de France 1 (DC-2014-2091, and written informed consent was obtained from each patient. Cohort characterization is summarized in Supplementary Table 6.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

Datasets are available in the ArrayExpress database at EMBL-EBI (www.ebi.ac.uk/arrayexpress). CSF-1R, H3K4me1, H3K4me3 ChIP-Seq comparing healthy donor monocyte and macrophage profiles (BC212: donor1; BC214: donor2; BC215: donor3), EGR1 ChIP-seq in healthy monocytes (BC275 and BC276) and CSF-1R ChIP-seq using Cterminal antibody in monocyte from healthy (BC270) and CMML (CMML1941: CMML#1982; CMML#2) monocytes are referenced as E-MTAB-6305 and CSF-1R ChIP-seq in THP1 WT clones, THP1 CRISPR clones and in CMML_2130 (CMML#3) and CMML_2609 (CMML#4) using N terminal antibody are referenced as E-MTAB-7756. Bed files of CSF-1R peaks shared by the three donors in monocytes (d0) and

macrophages (d3) are provided in Supplementary Data 15 and Supplementary Data 16. All other relevant data supporting the key findings of this study are available within the article and its Supplementary Information files or from the corresponding authors upon reasonable request. A reporting summary for this Article is available as a Supplementary Information file.

Received: 23 November 2017 Accepted: 10 April 2019

Published online: 26 April 2019

References

- Schlessinger, J. Receptor tyrosine kinases: legacy of the first two decades. *Cold Spring Harb. Perspect. Biol.* **6**, a008912–a008912 (2014).
- Carpenter, G. & Liao, H.-J. Receptor tyrosine kinases in the nucleus. *Cold Spring Harb. Perspect. Biol.* **5**, a008979–a008979 (2013).
- Chen, M.-K. & Hung, M.-C. Proteolytic cleavage, trafficking, and functions of nuclear receptor tyrosine kinases. *FEBS J.* **282**, 3693–3721 (2015).
- Wang, Y.-N. et al. COPI-mediated retrograde trafficking from the Golgi to the ER regulates EGFR nuclear transport. *Biochem. Biophys. Res. Commun.* **399**, 498–504 (2010).
- Gomes, D. A. et al. c-Met must translocate to the nucleus to initiate calcium signals. *J. Biol. Chem.* **283**, 4344–4351 (2008).
- Lin, S.-Y. et al. Nuclear localization of EGF receptor and its potential new role as a transcription factor. *Nat. Cell Biol.* **3**, 802–808 (2001).
- Lee, H.-J. et al. Tyrosine 370 phosphorylation of ATM positively regulates DNA damage response. *Cell Res.* **25**, 225–236 (2015).
- Lee, T.-H. et al. Vascular endothelial growth factor mediates intracrine survival in human breast carcinoma cells through internally expressed VEGFR1/FLT1. *PLoS Med.* **4**, e186 (2007).
- Matteucci, E., Bendinelli, P. & Desiderio, M. A. Nuclear localization of active HGF receptor Met in aggressive MDA-MB231 breast carcinoma cells. *Carcinogenesis* **30**, 937–945 (2009).
- Li, C., Iida, M., Dunn, E. F., Ghia, A. J. & Wheeler, D. L. Nuclear EGFR contributes to acquired resistance to cetuximab. *Oncogene* **28**, 3801–3813 (2009).
- Guilbert, L. J. & Stanley, E. R. Specific interaction of murine colony-stimulating factor with mononuclear phagocytic cells. *J. Cell Biol.* **85**, 153–159 (1980).
- Ries, C. H., Hoves, S., Cannarile, M. A. & Rüttinger, D. CSF-1/CSF-1R targeting agents in clinical development for cancer therapy. *Curr. Opin. Pharmacol.* **23**, 45–51 (2015).
- Lin, H. et al. Discovery of a cytokine and its receptor by functional screening of the extracellular proteome. *Science* **320**, 807–811 (2008).
- Li, W. & Stanley, E. R. Role of dimerization and modification of the CSF-1 receptor in its activation and internalization during the CSF-1 response. *EMBO J.* **10**, 277–288 (1991).
- Reedijk, M. et al. Tyr721 regulates specific binding of the CSF-1 receptor kinase insert to PI 3'-kinase SH2 domains: a model for SH2-mediated receptor-target interactions. *EMBO J.* **11**, 1365–1372 (1992).
- Jacquel, A. et al. Colony-stimulating factor-1-induced oscillations in phosphatidylinositol-3 kinase/AKT are required for caspase activation in monocytes undergoing differentiation into macrophages. *Blood* **114**, 3633–3641 (2009).
- Lee, P. S. et al. The Cbl protooncoprotein stimulates CSF-1 receptor multibiquitination and endocytosis, and attenuates macrophage proliferation. *EMBO J.* **18**, 3616–3628 (1999).
- Glenn, G. & van der Geer, P. CSF-1 and TPA stimulate independent pathways leading to lysosomal degradation or regulated intramembrane proteolysis of the CSF-1 receptor. *FEBS Lett.* **581**, 5377–5381 (2007).
- Barbetti, V. et al. Chromatin-associated CSF-1R binds to the promoter of proliferation-related genes in breast cancer cells. *Oncogene* **33**, 4359–4364 (2014).
- Zwaenepoel, O. et al. Functional CSF-1 receptors are located at the nuclear envelope and activated via the p110 isoform of PI 3-kinase. *FASEB J.* **26**, 691–706 (2012).
- Pham, T.-H. et al. Dynamic epigenetic enhancer signatures reveal key transcription factors associated with monocytic differentiation states. *Blood* **119**, e161–e171 (2012).
- Saeed, S. et al. Epigenetic programming of monocyte-to-macrophage differentiation and trained innate immunity. *Science* **345**, 1251086 (2014).
- Laslo, P. et al. Multilineage transcriptional priming and determination of alternate hematopoietic cell fates. *Cell* **126**, 755–766 (2006).
- Niedojadło, J. et al. Transcribed DNA is preferentially located in the perichromatin region of mammalian cell nuclei. *Exp. Cell Res.* **317**, 433–444 (2011).
- Kasza, A. et al. Transcription factors Elk-1 and SRF are engaged in IL1-dependent regulation of ZC3H12A expression. *BMC Mol. Biol.* **11**, 14 (2010).

26. Ogura, M. & Kitamura, M. Oxidant stress incites spreading of macrophages via extracellular signal-regulated kinases and p38 mitogen-activated protein kinase. *J. Immunol. Baltim. Md 1950* **161**, 3569–3574 (1998).
27. Joo, M. et al. Yin Yang 1 enhances cyclooxygenase-2 gene expression in macrophages. *AJP Lung Cell. Mol. Physiol.* **292**, L1219–L1226 (2007).
28. Selimoglu-Buet, D. et al. Characteristic repartition of monocyte subsets as a diagnostic signature of chronic myelomonocytic leukemia. *Blood* **125**, 3618–3626 (2015).
29. Deininger, M. W. N., Tyner, J. W. & Solary, E. Turning the tide in myelodysplastic/myeloproliferative neoplasms. *Nat. Rev. Cancer* **17**, 425–440 (2017).
30. Elizalde, P. V., Cordo Russo, R. I., Chervo, M. F. & Schillaci, R. ErbB-2 nuclear function in breast cancer growth, metastasis and resistance to therapy. *Endocr. Relat. Cancer* **23**, T243–T257 (2016).
31. Reif, R. et al. Activated ErbB3 translocates to the nucleus via clathrin-independent endocytosis, which is associated with proliferating cells. *J. Biol. Chem.* **291**, 3837–3847 (2016).
32. Williams, C. C. et al. The ERBB4/HER4 receptor tyrosine kinase regulates gene expression by functioning as a STAT5A nuclear chaperone. *J. Cell Biol.* **167**, 469–478 (2004).
33. Joyal, J.-S. et al. Subcellular localization of coagulation factor II receptor-like 1 in neurons governs angiogenesis. *Nat. Med* **20**, 1165–1173 (2014).
34. Chou, R.-H. et al. EGFR modulates DNA synthesis and repair through Tyr phosphorylation of histone H4. *Dev. Cell* **30**, 224–237 (2014).
35. Hu, Y. et al. 90-kDa ribosomal S6 kinase is a direct target for the nuclear fibroblast growth factor receptor 1 (FGFR1): role in FGFR1 signaling. *J. Biol. Chem.* **279**, 29325–29335 (2004).
36. Liu, H., Shi, B., Huang, C.-C., Eksarko, P. & Pope, R. M. Transcriptional diversity during monocyte to macrophage differentiation. *Immunol. Lett.* **117**, 70–80 (2008).
37. Komano, Y., Nanki, T., Hayashida, K., Taniguchi, K. & Miyasaka, N. Identification of a human peripheral blood monocyte subset that differentiates into osteoclasts. *Arthritis Res. Ther.* **8**, R152 (2006).
38. Geissmann, F. et al. Development of monocytes, macrophages, and dendritic cells. *Science* **327**, 656–661 (2010).
39. Merlevede, J. et al. Mutation allele burden remains unchanged in chronic myelomonocytic leukaemia responding to hypomethylating agents. *Nat. Commun.* **7**, 10767 (2016).
40. Mossadegh-Keller, N. et al. M-CSF instructs myeloid lineage fate in single haematopoietic stem cells. *Nature* **497**, 239–243 (2013).
41. Aucagne, R. et al. Transcription intermediary factor 1 γ is a tumor suppressor in mouse and human chronic myelomonocytic leukemia. *J. Clin. Invest.* **121**, 2361–2370 (2011).
42. Droin, N. M., Pinkoski, M. J., DeJardin, E. & Green, D. R. Egr family members regulate nonlymphoid expression of Fas ligand, TRAIL, and tumor necrosis factor during immune responses. *Mol. Cell. Biol.* **23**, 7638–7647 (2003).
43. Arber, D. A. et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood* **127**, 2391–2405 (2016).

Acknowledgements

This work was supported by INSERM, the Fondation ARC and the Ligue contre le cancer (labeled team). We are grateful to F. Ling and M. Gaudry who provided us sequencing primers for Luciferase cloning assessment, to M. Gastou for flow imaging and cellular compartment separation protocol, to F. Porteu, J.L. Villeval, and F. Louache for scientific discussions. We thank Gustave Roussy institute facilities for imaging (Sophie Salome-Desnoulz) and genomics, and the Imagine institute facilities for flow imaging.

Author contributions

L.B. designed, performed, analyzed the experiments, and drafted the manuscript, C.L. and K.D.M. performed bioinformatic analyses, J.R. provided assistance for cloning experiments, F.D.L. provided assistance for live cell imaging, M.D. for flow imaging, G.P. and S.S. for electron microscopy, A.I. and A.N. for ChIP-seq and RNA-seq, M.M. for managing patient samples, N.D. for qPCR experiments. E.S. and N.D. co-supervised the project and wrote the manuscript.

Additional information

Supplementary Information accompanies this paper at <https://doi.org/10.1038/s41467-019-09970-9>.

Competing interests: The authors declare no competing interests.

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Journal peer review information: *Nature Communications* thanks Terrence Furey and the other anonymous reviewer(s) for their contribution to the peer review of this work. Peer reviewer reports are available.

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Rôle dynamique de CSF1R nucléaire dans la lignée monocyttaire

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► Typiquement, les récepteurs à activité tyrosine kinase (RTK) sont des glycoprotéines transmembranaires qui, en réponse à la fixation d'un ligand, induisent des signaux intracellulaires stimulant la prolifération, la différenciation, et d'autres fonctions cellulaires. Chez l'homme, il existe 58 RTK classés en 20 sous-familles [1]. Ces récepteurs sont le plus souvent constitués d'une partie extracellulaire sur laquelle se fixe un ligand, d'un domaine juxta-membranaire ayant un rôle régulateur, d'un domaine portant l'activité kinase, et d'une région C-terminale. La fixation du ligand induit la dimérisation du récepteur et active sa fonction kinase par auto- ou trans-phosphorylation du domaine cytoplasmique [2]. Cette phosphorylation et le changement de conformation permettent le recrutement de protéines (kinases, phosphatases, protéines adaptatrices, etc.) impliquées dans la signalisation intracellulaire. Pour interrompre cette activité enzymatique, la cellule internalise le récepteur et le dégrade, généralement dans ses lysosomes, ou le recycle à la membrane plasmique après l'avoir inactivé.

Plusieurs de ces récepteurs sont également localisés dans le noyau (en anglais, *membrane receptor in nucleus*, ou MRIN). Des MRIN ont en effet été identifiés dans 12 des 20 sous-familles de RTK. Ils sont présents dans le noyau sous une forme entière (holorécepteur) ou sous la forme de molécule tronquée. Ils participent à la régulation transcriptionnelle et à la réparation de l'ADN. CSF1R (*colony stimulating*

factor 1 receptor) ou M-CSFR (*macrophage colony-stimulating factor receptor*) sont des MRIN. CSF1R joue un rôle essentiel dans le lignage monocyttaire et macrophagique. Ses ligands sont le CSF1 et l'interleukine-34 (IL-34). Il est anormalement exprimé par les cellules de nombreuses lignées cancéreuses d'origine épithéliale. C'est un RTK de classe III qui comporte deux domaines à activité kinase séparés par un domaine KI (*kinase insert*). Cette structure est caractéristique d'une famille dans laquelle on trouve aussi PDGF-R (*platelet-derived growth factor receptor*), KIT/SCFR (*mast/stem cell growth factor receptor*), et FLT3 (*Fms-like tyrosine kinase 3*). Seuls PDGF-R et CSF1R ont été détectés dans le noyau. CSF1R a d'abord été observé dans le noyau de macrophages murins et de cellules cancéreuses humaines. Cette localisation nucléaire a été attribuée à l'isoforme p110δ de la phosphoinositide 3-kinase (PI3K) et à la GTPase Rab5. Dans le noyau, CSF1R s'est montré capable de phosphoryler les protéines p27 et Akt (ou protéine kinase B) [3]. Dans des cellules cancéreuses humaines, CSF1R a aussi été détecté sur le promoteur des gènes *CCND1* (*cyclin D1*), *MYC*, *JUN* et *CSF1* [4], suggérant un rôle dans le contrôle transcriptionnel de la prolifération cellulaire.

En combinant plusieurs formes de microscopie, nous avons aussi détecté CSF1R dans le noyau des monocytes humains circulants et dans celui des macrophages issus de la différenciation de ces monocytes en réponse à un

ligand de CSF1R. C'est l'holorécepteur qui se retrouve dans le noyau de ces cellules et l'utilisation d'un ligand fluorescent a montré que le récepteur migre dans le noyau avec son ligand. Cette translocation nucléaire est bloquée par deux petites molécules inhibitrices de la fonction kinase de CSF1R, BLZ945 et GW2580. Par immunoprécipitation de la chromatine suivie de séquençage (ChIP-seq), nous avons détecté 4 980 « pics » CSF1R sur l'ADN des monocytes de sujets sains. Certains de ces pics étaient localisés sur le promoteur du gène *PU.1/SPI1* (*purine-rich nucleic acid-binding protein 1/SFFV provirus integration site-1*), codant un facteur de transcription de la différenciation myélo-monocytaire, et sur le site d'initiation de la transcription de *CSF2RB*, codant une des chaînes du récepteur du GM-CSF (*granulocyte monocyte-CSF*). Plus globalement, les motifs de l'ADN où les pics CSF1R ont été détectés correspondent aux sites de fixation des facteurs de transcription EGR (*early growth response*). Des ChIP-seq ciblant EGR1 ont montré une co-localisation des pics CSF1R et EGR1, le plus souvent dans les zones intergéniques, avec la marque H3K4me1 (*histone H3 mono-methylation at lysine 4*). Nous avons renouvelé ces expériences après avoir exposé les monocytes à CSF1, ligand de CSF1R, et avons alors observé une relocalisation de ce récepteur aux sites d'initiation de la transcription dès la sixième heure, un effet que nous avons également observé après 3 jours d'exposition à CSF1 (Figure 1). CSF1R est alors co-localisé avec la marque



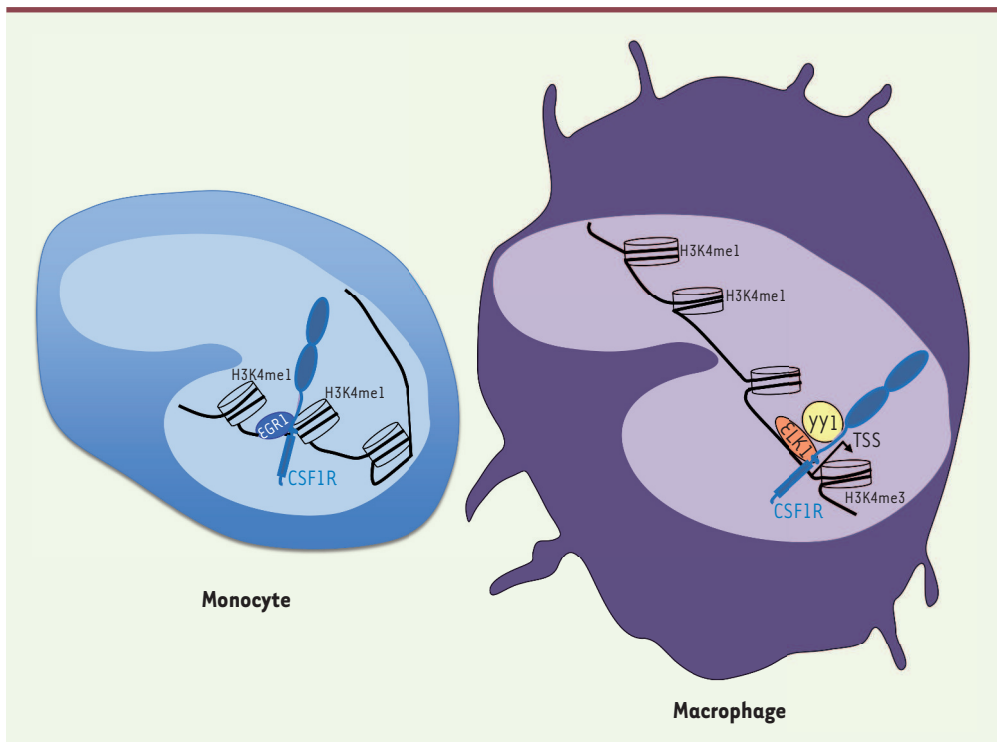


Figure 1. Les interactions de CSF1R avec la chromatine diffèrent dans les monocytes et dans les macrophages issus de la différenciation de ces monocytes. Dans les monocytes, CSF1R interagit avec EGR1, et est co-localisé avec H3K4me1 dans les régions intergéniques. Dans les macrophages, CSF1R interagit avec ELK1 ou YY1, et est co-localisé avec H3K4me3 aux sites initiateurs de la transcription (transcription start sites, ou TSS).

H3K4me3 (*histone H3 tri-methylation at lysine 4*). Les motifs de l'ADN où les pics CSF1R sont détectés correspondent aux sites de fixation des facteurs de transcription E2F4 (*E2F transcription factor 4*), ELK (*ETS transcription factor*), et YY1 (*ying yang 1*). Des expériences de co-immunoprécipitation ont confirmé l'interaction de CSF1R avec ELK1 et YY1. La leucémie myélo-monocytaire chronique est une hémopathie myéloïde clonale associée au vieillissement dans laquelle des monocytes s'accumulent dans la moelle osseuse et le sang. L'expression de CSF1R peut être diminuée dans les cellules du clone leucémique. Quand il est exprimé, CSF1R est encore détecté sur la chromatine, mais dans des régions distinctes de celles identifiées dans les monocytes de sujets sains [5].

La fonction de CSF1R ne se limite donc pas à l'induction de signaux intracellulaires à partir de la membrane plasmique, mais comporte également des rôles nucléaires. Cette découverte pourrait éclairer plusieurs aspects de la différenciation myélo-monocytaire

normale et pathologique. Pendant des années, l'engagement d'une cellule souche hématopoïétique dans une voie de différenciation a été attribué aux variations stochastiques de l'équilibre entre facteurs de transcription. En 2013, l'équipe dirigée par Michael Sieveke a montré que CSF1 induisait l'expression du gène *PU.1*, un puissant régulateur de la différenciation myéloïde, dans des cellules souches hématopoïétiques murines, suggérant un effet instructeur de facteurs extrinsèques sur leur différenciation [6]. La présence de CSF1R dans le noyau des cellules souches et progénitrices hématopoïétiques suggère que cet effet instructeur pourrait s'exercer également sur l'ADN. La forme nucléaire de CSF1R pourrait aussi contribuer à l'hétérogénéité des monocytes circulants. Depuis une dizaine d'années, une nomenclature internationale distingue trois populations de monocytes selon le taux d'expression des gènes *CD14* et *CD16* (*cluster of differentiation 14 and 16*): les monocytes classiques ($CD14^{++}/CD16^{-}$), intermédiaires ($CD14^{++}/CD16^{++}$), et non-classiques

($CD14^{-}/CD16^{++}$) constituent respectivement environ 85 %, 5 %, et 10 % des monocytes circulants. Les mécanismes à l'origine de cette hétérogénéité restent controversés, mais une partie au moins des monocytes intermédiaires et non-classiques dérivent des monocytes classiques [7]. Les mécanismes moléculaires de cette conversion, encore imparfaitement compris, impliquent notamment un axe miR150/TET3 (*microRNA150 / methylcytosine dioxygenase 3*), dérégulé par un mécanisme épigénétique dans la leucémie myélo-monocytaire chronique [8]. CSF1R est aussi impliqué dans cette conversion puisque, dans la leucodystrophie héréditaire diffuse (*hereditary diffuse leukodystrophy with axonal spheroids*), une microgliopathie rare due à des mutations constitutives inactivatrices du domaine tyrosine kinase de CSF1R, on observe une diminution du nombre de monocytes intermédiaires et non-classiques [9]. En outre, un anticorps bloquant CSF1R, utilisé dans le traitement de la polyarthrite rhumatoïde, entraîne une déplétion des monocytes non-classiques [10]. Il reste à

déterminer si les fonctions nucléaires de CSF1R jouent un rôle dans la conversion d'un monocyte classique en monocyte non-classique, et si la perturbation de ces fonctions observée dans la leucémie myélo-monocytaire chronique contribue également à la répartition anormale des populations monocytaires caractéristique de cette maladie. ♦

Dynamic role of nuclear CSF1R in the monocytic lineage

LIENS D'INTÉRÊT

Les auteurs déclarent n'avoir aucun lien d'intérêt concernant les données publiées dans cet article.

RÉFÉRENCES

1. Blume-Jensen P, Hunter T. Oncogenic kinase signalling. *Nature* 2001 ; 411 : 355-65.
2. Lemmon MA, Schlessinger J. Cell signaling by receptor tyrosine kinases. *Cell* 2010 ; 141 : 1117-34.
3. Zwaenepoel O, Tzenaki N, Vergetaki A, et al. Functional CSF-1 receptors are located at the nuclear envelope and activated via the p110δ isoform of PI 3-kinase. *FASEB J* 2012 ; 26 : 691-706.
4. Barbetti V, Morandi A, Tusa I, et al. Chromatin-associated CSF-1R binds to the promoter of proliferation-related genes in breast cancer cells. *Oncogene* 2014 ; 33 : 4359-64.
5. Bencheikh L, Diop MK, Rivière J, et al. Dynamic gene regulation by nuclear colony-stimulating factor 1 receptor in human monocytes and macrophages. *Nat Commun* 2019 ; 10 : 1935.
6. Mossadegh-Keller N, Sarrazin S, Kandalla PK, et al. M-CSF instructs myeloid lineage fate in single haematopoietic stem cells. *Nature* 2013 ; 497 : 239-43.
7. Patel AA, Zhang Y, Fullerton JN, et al. The fate and lifespan of human monocyte subsets in steady state and systemic inflammation. *J Exp Med* 2017 ; 214 : 1913-23.
8. Selimoglu-Buet D, Rivière J, Ghamlouch H, et al. A miR-150/TET3 pathway regulates the generation of mouse and human non-classical monocyte subset. *Nat Commun* 2018 ; 9 : 5455.
9. Hofer TP, Zawada AM, Frankenberger M, et al. Slan-defined subsets of CD16-positive monocytes: impact of granulomatous inflammation and M-CSF receptor mutation. *Blood* 2015 ; 126 : 2601-10.
10. Korkosz M, Bukowska-Strakova K, Sadis S, et al. Monoclonal antibodies against macrophage colony-stimulating factor diminish the number of circulating intermediate and nonclassical (CD14⁺CD16⁺/CD14⁺CD16⁺) monocytes in rheumatoid arthritis patient. *Blood* 2012 ; 119 : 5329-30.



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Stepwise GATA1 and SMC3 mutations alter megakaryocyte differentiation in a Down syndrome leukemia model

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Acute megakaryoblastic leukemia of Down syndrome (DS-AMKL) is a model of clonal evolution from a preleukemic transient myeloproliferative disorder requiring both a trisomy 21 (T21) and a *GATA1s* mutation to a leukemia driven by additional driver mutations. We modeled the megakaryocyte differentiation defect through stepwise gene editing of *GATA1s*, *SMC3*^{-/-}, and *MPL*^{W515K}, providing 20 different T21 or disomy 21 (D21) induced pluripotent stem cell (iPSC) clones. *GATA1s* profoundly reshaped iPSC-derived hematopoietic architecture with gradual myeloid-to-megakaryocyte shift and megakaryocyte differentiation alteration upon addition of *SMC3* and *MPL* mutations. Transcriptional, chromatin accessibility, and *GATA1*-binding data showed alteration of essential megakaryocyte differentiation genes, including *NFE2* downregulation that was associated with loss of *GATA1s* binding and functionally involved in megakaryocyte differentiation blockage. T21 enhanced the proliferative phenotype, reproducing the cellular and molecular abnormalities of DS-AMKL. Our study provides an array of human cell-based models revealing individual contributions of different mutations to DS-AMKL differentiation blockage, a major determinant of leukemic progression.

Introduction

Acute megakaryoblastic leukemias (AMKLs) are rare disorders that are more frequent in children (1, 2). Children with Down syndrome (DS) who have constitutive trisomy 21 (T21) have an increased risk of developing AMKL (3). While children with DS-AMKL have unique phenotypic and clinical features, this condition also represents a model of multistep leukemogenesis with key characteristics found in many leukemias (4).

DS-AMKL is always preceded by a clonal preleukemic state called transient myeloproliferative disorder (TMD) driven by *GATA1* mutations, mostly in exon 2 and resulting in the expression of an N-terminal deleted shorter protein, called *GATA1s* (5,

6). Usually, TMD spontaneously regresses, but 10% to 20% of these children will develop DS-AMKL within 5 years (7). This leukemia originates from the initial *GATA1s* clone with 2 or more acquired additional mutations (8, 9), including loss-of-function mutations in cohesin complex genes (e.g., *STAG2*, *RAD21*, *SMC1A/3*) and a mutation in a gene encoding a signaling molecule leading to RAS or JAK/STAT pathway constitutive activation (8, 9). *EZH2* or *SUZ12*, 2 members of PRC2, are frequently mutated in addition to the other mutations.

How *GATA1s* and these secondary mutations cooperate to induce AMKL and why this leukemia development requires a T21 and a fetal hematopoiesis remain unclear and need modeling approaches. Mouse models have been limited by only minor defects induced by *Gata1s* in late megakaryocyte (MK) differentiation (10–13). Thus, it was crucial to develop human models of DS-AMKL. An approach consisting of introducing *GATA1s* in induced pluripotent stem cells (iPSCs) or deriving iPSCs from T21 individuals with and without TMD has been developed, but was mostly focused on the erythroid cells (14–17). A more recent approach has been to edit the *GATA1s* and *STAG2* mutations in primary human CD34⁺ cells, including T21 CD34⁺ fetal liver cells, followed by xenotransplantation (18, 19). The latter approach was focused on hematopoiet-

► **Related Commentary:** <https://doi.org/10.1172/JCI161659>

Authorship note: TM and WV contributed equally to this work.

Conflict of interest: The authors have declared that no conflict of interest exists.

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Submitted: November 1, 2021; **Accepted:** May 13, 2022; **Published:** July 15, 2022.

Reference information: *J Clin Invest.* 2022;132(14):e156290.

<https://doi.org/10.1172/JCI156290>.

ic stem and progenitor cells (HSCP) and has clearly unveiled that the cooperation between *GATA1s* and *STAG2* mutations induces self-renewal and progression toward AMKL.

In contrast to erythroid differentiation (20, 21), the precise role of *GATA1s* on human MK differentiation is incompletely understood (17). It appears essential to better understanding how *GATA1s* precisely cooperates with T21 and additional mutations to induce MK differentiation blockage and proliferation, two key features of the leukemic process. Based on the consequences of cohesin mutations on HSPC (22–24), we hypothesized that *SMC3* haploinsufficiency may modify the chromatin occupancy of *GATA1s* and other transcription factors regulating late MK differentiation. We modeled the effects of different mutations implicated in the progression of TMD to AMKL on human hematopoiesis and more particularly on the MK differentiation. For this purpose, we sequentially introduced *GATA1s*, a gain-of-function *MPL* mutation (*MPL*^{W515K}) (25, 26) and a heterozygous inactivation of *SMC3* (*SMC3*^{+/-}), using CRISPR-Cas9 technology in T21 iPSC. Moreover, to understand the role of T21, we contrasted disomic 21 (D21) with T21 iPSCs. This approach provides insights at the cellular and molecular levels on how *GATA1s* alters MK differentiation alone or in cooperation with *SMC3* deficiency to deeply enhance differentiation alterations and increase proliferation.

Results

Sequential editing generates a panel of iPSC lines to investigate the different steps of DS-AMKL progression. To recapitulate DS-AMKL progression, we sequentially introduced by CRISPR/Cas9-mediated engineering *GATA1s* mutations (G), followed by an *MPL*^{W515K} mutation (M) and *SMC3* mutations (S), into the parental T iPSCs (Figure 1A).

First, we generated 2 Trisomy 21 *GATA1s* (TG) iPSC clones carrying *GATA1s* truncating mutations and confirmed that T MKs expressed both full-length (FL) and short *GATA1* isoforms, while TG mutant MKs expressed only *GATA1s* (Supplemental Figure 1, A, B, and R; supplemental material available online with this article; <https://doi.org/10.1172/JCI156290DS1>). Thereafter, the *MPL*^{W515K} gain-of-function mutation was introduced in a TG clone to generate 4 Trisomy 21 *GATA1s* *MPL*^{W515K/W515K} (TGM) clones (Supplemental Figure 1, C–F and S). To assess the functionality of the *MPL*^{W515K} mutation, we quantified the MK differentiation (% of CD41⁺CD42⁺ cells) from *MPL*^{WT/WT} and *MPL*^{W515K/W515K} iPSCs (Supplemental Figure 1, G and H). The MK differentiation obtained from the TGM in the presence of Stem Cell Factor (SCF) was Thrombopoietin (TPO) independent and abolished by ruxolitinib, a JAK1/JAK2 inhibitor, demonstrating that the *MPL*^{W515K} mutant was functional.

To assess the role of *SMC3* haploinsufficiency, we edited *SMC3* in the heterozygous state in T, TG, and TGM and we obtained 3 clones in each genetic context (TS, TGS, TGMS) (Supplemental Figure 1, I–K). *SMC3* haploinsufficiency was confirmed by Western blots (Supplemental Figure 1, L and M). Notably, intracellular flow cytometry analysis indicated a similar increase in *GATA1* protein levels in MKs from TG and TGMS as compared with T (Supplemental Figure 1, N and O).

To understand the role of T21, we derived an isogenic D *GATA1s* *MPL*^{W515K} (DGM) clone by a spontaneous loss of a chromosome 21 (Supplemental Figure 1V). Then we generated 2 isogenic

DGMS clones (Supplemental Figure 1, T and V). MKs from DGMS and TGMS presented similar levels of *GATA1* protein (Supplemental Figure 1, P and Q).

All iPSC clones were genetically characterized by karyotypic analyses (Supplemental Figure 1, U and V), fluorescence in situ hybridization (Supplemental Figure 1W), and Sanger sequencing (no off-targets in CRISPR/Cas9-edited iPSC clones; Supplemental Table 1). We obtained a panel of 20 different iPSC clones, presenting functionally validated mutations in *GATA1*, *MPL*, and *SMC3* that allowed us to study the contribution of each mutation, including T21, and those mutations' cooperation in the progression to DS-AMKL.

GATA1s and SMC3^{+/-} alter hematopoietic progenitors and MK yield. To investigate the hematopoietic differentiation of the different iPSC lines, we used a 2D cell culture with a sequential addition of cytokines (Figure 1B) and CD34⁺CD43⁺ hematopoietic progenitor cells (HPC) purified from the supernatant at day 13 of culture. While T, TS clones generated erythroid and myeloid colonies, TG clones showed increased myeloid, but abrogated erythroid, colony formation (Supplemental Figure 2, A and B), confirming previous data (16). The addition of *MPL*^{W515K/W515K} and/or *SMC3*^{+/-} had no significant effect (Supplemental Figure 2, A and B).

The MK colony-forming potential, the size of MK colonies, and the total number of generated CD41⁺CD42⁺ MKs were significantly increased in T21 only when *GATA1s* and *SMC3*^{+/-} were combined (Figure 1, C–E). T and TS showed typical micro-MK (Figure 1F), characteristic for the iPSC-derived MKs (iMKs). However, all genotypes presenting *GATA1s* showed the presence of large-sized basophilic dysplastic multilobulated MKs (Figure 1F). The predominant classes of ploidy were the 2N and 4N, as observed for MK derived from WT human iPSCs or embryonic stem (ES) cells (27). *GATA1s* increased the mean percentage of more than 4N MKs in synergy with S, but not M (Figure 1, F and G). These data support the idea that *GATA1s* cooperates with *SMC3* haploinsufficiency in T21 to alter MK differentiation, including increased clonogenic potential and proliferation of MK progenitors with accumulation at an immature stage and increased size and ploidy in some immature MKs.

Effects of SMC3^{+/-} and GATA1s on MK maturation. We then assessed cell-surface marker expression. While a fraction of CD41⁺ cells were CD42 negative, as revealed by an anti-CD42a antibody, the vast majority expressed the CD42 from all genotypes (Figure 2, A and B), including an independent D21 iPSC derived from a healthy individual (Supplemental Figure 3, A and B). This phenotype matches what is observed in the MK differentiation of iPSCs or primary CD34⁺ cells. However, MFI was decreased by *GATA1s* independently of the associated mutations, suggesting that *GATA1s* impaired terminal MK maturation (Figure 2, A and B). The CD41⁺CD42⁺ cell fractions that maintained CD34 expression were 30% and 10% in D21 and T21, respectively (Supplemental Figure 3C). CD34 expression was increased by *GATA1s* in T21 (TG) to 45% and further by *SMC3* haploinsufficiency to 55% (Figure 2, A and C). TMD and DS-AMKL megakaryoblasts are characterized by the expression of CD34, CD41, and CD42 antigens, but also other markers, such as KIT and CD7 (28, 29). While only a small fraction of D21 or T21 CD41⁺ cells expresses KIT (Supplemental Figure 3A), it is detected on over half of the *GATA1s* CD41⁺

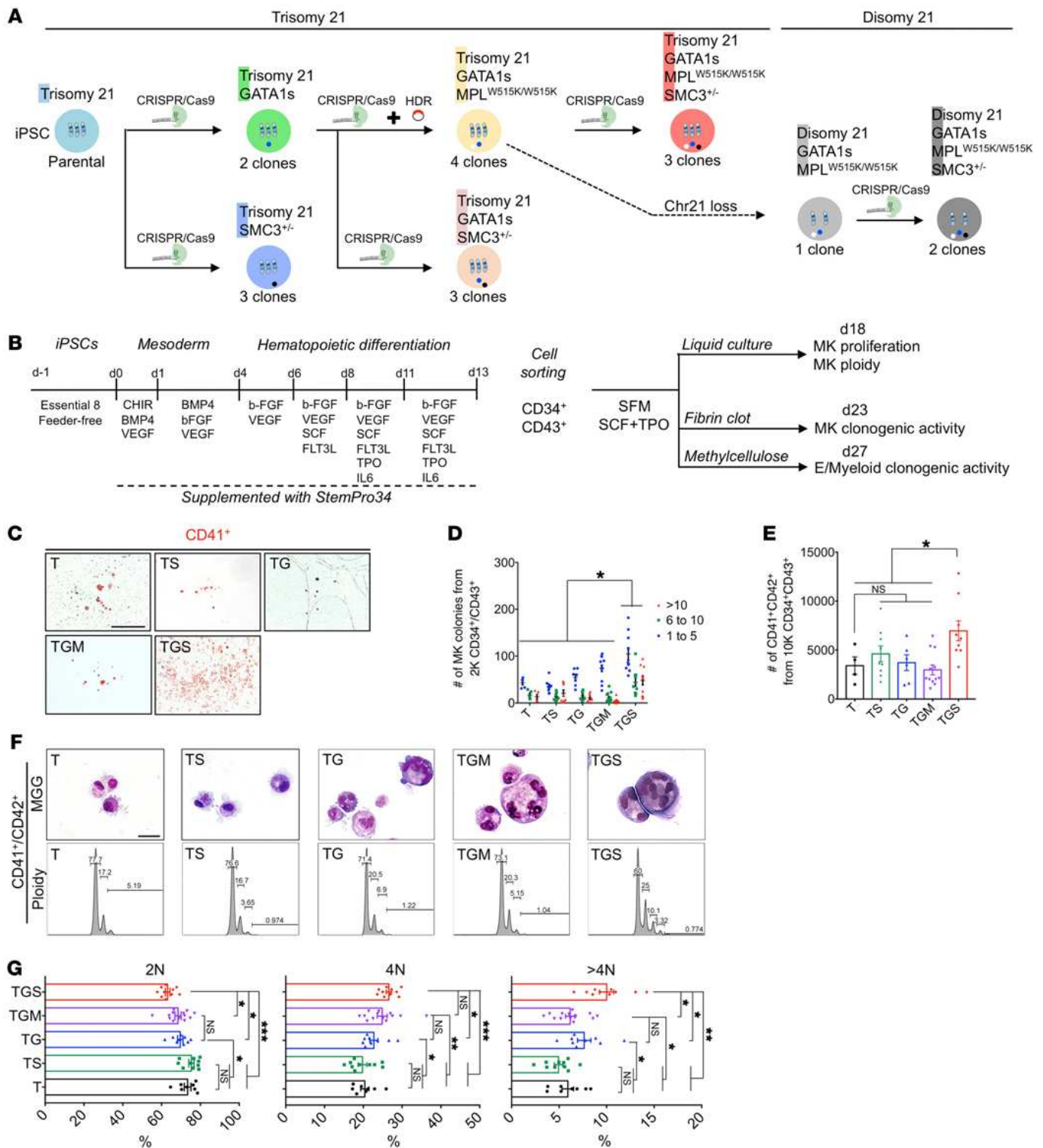


Figure 1. *GATA1s* cooperates with *SMC3*^{-/-} to increase MK clonogenic potential, proliferation, and polyploidization. (A) Schematic overview of the iPSC clones generated by a stepwise introduction of *GATA1s*, *MPL*^{W515K}, and *SMC3*^{-/-}, using CRISPR/Cas9. Bold letters highlight the abbreviation used hereafter. The number of clones obtained for each genotype and subjected to MK differentiation studies is indicated. The dashed arrow indicates the isogenic D iPSC clone harboring *GATA1s* and *MPL*^{W515K} mutations randomly obtained through loss of 1 chromosome 21. (B) Schematic overview of the hematopoietic differentiation method used and the subsequent MK phenotypic characterization. (C) Representative images of CFU-MK colonies. Scale bar: 500 μm. (D) Histogram of the number of CD41⁺ colonies obtained from 2000 CD34⁺CD43⁺ in fibrin clot assay. (E) Histogram of the number of CD41⁺CD42⁺ MKs obtained from 10,000 CD34⁺CD43⁺ in liquid culture assay. Data are represented as mean ± SEM; n = 3–4. The number of clones tested per genotype was as follows: T/parental = 1; TS = 3; TG = 2; TGM = 4; TGS = 3. (F) May-Grünwald-Giemsa (MGG) staining (E, upper panels) and ploidy plots (E, lower panels) of iMK according to the indicated genotypes. T and TS CD41⁺CD42⁺ showed typically mature micro-MKs with acidophilic cytoplasm, while TG, TGM, or TGS showed large polyploid immature MKs with basophilic cytoplasm. Scale bar: 50 μm. (G) Histograms of the percentages of 2N, 4N, and >4N of iMKs. Data are represented as mean ± SEM; n = 3 to 8. The number of clones tested per genotype was as follows: T/parental = 1; TS = 2; TG = 2; TGM = 4; TGS = 3. Statistical significance was determined using 1-tailed Mann-Whitney U test. *P < 0.05; **P < 0.01; ***P < 0.001.

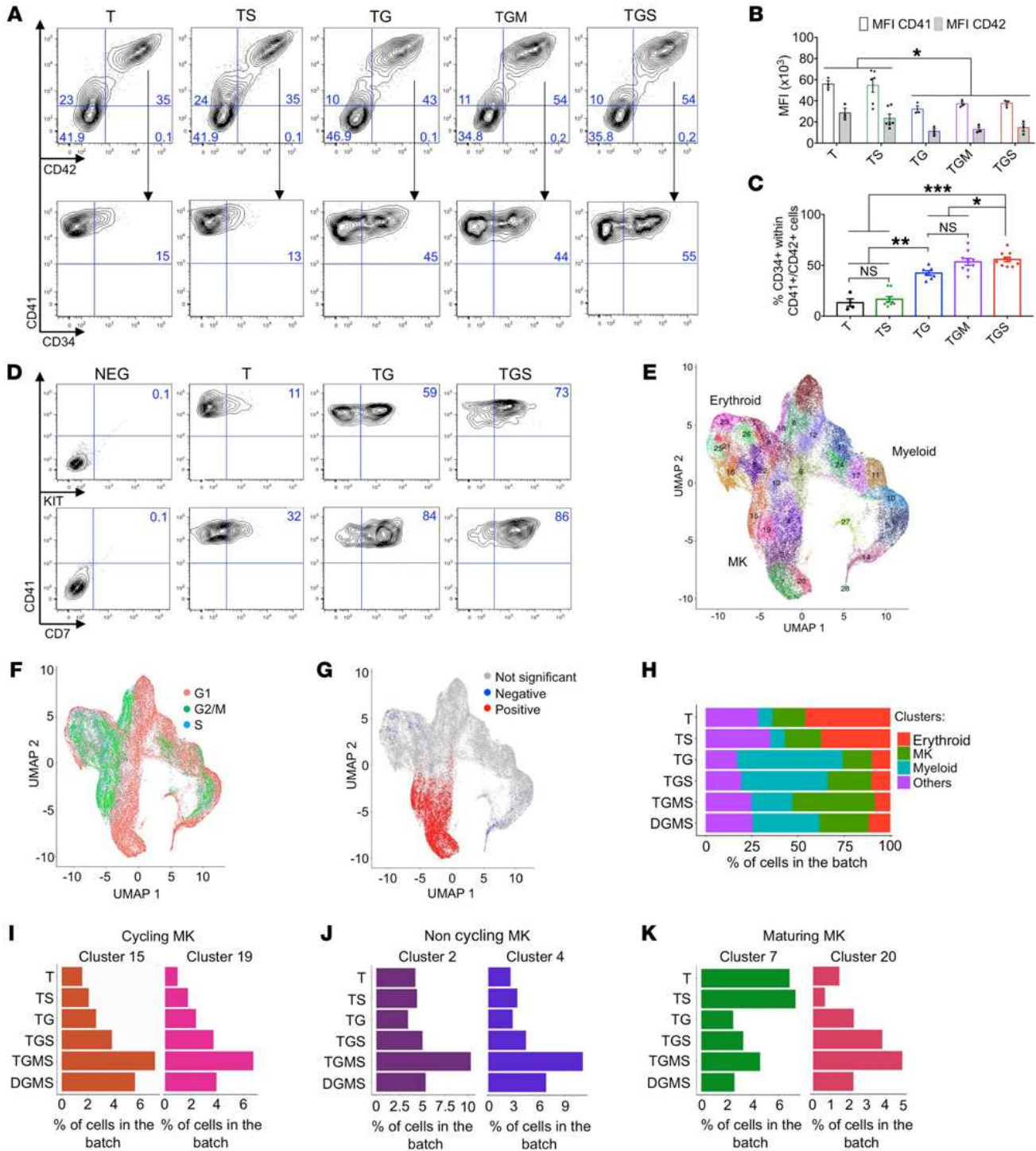


Figure 2. Assessment of iMK differentiation alterations. (A–D) Immunophenotypes of iMKs for the CD34, KIT, CD7, CD41, and CD42 markers found in DS-AMKL patient blasts. (A) Contour plots showing the expression of CD34, CD41, and CD42 markers. (B) Histogram shows the MFI of CD41 and CD42. (C) Histogram shows the percentage of CD34⁺CD41⁺ per total CD41⁺ population. Data in B and C are represented as mean ± SEM; n = 3–4. The number of clones tested per genotype was as follows: T/parental = 1; TS = 2; TG = 2; TGM = 3; TGS = 3. Statistical significance was determined using 1-tailed Mann-Whitney’s U test: *P < 0.05; **P < 0.01; ***P < 0.001. (D) Representative contour plots of KIT and CD7 expression in the iMK population from 2 independent experiments. (E and F) scRNA-Seq of CD43⁺ iPSC-derived hematopoietic cells at day 13 of culture. (E) UMAP integration of cells from all conditions. Clusters were defined using the Louvain algorithm and numbered and labeled with unique colors. (F) UMAP integration with cells colored according to the predicted cell-cycle stage (Seurat method). (G) UMAP integration with cells colored according to the enrichment in a MK signature. Red, cells are significantly enriched for the signature; blue, cells are significantly depleted for the signature; gray, no significant enrichment. (H) Bar plot shows the proportion of cells in the indicated hematopoietic lineages for each condition. (I) Bar plots of the proportion of cells in the 2 clusters of cycling MKs. (J) Bar plots of the proportion of cells in the 2 clusters of noncycling MKs. (K) Bar plots of the proportion of cells in the 2 clusters of maturing MKs. Cluster 7 represents normal maturing MKs. Cluster 20 represents an abnormal MK population.

cells and further increased by *SMC3* haploinsufficiency (Figure 2D). Similar results were observed for CD7 (Supplemental Figure 3A and Figure 2D). These results show that the *GATA1*s-induced blockage of MK differentiation at the megakaryoblast stage is worsened by *SMC3* haploinsufficiency.

To obtain a precise picture of the altered differentiation, we performed single-cell RNA-Seq (scRNA-Seq) for D, T, TS, TG, TGS, TGMS, and DGMS at an early differentiation time point (day 13). From 6800 to 14,001 cells with similar characteristics were analyzed per condition (Supplemental Figure 4A). UMAP clustering (<https://umap-learn.readthedocs.io/en/latest/clustering.html>) obtained by pooling cells from all the conditions provided 28 clusters (Figure 2E) for which cell-cycle status was inferred using Seurat (Figure 2F and Supplemental Figure 5A) and cells of the different clusters showed expression of MK, erythroid, and myeloid differentiation markers (Figure 2G, Supplemental Figure 4, B–D, and Supplemental Figure 5B). To identify all cells belonging to the MK, erythroid, and myeloid lineages, we computed scores for lineage-specific gene lists (Supplemental Figure 4E). T21 differentiation was clearly biased toward the erythroid lineage in comparison with D21 (Supplemental Figure 5C). The highest percentage of MK cells was observed in TGMS (Figure 2H). By quantifying the clusters along MK differentiation, we observed that the cycling, noncycling, and maturing MKs were similar between D21 and T21 (Supplemental Figure 5, D–F). Interestingly, a gradual increase for the cycling MKs (clusters 15 and 19) was observed in TS, TG, TGS, and TGMS as compared with T, with the highest percentage in TGMS (Figure 2I). Noncycling MK clusters (clusters 2 and 4) were more specifically amplified in TGMS as compared with T, TS, TG, and TGS (Figure 2J). Strikingly, 2 clusters were associated with the highest expression of maturing MK markers (clusters 7 and 20) (Figure 2K). Cluster 7 represented the vast majority of cells in T and TS, while cluster 20 was gradually increased in TG, TGS, and TGMS, representing approximately 50% of maturing MKs in TGMS.

Together, these data show that *GATA1*s and *SMC3*^{+/−} cooperated together and with T21 through (a) an increase in immature cycling MKs and (b) an altered MK differentiation with the predominance of a cluster of maturing MKs that represents a minority among D21 or T21 MKs. *MPL*^{W515K} markedly increased MK output at the expense of the myeloid lineage.

*GATA1*s impairs organelle development and proplatelet formation. To better understand the cellular defect in MK differentiation, we performed staining of vWF and CD63, respective markers of α -granules and lysosomes, both deriving from multivesicular bodies (Figure 3A). In contrast with T and TS MKs, TG MKs showed the presence of abnormally large sized α -granules, as attested by the vWF labeling (Figure 3A). Colabeling with the anti-CD63 antibody was as rare as in control MKs (10% of total MK), attesting that these large granules were not multivesicular bodies (Figure 3A). The anti-CD41 antibody was used to highlight demarcation membrane system (DMS) development (Figure 3B; adapted from ref. 30). In T and TS MKs, CD41 was localized at the cell surface and well distributed in the cytoplasm (Figure 3C) in more than 90% of the MKs. In contrast, in the presence of *GATA1*s, CD41 was detected in the center of the cell between the nuclear lobes and only very weakly at the cell surface in the

great majority of the cells (Figure 3C). Complete Z-stack analysis showed that this intracytoplasmic labeling was in continuity with the cell surface with usually only a single connection demonstrating the presence of a rudimentary DMS. This abnormality was even increased by *SMC3*^{+/−} with a more polarized labeling (Figure 3C). Ultrastructural studies confirmed that the *GATA1*s mutation was associated with the presence of very large cells (over 40 μ m diameter) with a large multilobulated uncondensed nucleus containing a majority of euchromatin and large atypical granules (Figure 3D). Only a pre-DMS could be detected, usually localized between the nuclear lobes, a defect that was increased by *SMC3*^{+/−}.

Then, we investigated the effects of *GATA1*s and the other mutations on proplatelet (PPT) formation. T and TS MKs were able to form PPTs (Figure 3E). Unexpectedly, *SMC3*^{+/−} increased the percentage of PPT-forming MKs, suggesting that, in the context of *GATA1*^{WT}, it favors MK maturation (Figure 3F). In contrast, MKs from all *GATA1*s conditions, including with *SMC3*^{+/−}, barely formed PPT (Figure 3, E and F).

Combined GATA1s and SMC3^{+/−} induce proliferative and DS-AMKL signatures. We then performed transcriptomic analyses. To study a pure population of MKs, we excluded cells positive for the CD33 myeloid marker and sorted CD41⁺CD42⁺CD33[−] cells using 2 consecutive sorting steps to achieve a purity greater than 98%, confirmed by flow cytometry reanalysis and cytology (Supplemental Figure 6, A–C). By principal component analysis (PCA), a clear clustering of samples according to *GATA1*s or *SMC3*^{+/−} status was found (Supplemental Figure 6D) with a large number of deregulated genes (DEGs) in TG versus T and TS, respectively (adjusted $P < 0.05$; Supplemental Figure 6E). *MPL*^{W515K} did not influence the clustering and had little impact on gene expression (Supplemental Figure 6, D and E). In contrast, TGS importantly increased the number of DEGs when compared with T, TS, and TG, respectively (Supplemental Figure 6E).

The genes commonly upregulated by TGS versus T, TS, or TG were enriched in signatures associated with cell-cycle activity and DNA replication (Supplemental Figure 6F), including MYC signatures (Figure 4, A and B) and MYC target genes (e.g. *PAICS*, *MYBPIA*, *BOPI*, *ATIC*, and *CDK4*) (Figure 4C). Importantly, Gene Set Enrichment Analyses (GSEAs) indicated that the gene signature characterizing DS-AMKL versus DS-TMD blast patients (31) was also enriched in the TGS versus TG MKs (Figure 4D). Accordingly, the gene signature of DS-TMD versus DS-AMKL patients was enriched in TG versus TGS MKs (Figure 4D). These results support the idea that *SMC3* haploinsufficiency acquisition, in the context of T21 and *GATA1*s, leads to increased proliferation properties and reproduces the molecular progression observed from TMD to DS-AMKL in patients.

SMC3^{+/−} cooperates with GATA1s to impair GATA1 target and MK maturation gene expression. We subsequently analyzed *GATA1* target genes involved in MK maturation. Gene Ontology and GSEA analyses indicated that *GATA1* target genes (32) are downregulated in TG versus T (Figure 4, E and F). *SMC3*^{+/−} further decreased expression of *GATA* targets when associated with *GATA1*s (Figure 4, E and G), but had no significant effect on *GATA1* targets in a *GATA1*^{WT} context (Figure 4F). Genes involved in DMS development, such as *GP1BA* and *PACSIN2* (Figure 4, H and I), and genes encoding the RAB family proteins controlling membrane traf-

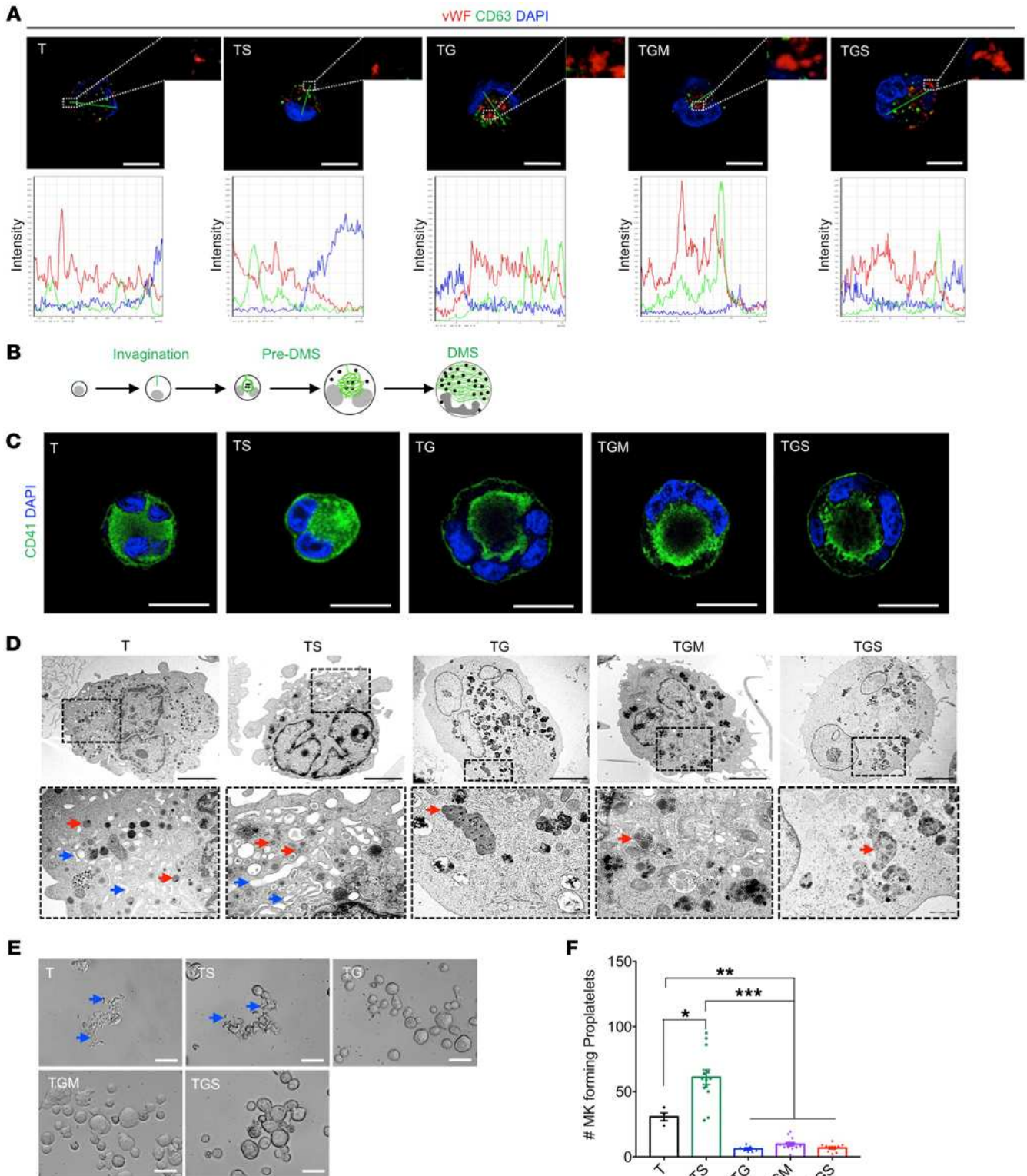


Figure 3. Assessment of MK maturation and platelet formation. (A) Confocal analyses of vWF and CD63 expression in MK (left panel) and colocalization analyses (right panel). Scale bars: 50 μ m. (B) Schematic overview of the normal development steps of DMS. (C) Confocal analysis of CD41 marker distribution in iMKs. Scale bars: 50 μ m. (D) Ultrastructural characterization of the iMK according to the compared genotypes. A representative MK is shown for each condition (upper panels), a part of which (dotted square) is enlarged (lower panels). More than 80% of T and TS MKs showed maturity characteristics associated with condensed nuclei, a well-developed DMS (blue arrows), and the presence of normal α -granules (red arrows). More than 90% of TG, TGM, and TGS MKs showed blockages in maturation characterized by the presence of uncondensed nuclei majorly composed of euchromatin and the absence of DMS formation, with endosomes presenting an abnormal accumulation of granules (red arrows). Scale bars: 5 μ m. (E) Representative microphotographs of CD41⁺CD42⁺ iMKs under PPT formation assay. PPT-forming MKs are highlighted with blue arrows in the T and TS conditions. Scale bars: 50 μ m. (F) Histogram of the number of PPT-forming MKs in the compared conditions. Data are represented as mean \pm SEM; $n = 3-4$. The number of clones tested per genotype was as follows: T/parental = 1; TS = 3; TG = 2; TGM = 4; TGS = 3. Statistical significance was determined using 1-tailed Mann-Whitney's *U* test. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

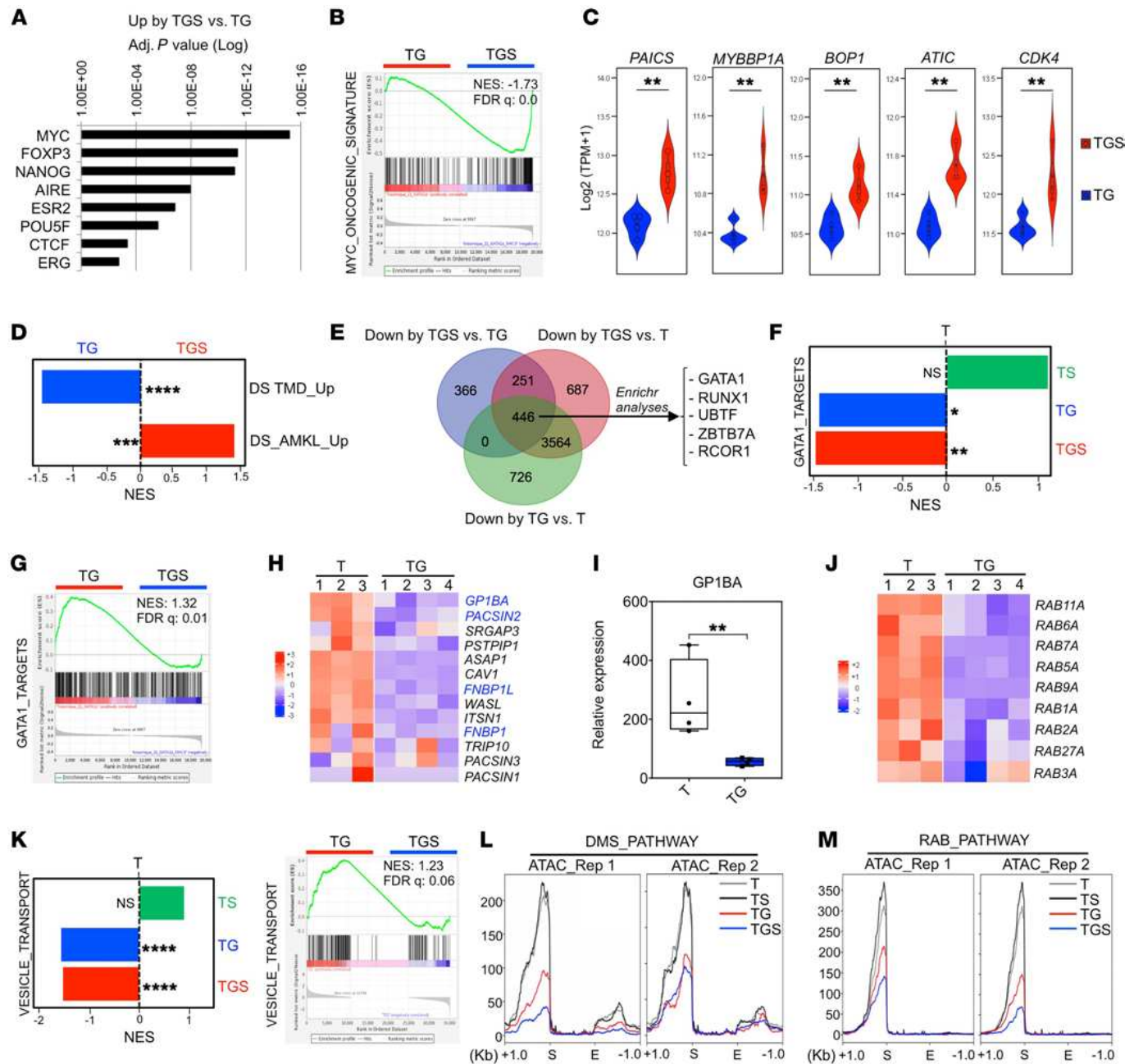


Figure 4. *GATA1s* cooperates with *SMC3^{-/-}* to acquire DS-AMKL features in iMK. (A) Transcriptional signature (transcription factor protein-protein interactions) of the upregulated genes in TGS versus TG iMKs. (B) GSEA of the MYC oncogenic signature in TGS versus TG. (C) Violin plots showing expression (GSEA) of the MYC target genes in TGS versus TG. ***P* < 0.01. (D) GSEA of the genes upregulated in DS-TMD compared with DS-AMKL or the genes upregulated in DS-AMKL compared with DS-TMD (31) patients in TGS versus TG iMKs. ****FDR* < 0.01; *****FDR* < 0.001. (E) Venn diagram showing the overlap of downregulated genes in the indicated comparisons. The top transcriptional signatures from the commonly downregulated genes in the compared conditions are annotated with an adjusted *P* < 0.000001 (Encode and ChEA Consensus TFs from CHIP-X). (F) GSEA for *GATA1* gene targets (32) in TS versus T, TG versus T, and TGS versus TG iMK. NES, normalized enrichment score. **FDR* < 0.25; ***FDR* < 0.05. (G) GSEA for *GATA1* gene targets (32) in TGS versus TG iMKs. (H) Heatmap showing the expression of genes encoding the F-BAR domain-containing proteins in T versus TG iMKs. Genes known to be direct targets of *GATA1* (32) are marked in blue. (I) RT-qPCR analysis of *GP1BA* relative expression in T versus TG iMKs. ***P* < 0.01. (J) Heatmap showing the expression of the RAB family genes in T versus TG iMKs. (K) GSEA for vesicle-mediated transport (GO term GO:0016192) in T versus TG or TG versus TGS iMKs. *****FDR* < 0.001. (L) Profile plots of ATAC signal on DMS genes comparing T, TS, TG, and TGS iMKs. The start (S) and the end (E) of the genes were plotted across a 1 kb flanking window; the y axis indicates depth per million mapped reads. Left panel: replicate (Rep 1); right panel: replicate 2. (M) Profile plots of ATAC signal on RAB family genes comparing T, TS, TG, and TGS iMKs.

ficking and granule movement were downregulated in TG versus T MKs (Figure 4J). In addition, TG MKs were depleted in genes involved in vesicle transport compared with T MKs, and addition of *SMC3^{-/-}* further depleted this signature (Figure 4K).

In view of these marked differences between *GATA1* and *GATA1s*, we hypothesized that *GATA1* and *GATA1s* may modify chromatin accessibility at key differentiation gene loci. Addition of *GATA1s* (TG), but not *SMC3^{-/-}* (TS), significantly reduced the

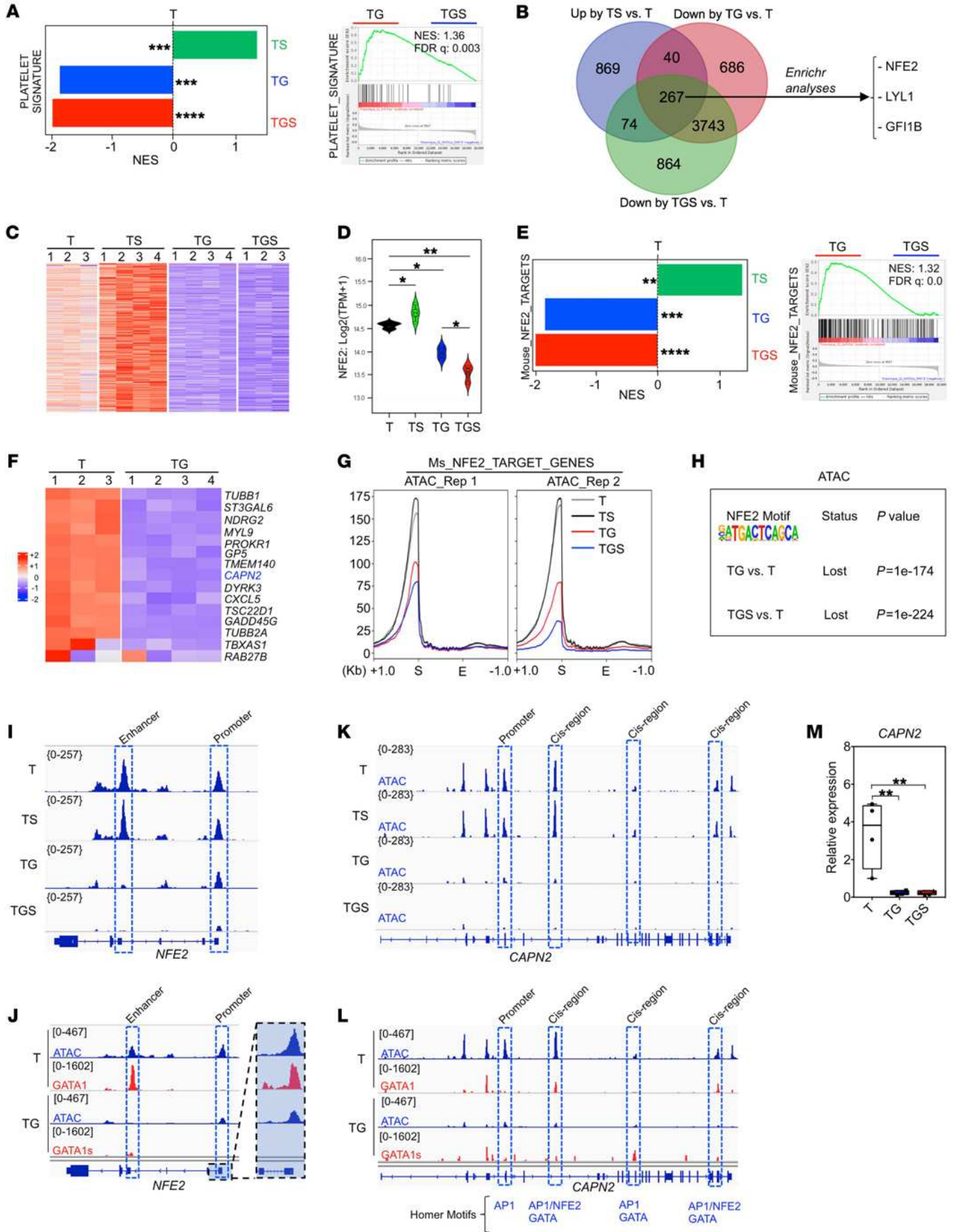


Figure 5. *GATA1s* cooperates with *SMC3*^{-/-} to downregulate the *NFE2* transcriptional program. (A) GSEA for platelet genes (47) in the indicated comparisons. ***FDR < 0.01; ****FDR < 0.001. (B) Venn diagram showing the overlap of up- and downregulated genes in the indicated comparisons. The top transcriptional signatures from the commonly downregulated genes in TG or TGS and upregulated in TS compared with T are annotated with an adjusted *P* < 0.0001 (Enrichr submissions TF-gene cooccurrence). (C) Heatmap showing the expression of the 267 genes that are common in all comparisons (up by TS versus T, down by TG versus T, down by TGS versus T) shown in the Venn diagram in B. (D) Violin plot showing *NFE2* expression in the indicated conditions. **P* < 0.05 and ***P* < 0.01. (E) GSEA for *NFE2* target genes (39) in TS versus T, TG versus T or TGS versus T (left panel), and TGS versus TG (right panel) iMKs. **FDR < 0.05; ***FDR < 0.01; ****FDR < 0.001. (F) Heatmap showing the expression of *NFE2* target genes that are downregulated in TG compared with T. *CAPN2* gene marked in blue is also known as a direct target of *GATA1*. (G) Profile plots of ATAC signal from *NFE2* target genes (Zang et al., 2016) comparing T, TS, TG, and TGS iMKs. The start (S) and the end (E) of the genes were plotted across a 1 kb flanking window; the y axis indicates depth per million mapped reads. Left: replicate 1; right, replicate 2. (H) Relative loss of the *NFE2* motif in ATAC-Seq data from the indicated comparisons. (I) ATAC-Seq profile on *NFE2* in T, TS, TG, and TGS. (J) Track depicting CUT&TAG-Seq for *GATA1* and *GATA1s* and ATAC-Seq on the *NFE2* locus in T or TG iMKs. (K) ATAC-Seq profile on *CAPN2* in T, TS, TG, and TGS. (L) Track depicting CUT&TAG-Seq for *GATA1* and *GATA1s* and ATAC-Seq on the *CAPN2* locus in T or TG iMKs. (M) RT-qPCR analysis of *CAPN2* expression in T, TS, TG, and TGS iMK.

global intensity of ATAC-Seq signals compared with T (Supplemental Figure 7A). Combination of *GATA1s* and *SMC3*^{-/-} showed further reduction in peak intensity (Supplemental Figure 7A). Lower accessibilities in TG versus T and in TGS versus T were seen in promoter, intergenic, and intronic regions, while the gained sites were mainly intergenic and intronic (Supplemental Figure 7B). The peak distribution was different in *GATA1*^{WT} (TS versus T) (Supplemental Figure 7B). ATAC profiles on DMS and RAB pathway genes showed a decreased accessibility at their transcription starting sites in TG and even more in TGS versus T (Figure 4, L and M). Together, combined RNA-Seq and ATAC-Seq profiles show that *GATA1s* in T MKs represses *GATA1* targets as well as genes involved in organelle formation, a phenotype that was further enhanced by addition of *SMC3*^{-/-}, supporting a model of cooperation leading to a failure of the *GATA1*-driven transcriptional program.

Molecular signatures of progression identify inhibition of the NFE2 program. Since *SMC3* haploinsufficiency-induced PPT formation in *GATA1*^{WT} was abrogated in a *GATA1s* context (Figure 3, E and F), we searched for molecular signatures enriched in TS but depleted in TG and TGS contexts. This included platelet gene signatures (Figure 5A). Enrichment analysis of the 267 common genes suggested that the *NFE2*-regulated program was inhibited (Figure 5, B and C). Notably, while *NFE2* is a well-characterized target of *GATA1* in terminal MK differentiation (33), it is not deregulated by *Gata1s* in mouse MKs (34). Here, *NFE2* expression was profoundly downregulated in a stepwise manner (Figure 5D). GSEA and heatmap analysis showed a depletion in *NFE2* targets in TG versus T MKs (Figure 5, E and F) and TGS versus TG MKs (Figure 5E). In contrast, *SMC3*^{-/-} in a *GATA1*^{WT} (TS) showed an increased expression of both *NFE2* and *NFE2* target genes (Figure 5, D and E), supporting the enhanced PPT formation.

ATAC-Seq profiles over TSS of *NFE2* target genes showed a gradually lower accessibility in TG and TGS compared with either T or TS MKs (Figure 5G). DNA-binding motif analyses indicated that *GATA1s* led to a relative loss of chromatin accessibility at *NFE2* (*P* = 1 × 10⁻¹⁷⁴, Figure 5H) as well as of other AP1 family and *GATA* motifs (Supplemental Figure 7C) and gain in chromatin accessibility at *RUNX* and *ETS* motifs (Supplemental Figure 7D) in both TG and TGS (Supplemental Figure 7, E and F). The *ETS* and *RUNX* motifs as well as AP1 family motifs were further lost in TGS compared with TG (Supplemental Figure 7G), while no motif was significantly gained (Supplemental Figure 7H).

Importantly, there was a good correlation between *NFE2* gene expression and its chromatin accessibility with a marked decreased accessibility at its promoter as well as cis-regulatory enhancer by *GATA1s* (Figure 5I). The CUT&Tag approach (35) showed a direct binding of *GATA1* at both the *NFE2* promoter and enhancer (T21 context) with a strong reduction in *GATA1s* binding (TG context; Figure 5J). Notably, a similar observation was made for *CAPN2* (Figure 5, K–M), which was previously demonstrated to be altered by *Gata1s* in mouse MK (12). Our integrative approach revealed that *NFE2* is a direct target of *GATA1* in human MKs, and that *GATA1s* plus *SMC3* mutation leads to a stepwise reduction in *NFE2* expression associated with a global inhibition of the *NFE2*-mediated transcriptional program.

NFE2 is involved in the MK development defect induced by GATA1s. To assess the role of *NFE2* downregulation by *GATA1s*, we transduced empty or *NFE2*-encoding lentiviral vector into T and TG iPSC-derived hematopoietic cells. Transduced CD34⁺CD43⁺ HPCs were sorted according to GFP expression and induced into MK differentiation (Figure 6A). Ectopic *NFE2* was confirmed by quantitative reverse-transcriptase PCR (RT-qPCR) in T and TG (Figure 6B). Known *NFE2* target genes that were strongly downregulated upon *GATA1s* acquisition were significantly upregulated in *NFE2*-transduced compared with empty vector-transduced TG MKs (Figure 6C). *NFE2* expression in TG cells completely restored levels of *CAPN2*, *TBXAS1*, and *RAB27B* and partially restored levels of *TUBB1*.

NFE2 expression did not influence the clonogenic potential of either MK or HPC progenitors in T or TG (Figure 6D, Supplemental Figure 8, A and B), supporting the idea that *NFE2* does not regulate MK progenitor proliferation. Also, *NFE2* reexpression had no effect on the ploidy (Figure 6E and Supplemental Figure 8C) or the percentages of CD41⁺CD42⁺ and CD34⁺ cells (Supplemental Figure 8, D–F). However, *NFE2* expression significantly decreased the proportion of large megakaryoblasts with abnormal α -granules and a pre-DMS, the maturation signature defect of TG MKs (Figure 6, F and G). Furthermore, ectopic *NFE2* induced expression of β_1 -tubulin located along the cytoplasmic membrane in 30% of TG MKs (Figure 6H) and partly rescued PPT formation by TG MK (Figure 6, I and J).

Contribution of the T21 to the cooperation between GATA1s and SMC3^{-/-}. To characterize the contribution of T21 in the phenotype described above, we generated an isogenic DGM clone and further introduced the *SMC3*^{-/-} mutation to obtain DGMS clones (Figure 1A and Supplemental Figure 1, T and V).

The percentage of CD41⁺CD42⁺ MKs generated in isogenic D and T conditions was close and above 50% (Supplemental Figure 9A and Figure 7A). Unexpectedly, the percentage of CD34⁺CD41⁺ cells

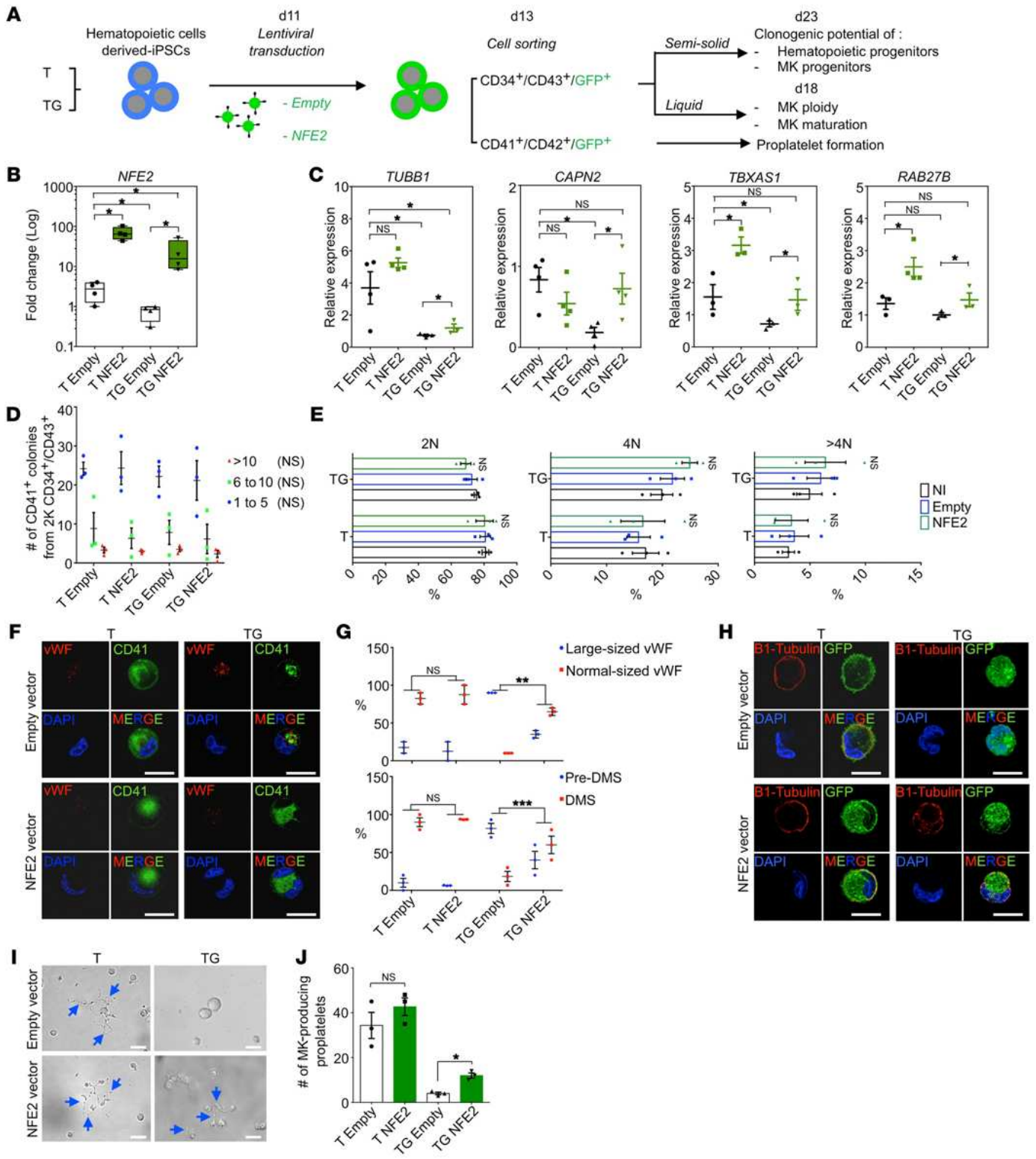


Figure 6. NFE2 overexpression in TG MK partially rescues maturation and PPT defects. (A) Schematic overview of NFE2 overexpression strategy in T and TG iMKs and of subsequent phenotypic studies. (B) Validation by RT-qPCR of NFE2 overexpression in T and TG iMKs. (C) RT-qPCR analyses of the known NFE2 target gene expression with empty or NFE2 lentiviral vectors. (D) Effect of NFE2 overexpression on the clonogenic potential of T and TG iMKs. (E) Effect of NFE2 overexpression on the ploidization of T and TG iMKs. (F) Confocal analyses of NFE2 overexpressing MK in T and TG for CD41 and vWF expression. Scale bars: 50 μm. (G) Histograms of the percentages of MKs with normal or large-sized vWF (upper panel) and the percentages of MKs with pre-DMS or DMS (lower panel) according to the absence (empty) or presence of NFE2 lentiviral vector. Quantifications were performed on 20 MKs from 3 independent experiments. (H) Confocal analyses of β₁-tubulin expression in T- and TG-derived MKs according to the absence (empty) or presence of NFE2 lentiviral vector. Scale bars: 50 μm. (I) Representative microphotographs of CD41⁺CD42⁺ MKs under PPT formation assay. Note the presence of PPT-forming MKs (blue arrows) in the absence or presence of NFE2 lentivector for the T, while in TG, the presence of PPT-forming MK was observed only in the presence of NFE2 lentivector (blue arrows). Scale bars: 50 μm. (J) Histogram of the number of PPT-forming MK shown in I according to the compared genotypes. Data are represented as mean ± SEM; n = 3. Statistical significance was determined using 1-tailed Mann-Whitney's U test. *P < 0.05; **P < 0.01; ***P < 0.001.

was significantly higher in isogenic clones, nearly 80% of the CD41⁺ cells reproducing the differences previously observed between T21 and D21 MKs (Supplemental Figure 3C, Supplemental Figure 9A, and Figure 7B). This was correlated with a higher chromatin accessibility at the CD34 promoter in TG and TGMS versus T, which was even higher in DGMS (Figure 7C). CUT&Tag for GATA1 revealed a higher binding of GATA1s compared with GATA1 at a cis-intronic region of *CD34* (Figure 7D). At the cellular level, the CD34⁺ fraction of CD41⁺CD42⁺ MKs in TG or DGMS corresponded to small immature blast cells while the CD34⁺ fraction included the large polyploid megakaryoblasts (Figure 7E). Therefore, we hypothesized that CD34 expression is aberrantly upregulated upon differentiation in GATA1s MKs. To verify this hypothesis, CD34⁺CD41⁺CD42⁺ MKs from T, TG, and DGMS were sorted and seeded with SCF and TPO to investigate phenotypes after 2 days of culture (Figure 7F). While T MKs remained CD34⁺CD41⁺CD42⁺, some TG MKs, and even more so DGMS MKs, reacquired CD34 expression (Figure 7F). These results support the idea that aberrant reacquisition of CD34 expression is controlled by GATA1s in both T and D MKs, with a higher expression in the latter context. Notably, there was no difference in the ploidy between T and D, with a similar increase in the mean percentage of more than 4N MKs by *SMC3*^{-/-} (Supplemental Figure 9B). MK morphological abnormalities, including α -granules and DMS, were identical at confocal and electron microscopy for DGMS and TGMS MKs (Supplemental Figure 9, C and D). PPT formation was similarly abrogated in DGM and DGMS versus TGM and TGMS (Supplemental Figure 9, E and F). The lack of significant enrichment of platelet signature gene lists further supported the proximity between DGMS and TGMS MK maturation defects (Supplemental Figure 9G). Therefore, the data show that T21 is not directly involved in the MK maturation defect induced by GATA1s and its cooperation with *SMC3*^{-/-}.

We subsequently studied whether T21 may affect HPC proliferation. DGM and TGM CD34⁺CD43⁺ cells showed similar MK clonogenic potential (Figure 7, G and H). In contrast, TGMS HPCs had higher MK clonogenic potential than their DGMS counterparts, with a higher proliferative capacity highlighted by an increased number of MK colonies composed of more than 10 cells and by an increased MK yield in liquid cultures (Figure 7, G-I). Of note, some rare very large TGMS colonies composed of more than 1000 MKs could be observed.

Differential gene expression analyses revealed 495 up- and 289 downregulated genes in TGMS versus DGMS (Supplemental Figure 6, G and H). More specifically, GSEA showed enrichment of a MYC signature in both TGS and TGMS versus DGMS MKs as well as TGMS versus TGM MKs (Figure 7J), indicating that *SMC3*^{-/-} cooperated with T21 and *GATA1*s to enhance the MYC-induced proliferation program. Importantly, GSEA with a DS-AMKL gene list showed signatures significantly enriched in TGMS versus DGMS iMKs (Figure 7K). Among the genes enriched in TGMS versus DGMS and in TG versus DGMS MKs was MYB (Supplemental Figure 9, H and I), a regulator of myeloid leukemogenesis (36, 37). scRNA-Seq between DGMS and TGMS showed that T21 increased MK output as well as all MK-related clusters, including the abnormal cluster 20 (Figure 2, I-K). Together, the results indicate that T21 contributes to the molecular signature of DS-AMKL and cooperates with *GATA1*s and *SMC3*^{-/-} in part through positive regulation of a MYC and MYB proliferative program.

Pharmacological inhibition of MYC reduces megakaryoblast expansion and enhances their differentiation. To investigate whether MYC inhibition can target the proliferating megakaryoblasts, hematopoietic progenitors from T, TG, TGMS, and DGMS were treated with JQ-1 inhibitor in a dose-dependent manner for 5 days (Supplemental Figure 10, A-E). JQ-1 decreased the percentage of CD41⁺CD42⁺ cells, while it increased the percentage of CD34⁺ cell fraction within T, TG, TGMS, and DGMS MK populations as compared with the nontreated conditions (Figure 8, A-C). Notably, even very low concentration of JQ-1 (50 nM) dramatically decreased the absolute number of TG but also TGMS or DGMS megakaryoblasts (Figure 8D). In parallel, JQ-1 restored the expression levels of CD41 and CD42 in TG, TGMS, and even more in DGMS compared with the respective nontreated conditions (Figure 8, E and F). To precisely determine the impact of JQ-1 inhibitor on proliferation and differentiation, we evaluated the expression of CD41 and CD42 according to the number of divisions using a violet dye cell tracker after 2 days of treatment (Figure 8G). JQ-1 significantly decreased the percentage of total cells, but also the percentage of CD41⁺CD42⁺ cells at division 4 in TGMS and DGMS as compared with the nontreated conditions (Figure 8, H and I). Importantly, JQ-1 induced a dramatic decrease in the absolute number of TGMS MK cells from division 2 until division 4 and even more marked in TGMS than DGMS (Figure 8J). Furthermore, JQ-1 did not yet influence the MFI of CD42 at the day 2 early time point whatever the number of divisions. However, it increased the MFI of CD41 through division 2 in TGMS at a level close to that observed in the nontreated DGMS (Figure 8, K and L). These results show that JQ1 has a much more rapid and significant effect in TGMS than in DGMS.

Together, these results strongly suggest that the MYC transcriptional program plays a key role in acquiring the leukemic features of DS megakaryoblasts and can be pharmacologically targeted.

Discussion

We modeled the defect in MK differentiation observed in human DS-AMKL through stepwise introduction of *GATA1* (*GATA1*s), *MPL* (*MPL*^{W515K}), and *SMC3* haploinsufficiency (*SMC3*^{-/-}) mutations in T21 iPSCs to characterize the contribution of individual alterations and how they cooperate to alter hematopoiesis and impose MK differentiation blockage. We showed that *GATA1*s impairs MK differentiation and that *SMC3*^{-/-} enhances this phenotype by synergistically acting with *GATA1*s to induce an even more profound failure of the *GATA1*-dependent MK differentiation program. These differentiation alterations were independent of T21, supporting the recent observation that T21 is not mandatory for inducing DS-AMKL by the combined *GATA1* and *STAG2* mutations (19). However, T21 cooperates with *SMC3*^{-/-} to increase proliferation. Also, *MPL*-activating mutation further enhanced MK output, including through induction of growth factor independence, but did not affect differentiation, as seen with *FLT3* mutation in another iPSC model of AML (38).

These data uncovered the importance of using human cells for the development of DS-AMKL models. Indeed, murine *Gata1*s models revealed increased proliferation of yolk sac and fetal liver MKs with minimal differentiation alteration and the

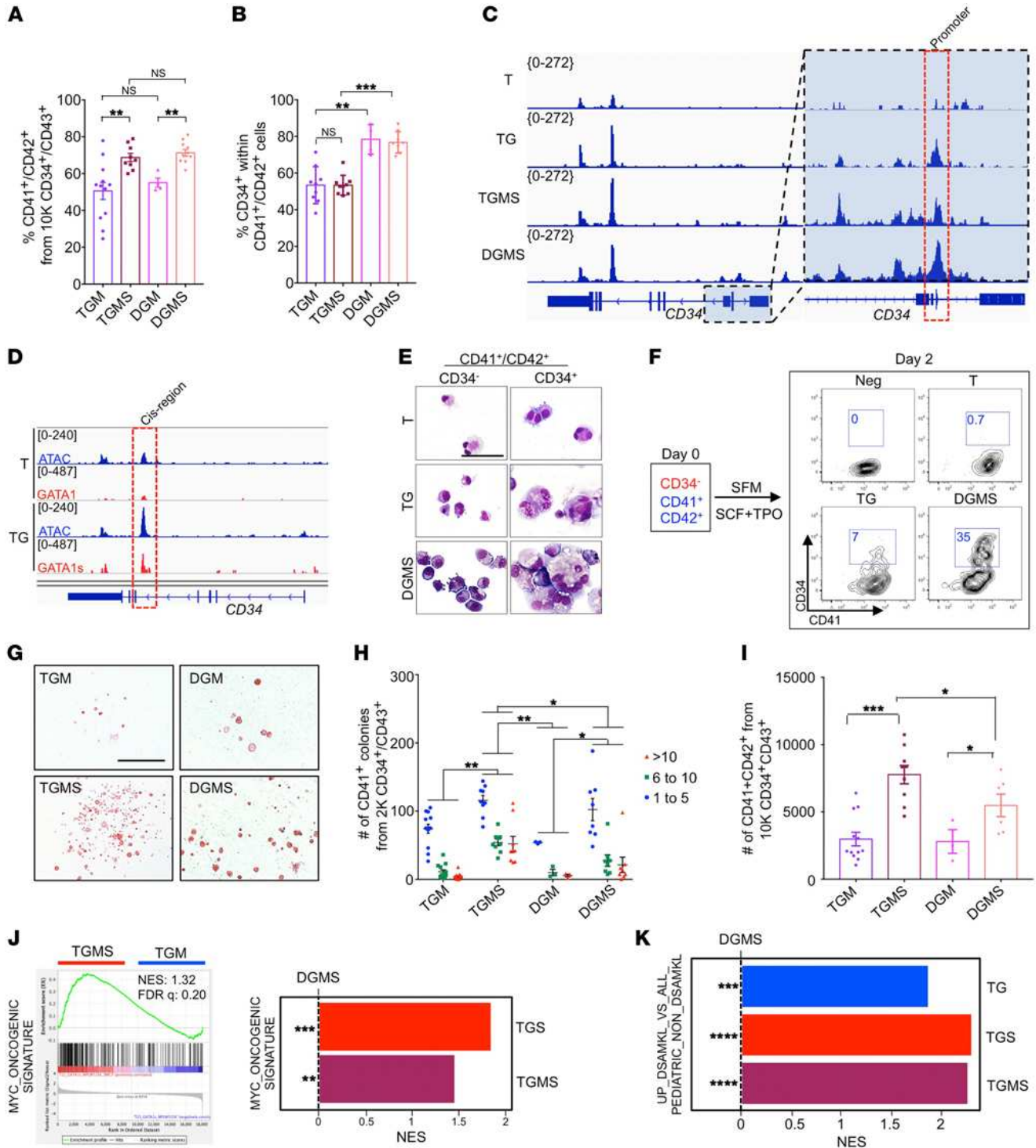


Figure 7. T21 acts in synergy with GATA1s and *SMC3*^{-/-} to increase MK proliferation and to acquire DS-AMKL features. (A) Histogram of the mean percentage of CD41⁺CD42⁺ cells. (B) Percentage of CD34⁺CD41⁺ within the CD41⁺ MK population. Data are represented as mean ± SEM; n = 3 to 4. The number of clones tested per genotype was as follows: TGM = 4; TGMS = 3; DGM = 1; DGMS = 2. (C) ATAC-Seq profile on *CD34* in T, TG, TGMS, and DGMS. (D) Track depicting CUT&TAG-Seq for GATA1 and GATA1s and ATAC-Seq at the *CD34* locus in T or TG iMKS. (E) May-Grünwald-Giemsa coloration of CD34⁻ or CD34⁺ fractions within the CD41⁺CD42⁺ cell population in T, TG, and DGMS. Scale bar: 50 μm. (F) Contour plots show CD34 expression in T, TG, and DGMS iMKS after 2 days of culture. Negative control (Neg CTL): MKs incubated without CD34 antibody. (G) Representative microphotographs of CFU-MK colonies in fibrin clot assay for TGM, TGMS, DGM, and DGMS. Scale bar: 500 μm. (H) Mean number of CFU-MK colonies obtained from 2000 CD34⁺CD43⁺ in fibrin clot assay. (I) Mean number of CD41⁺CD42⁺ MKs from 10,000 CD34⁺CD43⁺ in liquid cultures. (J) GSEA for the *MYC* oncogenic signature comparing indicated conditions. (K) GSEA for the upregulated genes in DS-AMKL versus non-DS AMKL pediatric patients in TGS versus DGMS or TGMS versus DGMS iMKS. Statistical significance was determined using 1-tailed Mann-Whitney *U* test. **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

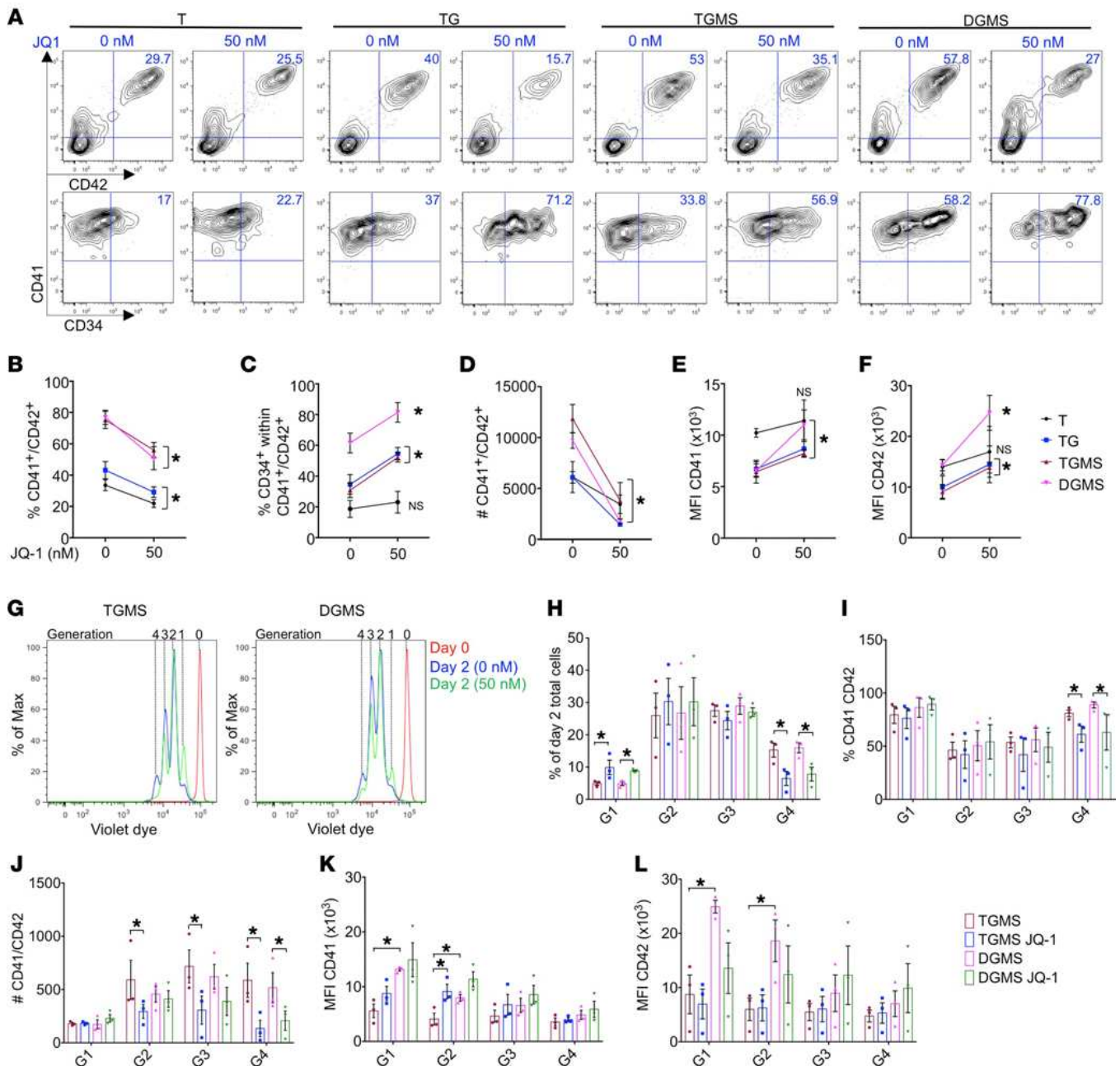


Figure 8. Assessment of MYC inhibition on iMK proliferation and differentiation. (A–F) Immunophenotypes of iMKs for the CD34, CD41, and CD42 markers found after JQ1 treatment. (A) Contour plots showing the expression of CD34, CD41, and CD42 markers. (B) Before-and-after graph shows the percentage of CD41⁺CD42⁺. (C) Before-and-after graph shows the percentage of CD34⁺ cells within the total CD41⁺CD42⁺ cell population. (D) Before-and-after graph shows the absolute number of CD41 and CD42. (E) Before-and-after graph shows the MFI of CD41. (F) Before-and-after graph shows the MFI of CD42. Data in B through F are represented as mean ± SEM; n = 3. Conditions with or without JQ-1 are compared, and statistical significance was determined using 1-tailed Mann-Whitney’s U test. *P < 0.05. (G) Representative flow cytometry plots of a violet dye experiment at day 2 of culture in the TGMS versus DGMS. Red, day 0 of culture; blue, dimethyl sulfoxide control; green, JQ-1. The number of generations is indicated at the top of the plot. (H) Histogram of the percentage of day 2 total cells per generation (G) in the indicated conditions. (I) Histogram shows the percentage of CD41⁺CD42⁺ per generation in the indicated conditions. (J) Histogram shows the absolute number of CD41⁺CD42⁺ in the indicated conditions per generation. (K) Histogram shows the MFI of CD41 per generation in the indicated conditions. (L) Histogram shows the MFI of CD42 per generation in the indicated conditions. Data are represented as mean ± SEM; n = 3. Conditions with or without JQ-1 are compared, and statistical significance was determined using the Kruskal-Wallis test. *P < 0.05.

generation of mature PPT-forming MKs (10). In contrast, the present study showed that GATA1s markedly affects the MK differentiation of both D21 and T21 human iPSCs, inducing the emergence of megakaryoblasts with major impairments in DMS, α-granules, and platelet formation.

Molecularly, numerous GATA1 target genes involved in MK differentiation were downregulated in GATA1s MKs (e.g., *GPIBA*, *PACSLN2*, *RAB* genes and *TUBB1*, respectively, involved in DMS, granule, and PPT formation). Previously, only 2 GATA1 targets (*GPIBA* and *CAPN2*) were reported as downregulated in *Gata1s* murine

models (34, 12). We found that NFE2 and its transcriptional program were profoundly inhibited in human iMKs, which is consistent with the essential role of NFE2 in the regulation of late MK differentiation (33, 39, 40). This resulted from a lower binding of GATA1s to *NFE2* regulatory elements and from further reduction of chromatin accessibility at *NFE2* and *NFE2* target loci upon addition of *SMC3*^{-/-} with a concomitant gain in accessibility at ETS motifs (e.g., *FLI1* and *ERG*). Interestingly, *SMC3*^{-/-} has the opposite effect in a *GATA1*^{WT} background by promoting MK differentiation with an increased PPT formation associated with an enhanced expression of genes involved in MK terminal differentiation, including an NFE2 signature. Therefore, this reveals a functional interplay between cohesin and GATA1 that is dependent of the chromatin organization shaped by GATA1 or GATA1s. The positive effect of *SMC3*^{-/-} on *GATA1*^{WT} MK maturation was unexpected, as it was reported that a cohesin defect blocks terminal erythroid and myeloid differentiation. However, the precise effect of cohesin mutations on MK maturation was not previously studied, apart from the report of small hypoploid MKs in *Stag2*-deficient mice (24). Together with the functional rescue of PPT formation upon NFE2 ectopic expression, these results emphasized the key role of NFE2 in GATA1s-induced MK differentiation blockage that is further enhanced upon cooperation between *GATA1s* and *SMC3*^{-/-}. Thus, the MK differentiation defect induced by GATA1s results from the altered expression of (a) genes directly regulated by GATA1 and (b) genes indirectly regulated through NFE2. Although murine models of *Gata1s* in combination with T21 and cohesin mutation have not been reported to date, the differential impact of GATA1s on *NFE2* expression in humans and mice may represent a molecular basis for incomplete DS-AMKL modeling in mice.

The predominant effect of endogenous *Gata1s* on mouse hematopoiesis is the induction of hyperproliferation of MK progenitors (10). In this study, we did not observe a significant increase in the number and proliferation of MK progenitors nor on the output of MKs defined as CD41⁺CD42⁺ cells upon engineering of GATA1s. This is consistent with the effect of *Gata1s* on murine ES cells (13) and the recent observation showing that the increased MK output generated by GATA1s is due to a shift in HSC differentiation toward the MK lineage rather than to a direct effect on MK progenitors (18, 19). However, our results showed a marked synergy between *SMC3*^{-/-} and GATA1s on both proliferation (e.g., increased MK progenitor number and size of MK colonies) and ploidization. This dual effect could be explained by the enrichment in a MYC program when combining T21, GATA1s, and *SMC3*^{-/-}, also seen in other models of *Smc3* haploinsufficiency (41). Indeed, a requirement for a transient MYC activity was reported for MK ploidization (42, 43), while its sustained activity is associated with impaired maturation (42). Notably, the *SMC3*^{-/-}-dependent gain in RUNX1/ETS binding site accessibility in T21 MKs may synergize with the previously reported loss of interaction between RB1 and GATA1s (44). An increased level of MYC and of MYC activity in DS-AMKL in comparison with other pediatric AMKL has been previously reported (45), and recently it has been reported that it is related to an excess of RUNX1A isoform (46). Here, we showed that low-dose BET inhibitor treatment inhibits both proliferation and survival of megakaryoblasts. Our data suggest that the MYC pathway is a major and targetable pathway in DS-AMKL and are consistent with a recent study showing that MYC inhibition induces apoptosis of DS-AMKL blasts (46).

While allowing reproducible cellular and molecular analyses from the same clone, the current protocol used to obtain iPSC-derived hematopoiesis does not recapitulate a definitive hematopoiesis with apparent lack of true HSC. This precludes efficient engraftment into immunodeficient mice and clearly represents a limitation in our current model. Indeed, *SMC3* haploinsufficiency did not induce a strong in vitro self-renewal of HSPC nor the engraftment into NSG immunodeficient mice (data not shown) as opposed to what is seen with human primary fetal *GATA1s* *STAG2*^{KO} HSPCs (19). Successful engraftment of hematopoietic cells derived from AML or high-grade MDS-iPSC or using an approach similar to ours showed that the sequential introduction of mutations involved in AML (*ASXL1*, *SRSF2*, *NRAS*) allowed low burden in vivo engraftment, but without the capacity of serial transplantations (38). Whether bona fide leukemic engraftment of an iPSC model of DS-AMKL will require more drastic consequences on HSPC self-renewal than *SMC3*^{-/-}, such as mutation of *STAG2* presenting only one transcribed allele located on the active X chromosome or a fourth alteration (e.g. targeting the PRC2 complex) (8, 9) or different culture or recipient conditions, remains to be determined. This aspect clearly represents a limitation in our modelization. Nonetheless, such a stepwise introduction of driver mutations of DS-AMKL reproduces the MK leukemic differentiation blockage, sheds light on its molecular mechanism, and paves the way for future studies on other leukemogenesis processes necessary for inducing self-renewal.

In summary, the present in-depth cellular and molecular characterizations of the iPSC-based model contribute to better understanding the progression from TMD to AMKL seen in children with Down syndrome. Specifically, we demonstrated that the low expression of *NFE2* is critical for the induction of MK dysplasia by GATA1s, uncovering a molecular basis of the cooperation among cohesin mutation, GATA1s, and T21 in the MK differentiation defect of DS-AMKL.

Methods

Further information can be found in the Supplemental Methods.

iPSC generation and expansion. The trisomic 21 iPSC line (T21.2; 47XY, +21) was a gift from Stella Chou and Mitchell Weiss. It was derived as previously described (14) through reprogramming of fetal stromal cells after transduction with pMXs-based retroviral supernatant encoding human OCT4, SOX2, KLF4, or MYC. iPSCs were maintained in Essential 8 or Essential 8 Flex Medium (Gibco; Thermo Fisher Scientific) on plates coated with N-truncated human recombinant vitronectin (Gibco; Thermo Fisher Scientific). Cell passages were performed mechanically or by using the StemPro Accutase Solution (Gibco; Thermo Fisher Scientific). A mycoplasma screening was routinely performed, according to the manufacturer's instructions (MilliporeSigma). A list of manufacturers with the catalog number of each product is provided in Supplemental Table 2.

iPSC genome editing. In order to obtain *GATA1s* mutation, oligo encoding sgRNA targeting exon 2 of the gene was inserted into pX330 GFP plasmid (Addgene), while for *SMC3*^{-/-} mutation, sgRNA targeting exon 9 was inserted into lenti-CRISPR V2 GFP plasmid (provided in house). For each experiment, 5 µg of GFP-expressing vector was electroporated into 1 × 10⁶ iPSCs with Human Stem Cell Nucleofector Kit 1 (Lonza) following the manufacturer's instructions. Seventy-two hours

later, the GFP-expressing cells were sorted by FACS and seeded at low density. From 9 to 11 days after seeding, 10 or 40 individual clones were picked manually for *GATA1*s or *SMC3*^{-/-}, respectively, where two-thirds of each colony were reserved for genomic DNA extraction. For *GATA1*s, DNA was amplified by PCR using the primers listed in Supplemental Table 3. *GATA1* PCR amplicons were submitted to Sanger sequencing (Eurofins), and 2 clones harboring a frame shift with a premature stop codon in exon 2 were selected. For *SMC3*^{-/-} clone genotyping, 500 ng of purified PCR amplicons were incubated with BanII restriction enzyme (NEB) for 2 hours at 37°C. The genotype was determined thereafter by 2% agarose gel migration. To identify *SMC3* indels of heterozygous mutated clones, the nondigested band was submitted to Sanger sequencing (Eurofins).

For *MPL*^{W515K/W515K} knockin, an sgRNA with a PAM corresponding to W515 of the *MPL* gene was selected and cloned into the lenti-CRISPR V2 GFP. The 5' and 3' homology arms of 700 bp of *MPL* surrounding a PGK cherry selection cassette were cloned into pUC57 vector (GenScript). Both CRISPR and donor vectors were cotransfected at a ratio of 2 µg:6 µg, respectively, into 3 × 10⁶ iPSCs using the Human Stem Cell Nucleofactor Kit 1 (Lonza). Six days later, cherry-positive expression selection by FACS was performed for clonal expansion. The genotypes of 12 cherry-positive clones were determined by PCR screening of genomic DNA and validated by Sanger sequencing (Eurofins). To delete the PGK-cherry cassette, correctly targeted clones were transfected with flippase-expressing plasmid, and 48 hours later, cells without cherry expression were sorted and expanded. PGK cherry cassette excision was confirmed by PCR.

The guide RNA design and off-target identification were determined using CRISPOR software (<http://crispor.tefor.net>). Potential indels were screened considering 1 to 4 mismatches by PCR/sequencing. For each edited gene, off-target loci containing a CFD score greater than 0.1 were selected. Off-target regions of each sgRNA used have been amplified by PCR and verified by Sanger sequencing (Eurofins). Karyotyping analyses of all targeted clones were performed by G-banding in a cytogenetics facility (Hôpital Antoine Béchère). All oligos of the different sgRNAs targeting *GATA1*, *SMC3*, and *MPL* genes and those used for PCR amplification are listed in Supplemental Table 3.

Hematopoietic differentiation from iPSC clones. Clumps from iPSC colonies were seeded on Geltrex-coated plates in Essential 8 medium at day -1. The starting cell concentration was at a 20% confluency range that corresponded to approximately 500 to 1000 single cells from 1 iPSC clone per experiment. At day 0, StemPro-34 SFM (Gibco; Thermo Fisher Scientific) xeno-free medium was used, supplemented with 1% penicillin/streptomycin (v/v; Gibco; Thermo Fisher Scientific), 1% L-glutamine (v/v; Gibco), 0.04 mg/mL of 1-thioglycerol (MilliporeSigma), and 50 mg/mL of ascorbic acid (MilliporeSigma). This medium

was retained until day 13 and supplemented with different small molecules, cytokines, and growth factors in a time-dependent manner, as described in Supplemental Table 4. At day 13, CD34⁺CD43⁺ HPC-like cells of the supernatant were sorted (see Supplemental Methods) and seeded to undergo MK differentiation in a serum-free medium containing 25 ng/ml hSCF (Biovitrum) and 25 ng/ml hTPO (Kirin Brewery). Functional validation experiments of the *MPL*^{W515K/W515K} were performed by adding or not adding 1 µM ruxolitinib (Euromedex). All incubations were performed at 37°C, 5% CO₂. A list of manufacturers with the catalog number of each product is provided in Supplemental Table 2.

Statistics. Statistical analyses were performed with GraphPad Prism software 70.a. Comparisons between different groups were performed using a 1-tailed unpaired Mann-Whitney *U* test or 1-way ANOVA Kruskal-Wallis test. Data are represented as mean ± SEM. For all analyses, *P* < 0.05 was considered statistically significant.

Data access. RNA-Seq, scRNA-Seq, ATAC-Seq, and CUT&Tag raw data were deposited in ArrayExpress (E-MTAB-11032, E-MTAB-11033, E-MTAB11036, E-MTAB-11044).

Author contributions

BA, HR, TM, and WV designed the study. BA, FB, S Mazzi, VD, AS, YM, ZA, MV, IA, MC, N Debili, and N Droin performed gene editing and cellular and molecular analyses. SS performed electron microscopy. RP, PR, CC, RD, GT, OH, IP, S Malinge, and ES contributed essential reagents and expertise. ER, FB, and MA performed the bioinformatics analyses with help from BA, FB, TM, and WV. BA, TM, and WV drafted the manuscript. All authors approved the final manuscript.

Acknowledgments

We thank Stella Chou (Children's Hospital of Philadelphia) and Mitchell Weiss (St. Jude Children's Research Hospital) for providing the T21 iPSCs (14). We also thank Heike Pahl (University of Freiburg Medical Center) for providing the NFE2 vector (pLeGO iG NFE2). This work was supported by Institut National Du Cancer (2016-1-PL BIO-04-1), Ligue Contre le Cancer (Equipe Labelisée: TM, WV, HR, IP), and Sites de Recherche Intégrée sur le Cancer-SOCRATE (SIRIC-SOCRATE) (INCa-DGOS-INSERM 12551). TM is a member of the OPALE Carnot Institute and of the PEDIAC Consortium (INCA_15670, www.programme-pediatic.com).

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- Lopez CK, et al. Pediatric acute megakaryoblastic leukemia: multitasking fusion proteins and oncogenic cooperations. *Trends Cancer*. 2017;3(9):631-642.
- McNulty M, Crispino JD. Acute megakaryocytic leukemia. *Cold Spring Harb Perspect Med*. 2020;10(2):a034884.
- Roberts I, et al. The impact of trisomy 21 on foetal haematopoiesis. *Blood Cells Mol Dis*. 2013;51(4):277-281.
- Garnett C, et al. *GATA1* and cooperating muta-

- tions in myeloid leukaemia of Down syndrome. *JUBMB Life*. 2020;72(1):119-130.
- Wechsler J, et al. Acquired mutations in *GATA1* in the megakaryoblastic leukemia of Down syndrome. *Nat Genet*. 2002;32(1):148-152.
- Roberts I, et al. *GATA1*-mutant clones are frequent and often unsuspected in babies with Down syndrome: identification of a population at risk of leukemia. *Blood*. 2013;122(24):3908-3917.
- Bhatnagar N, et al. Transient abnormal myelopoiesis and AML in down syndrome: an update. *Curr*

Hematol Malig Rep. 2016;11(5):333-341.

- Yoshida K, et al. The landscape of somatic mutations in Down syndrome-related myeloid disorders. *Nat Genet*. 2013;45(11):1293-1299.
- Labuhn M, et al. Mechanisms of progression of myeloid preleukemia to transformed myeloid leukemia in children with Down syndrome. *Cancer Cell*. 2019;36(2):123-138.
- Li Z, et al. Developmental stage-selective effect of somatically mutated leukemogenic transcription factor *GATA1*. *Nat Genet*. 2005;37(6):613-619.

11. Malinge S, et al. Increased dosage of the chromosome 21 ortholog Dyrk1a promotes megakaryoblastic leukemia in a murine model of Down syndrome. *J Clin Invest*. 2012;122(3):948–962.
12. Elagib KE, et al. Calpain 2 activation of P-TEFb drives megakaryocyte morphogenesis and is disrupted by leukemogenic GATA1 mutation. *Dev Cell*. 2013;27(6):607–620.
13. Juban G, et al. Oncogenic Gata1 causes stage-specific megakaryocyte differentiation delay. *Haematologica*. 2021;106(4):1106–1119.
14. Chou ST, et al. Trisomy 21-associated defects in human primitive hematopoiesis revealed through induced pluripotent stem cells. *Proc Natl Acad Sci U S A*. 2012;109(43):17573–17578.
15. Maclean GA, et al. Altered hematopoiesis in trisomy 21 as revealed through in vitro differentiation of isogenic human pluripotent cells. *Proc Natl Acad Sci U S A*. 2012;109(43):17567–17572.
16. Byrska-Bishop M, et al. Pluripotent stem cells reveal erythroid-specific activities of the GATA1 N-terminus. *J Clin Invest*. 2015;125(3):993–1005.
17. Banno K, et al. Systematic cellular disease models reveal synergistic interaction of Trisomy 21 and GATA1 mutations in hematopoietic abnormalities. *Cell Rep*. 2016;15(6):1228–1241.
18. Wagenblast E, et al. Functional profiling of single CRISPR/Cas9-edited human long-term hematopoietic stem cells. *Nat Commun*. 2019;10(1):4730.
19. Wagenblast E, et al. Mapping the cellular origin and early evolution of leukemia in Down syndrome. *Science*. 2021;373(6551):eabf6202.
20. Ling T, et al. Chromatin occupancy and epigenetic analysis reveal new insights into the function of the GATA1 N terminus in erythropoiesis. *Blood*. 2019;134(19):1619–1631.
21. Chlon TM, et al. Global transcriptome and chromatin occupancy analysis reveal the short isoform of GATA1 is deficient for erythroid specification and gene expression. *Haematologica*. 2015;100(5):575–584.
22. Mazumdar C, et al. Leukemia-associated cohesin mutants dominantly enforce stem cell programs and impair human hematopoietic progenitor differentiation. *Cell Stem Cell*. 2015;17(6):675–688.
23. Sasca D, et al. Cohesin-dependent regulation of gene expression during differentiation is lost in cohesin-mutated myeloid malignancies. *Blood*. 2019;134(24):2195–2208.
24. Viny AD, et al. Cohesin members Stag1 and Stag2 display distinct roles in chromatin accessibility and topological control of HSC self-renewal and differentiation. *Cell Stem Cell*. 2019;25(5):682–696.
25. Pardanani AD, et al. MPL515 mutations in myeloproliferative and other myeloid disorders: a study of 1182 patients. *Blood*. 2006;108(10):3472–3476.
26. Defour JP, et al. Oncogenic activation of MPL/thrombopoietin receptor by 17 mutations at W515: implications for myeloproliferative neoplasms. *Leukemia*. 2016;30(5):1214–1216.
27. Bluteau O, et al. Developmental changes in human megakaryopoiesis. *J Thromb Haemost*. 2013;11(9):1730–1741.
28. Langebrake C, et al. Immunophenotype of Down syndrome acute myeloid leukemia and transient myeloproliferative disease differs significantly from other diseases with morphologically identical or similar blasts. *Klin Padiatr*. 2005;217(3):126–134.
29. Wang L, et al. Acute megakaryoblastic leukemia associated with trisomy 21 demonstrates a distinct immunophenotype. *Cytometry B Clin Cytom*. 2015;88(4):244–252.
30. Eckly A, et al. Biogenesis of the demarcation membrane system(DMS) in megakaryocytes. *Blood*. 2014;123(6):921–930.
31. Bourquin JP, et al. Identification of distinct molecular phenotypes in acute megakaryoblastic leukemia by gene expression profiling. *Proc Natl Acad Sci U S A*. 2006;103(9):3339–3344.
32. Tijssen MR, et al. Genome-wide analysis of simultaneous GATA1/2, RUNX1, FLI1, and SCL binding in megakaryocytes identifies hematopoietic regulators. *Dev Cell*. 2011;20(5):597–609.
33. Shivdasani RA, et al. Transcription factor NF-E2 is required for platelet formation independent of the actions of thrombopoietin/MGDF in megakaryocyte development. *Cell*. 1995;81(5):695–704.
34. Muntean AG, Crispino JD. Differential requirements for the activation domain and FOG-interaction surface of GATA-1 in megakaryocyte gene expression and development. *Blood*. 2005;106(4):1223–1231.
35. Kaya-Okur HS, et al. CUT&Tag for efficient epigenomic profiling of small samples and single cells. *Nat Commun*. 2019;10(1):1930.
36. Zuber J, et al. An integrated approach to dissecting oncogene addiction implicates a Myb-coordinated self-renewal program as essential for leukemia maintenance. *Genes Dev*. 2011;25(15):1628–1640.
37. Takao S, et al. Convergent organization of aberrant MYB complex controls oncogenic gene expression in acute myeloid leukemia. *Elife*. 2021;10:e65905.
38. Wang T, et al. Sequential CRISPR gene editing in human iPSCs charts the clonal evolution of myeloid leukemia and identifies early disease targets. *Cell Stem Cell*. 2021;28(6):1074–1089.
39. Zang C, et al. NF-E2, FLI1 and RUNX1 collaborate at areas of dynamic chromatin to activate transcription in mature mouse megakaryocytes. *Sci Rep*. 2016;6:30255.
40. Potts KS, et al. Membrane budding is a major mechanism of in vivo platelet biogenesis. *J Exp Med*. 2020;217(9):e20191206.
41. Rivas MA, et al. Smc3 dosage regulates B cell transit through germinal centers and restricts their malignant transformation. *Nat Immunol*. 2021;22(2):240–253.
42. Takayama N, et al. Transient activation of c-MYC expression is critical for efficient platelet generation from human induced pluripotent stem cells. *J Exp Med*. 2010;207(13):2817–2830.
43. Muñoz-Alonso MJ, et al. MYC accelerates p21CIP-induced megakaryocytic differentiation involving early mitosis arrest in leukemia cells. *J Cell Physiol*. 2012;227(5):2069–2078.
44. Kadri Z, et al. Direct binding of pRb/E2F-2 to GATA-1 regulates maturation and terminal cell division during erythropoiesis. *PLoS Biol*. 2009;7(6):e1000123.
45. Klusmann JH, et al. Developmental stage-specific interplay of GATA1 and IGF signaling in fetal megakaryopoiesis and leukemogenesis. *Genes Dev*. 2010; 24(15):1659–1672.
46. Gialesaki S, et al. RUNX1 isoform disequilibrium in the development of trisomy 21 associated myeloid leukemia [preprint]. <https://doi.org/10.1101/2022.03.07.483334>. Posted on bioRxiv March 7, 2022.
47. Gnatenko DV, et al. Transcript profiling of human platelets using microarray and serial analysis of gene expression. *Blood*. 2003;101(6):2285–2293.

LYMPHOID NEOPLASIA

Structural variation cooperates with permissive chromatin to control enhancer hijacking–mediated oncogenic transcription

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KEY POINTS

- We describe a multimodal approach for quantitative analysis of transcriptional and structural impacts imposed by structural variants.
- Enhancer hijacking cooperates with permissive chromatin to activate oncogenic transcription in leukemic genomes.

Structural variants (SVs) involving enhancer hijacking can rewire chromatin topologies to cause oncogene activation in human cancers, including hematologic malignancies; however, because of the lack of tools to assess their effects on gene regulation and chromatin organization, the molecular determinants for the functional output of enhancer hijacking remain poorly understood. Here, we developed a multimodal approach to integrate genome sequencing, chromosome conformation, chromatin state, and transcriptomic alteration for quantitative analysis of transcriptional effects and structural reorganization imposed by SVs in leukemic genomes. We identified known and new pathogenic SVs, including recurrent t(5;14) translocations that cause the hijacking of *BCL11B* enhancers for the allele-specific activation of *TLX3* in a subtype of pediatric leukemia. Epigenetic perturbation of SV-hijacked *BCL11B* enhancers impairs *TLX3* transcription, which are required for the growth of t(5;14) leukemia cells. By CRISPR engineering of patient-derived t(5;14) in isogenic leukemia cells, we uncovered a new mechanism whereby the transcriptional output of SV-induced *BCL11B* enhancer hijacking is dependent on the loss

of DNA hypermethylation at the *TLX3* promoter. Our results highlight the importance of the cooperation between genetic alteration and permissive chromatin as a critical determinant of SV-mediated oncogene activation, with implications for understanding aberrant gene transcription after epigenetic therapies in patients with leukemia. Hence, leveraging the interdependency of genetic alteration on chromatin variation may provide new opportunities to reprogram gene regulation as targeted interventions in human disease.

Introduction

Cancer arises through the disruption of transcriptional programs by both genetic and epigenetic alterations.¹ Genetic alterations change the DNA sequence composition, whereas epigenetic aberrations disrupt chromatin modification and/or structure. Certain oncogenic mutations are capable of inducing chromatin state switches for transformation,² whereas permissive chromatin also allows stochastic oncogene activation or lineage plasticity.³ How the interdependence of genetic alteration and chromatin variation functions to modulate gene expression in a cancer genome remains poorly understood.

Structural variants (SVs), which are sequence differences larger than 50 bp from the reference genomes, contribute to human disease. However, dissecting their functional impact remains difficult.⁴ The challenges are to distinguish SV cancer drivers from nonfunctional mutations and to elucidate the underlying mechanisms. Pathogenic SVs can disrupt genome architecture, leading to reorganized chromatin domains that affect gene regulation.⁵⁻¹⁰ In particular, “enhancer hijacking” is a common mechanism by which noncoding SVs reposition transcriptional enhancers to the vicinity of new cognate gene(s), causing rewired chromatin interactions for oncogene activation. Although this mechanism helps explain the correlation between enhancer activity and aberrant gene expression, the molecular

determinants that control the functional output of enhancer hijacking remain largely unexplored.

A major hurdle is the lack of tools to identify SVs with pathogenic potential. Here, we developed a multimodal approach by combining genome sequencing, chromatin state, and sequence-based deep learning to quantitatively evaluate the transcriptional effects and structural reorganization imposed by SVs in human leukemia. Our approach revealed known and new SVs, including the recurrent t(5;14) translocations (TL) that cause the hijacking of *BCL11B* enhancers to activate the *TLX3* proto-oncogene. Modeling t(5;14) by genome editing uncovered the interdependency of genetic alteration on permissive chromatin for enhancer hijacking-mediated gene activation in cancer genomes.

Materials and methods

Patient samples

Primary leukemia samples were collected for diagnosis and deidentified for our study. This study was approved by the institutional review board at the University of Texas Southwestern Medical Center (IRB STU 122013-023).

Cell culture

Leukemia cell lines were cultured in RPMI 1640 or Iscove modified Dulbecco medium, supplemented with 10% or 20% fetal bovine serum and 1% penicillin/streptomycin. Cells were incubated at 37°C in 5% CO₂. 5-Aza-2'-deoxycytidine (5-azaD) was added to Jurkat and HuT78 cells at 5 μM for 7 days. UNC1999 was added to HEK293T cells at 5 μM for 7 days.

Whole genome sequencing

Genomic DNA from 38 leukemia samples and 12 cell lines were processed for whole genome sequencing (WGS). Paired-end 100 bp reads were aligned to GRCh37 using SpeedSeq. SVs were identified by LUMPY,¹¹ DELLY2,¹² and SvABA.¹³

3D regulatory impact score by Orca-Leukemia

The deep learning-based 3D genome sequence model was trained by Orca¹⁴ in 3 modes using matched Hi-C and H3K27ac chromatin immunoprecipitation (ChIP)-seq data for acute myeloid leukemia (AML) (THP-1, K562, and KBM7),¹⁵ T-acute lymphoblastic leukemia (ALL) (Jurkat, early T-cell precursor (ETP), and non-ETP),^{16,17} and B-ALL (NALM6 and GM12878).¹⁸ The training, validation, and application of the Orca-Leukemia model are described in supplemental Methods, available on the *Blood* website.

Hi-C

In situ Hi-C¹⁸ was performed using *MboI*. Trimmed reads were mapped and processed using HiC-Pro,¹⁹ and SV-rearranged alleles were visualized using NeoLoopFinder.⁷

RNA-seq, ChIP-seq, ATAC-seq, and 4C-seq

RNA extraction and quantitative reverse transcription-polymerase chain reaction (PCR) were performed as previously described.²⁰ RNA-seq data were processed and analyzed as described²¹ using STAR v2.6.1b²² and DESeq2.²³ Gene set enrichment analyses were conducted on Gene Set Enrichment Analysis v4.0.²⁴ ChIP-seq was performed using antibodies for H3K27ac (Abcam #ab4729), TLX3 (Sigma #HPA030504), and MYB (Abcam #ab45150). Genomic annotation of ChIP-seq

peaks was performed using HOMER v4.9.²⁵ Enrichment analysis was performed by GREAT v4.0.4.²⁶ Assay for transposase-accessible chromatin (ATAC)-seq was performed as previously described.²⁰ 4C-seq libraries were prepared using *DpnII* and *Csp6I* and analyzed using pipe4C.²⁷

Bisulfite sequencing

Genomic DNA was bisulfite-converted and used for PCR with EpiMark Hot Start Taq (NEB) following the manufacturer's protocols. PCR amplicon libraries were prepared using the NEBNext Ultra II DNA kit (NEB) and sequenced.

Competition-based cell proliferation

DND-41 cells were transduced with *Renilla* luciferase short hairpin RNAs (shRNAs) (shRen) or *TLX3*-targeting shRNAs (shTLX3) using the pLKO.1-U6-shRNA-hPGK-PuroR-IRES-TagBFP lentiviral vector. Equal numbers of shRNA-containing and untransduced DND-41 cells were mixed, and the fraction of blue fluorescent protein (BFP)-expressing cells was quantified weekly for 3 weeks.

CRISPR-based epigenetic perturbation

CRISPRi stable DND-41 cells were generated as described²⁸ using TRE3G-dCas9-KRAB-mCherry, Tet-On-3G-rtTA-BFP, and sgRNA-2xMS2-MCP-KRAB-zsGreen1 vectors. Cells were induced with 1 μg/mL doxycycline for 72 hours, and triple-positive cells were processed for quantitative reverse transcription-PCR. For CRISPRa, HEK293T cells were transfected with dCas9-p300 (Addgene #61357) and sgRNA-2xMS2-MCP-VP64-mCherry²⁰ using Lipofectamine 3000.

Generation of t(5;14) isogenic cells

Cells were electroporated or transfected with Cas9 and sgRNA-expressing plasmids targeting UT242-associated break points. Single-cell-derived clones harboring t(5;14) were generated by limiting dilution.

Results

SVs are enriched in noncoding leukemic genomes

We performed WGS of 38 primary leukemia samples and 12 cell lines (supplemental Figure 1A; supplemental Table 1) and identified candidate SVs using LUMPY,¹¹ DELLY2,¹² and SvABA,¹³ followed by filtering of known genomic polymorphisms²⁹ (Figure 1A). SVs detected by at least 2 software programs (supplemental Figure 1B) were stratified into 4 groups, including deletions (DEL), duplications (DUP), inversions (INV), and TL. In total, we identified 4133 SVs (1-107 DEL, 4-70 DUP, 1-46 INV, and 2-95 TL per sample; Figure 1B). Across all samples, 77% DELs, 74% DUPs, 57% INVs, and 58% TLs were between 0.1 and 10 kb in size (Figure 1C). All SVs were enriched at the promoter regions, whereas DELs, INVs, and TLs were depleted within coding sequences (Figure 1D). Of note, most SVs (72%) were located within the noncoding genome (Figure 1E). The number of SVs detected per sample correlated with p53 mutation but not with other features including age, gender, leukemia subtype, and WGS coverage (supplemental Figure 1C-I).

A recurrent TL involving chromosomes 5 and 14, or t(5;14), was identified in 2 T-lymphoid/myeloid mixed-phenotype acute

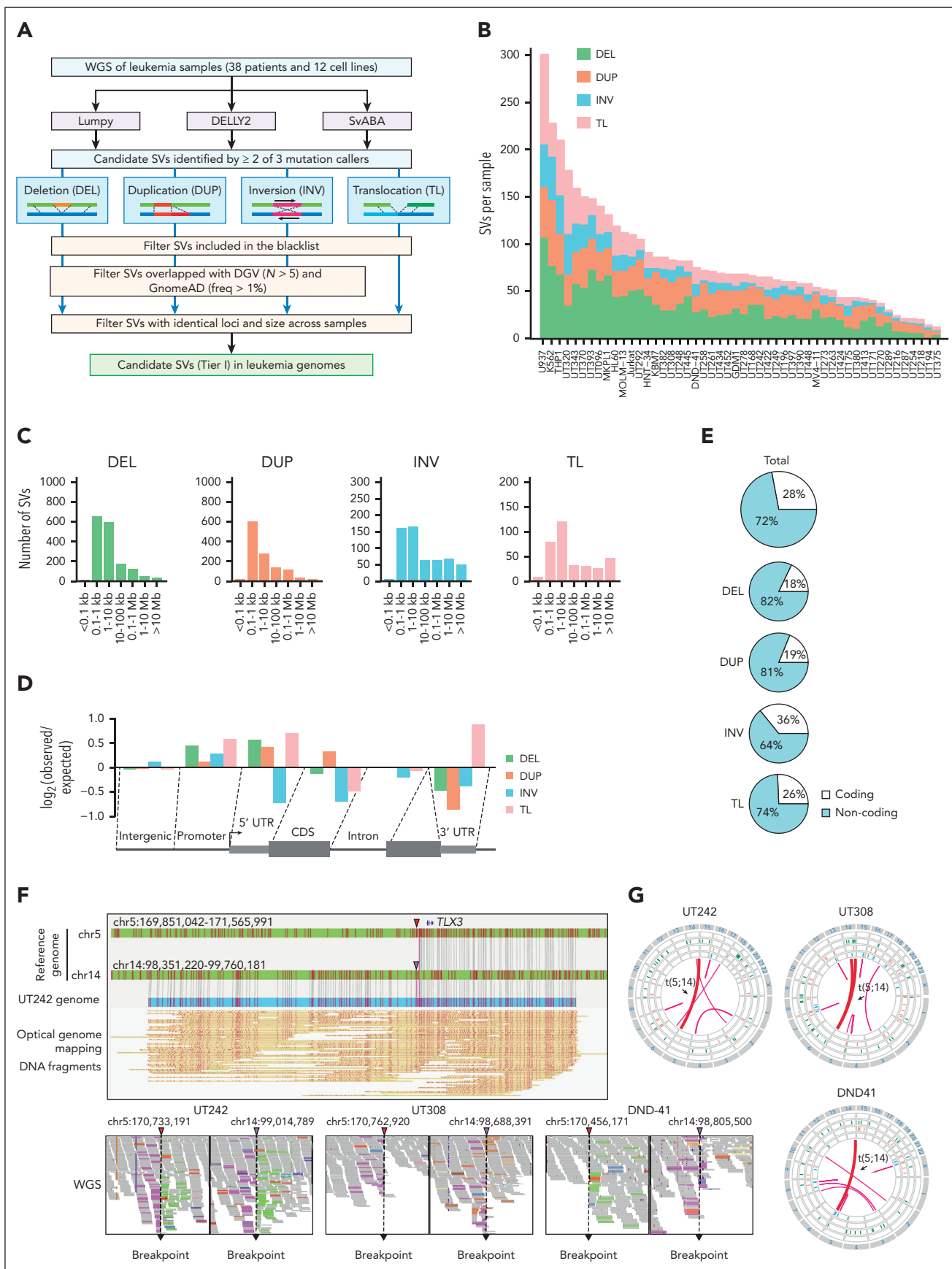


Figure 1.

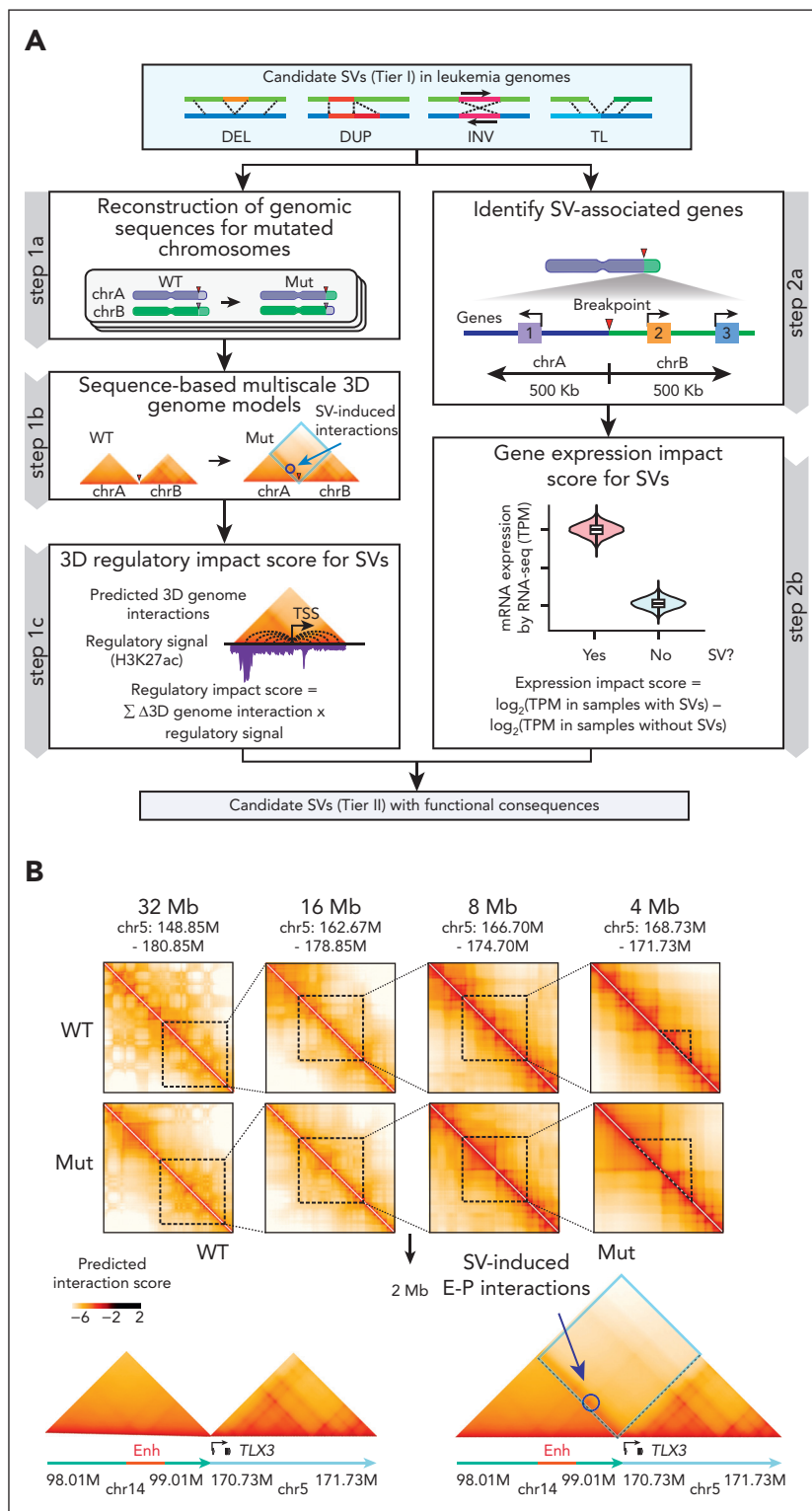


Figure 2.

Figure 1. Identification of SVs in leukemia genomes. (A) Schematic overview of the analytical pipeline to identify SVs from WGS. (B) Bar plot depicting the number of tier I SVs identified per sample. Each color within each bar represents a unique class of SV. (C) Bar plots showing the size distribution of SVs (distance between 2 breakpoints). For TL, only intrachromosomal TL are included for analysis. (D) Bar plots showing relative enrichment or depletion of SVs within genomic region. Positive and negative \log_2 observed/expected ratios indicate enrichment and depletion of SVs, respectively. (E) Pie charts showing the distribution of total SVs and individual SV classes in the coding vs noncoding genome. (F) Identification of a representative t(5;14) TL by WGS and OGM in UT242. Coordinates correspond to the human hg19 genome assembly. Arrowheads indicated so-called chromosomal break points. The piled WGS paired reads supporting the detected TL are indicated on the bottom. The pink and green colored reads indicate the forward and reverse orientations, respectively. (G) Circos plots of recurrent t(5;14) in independent samples.

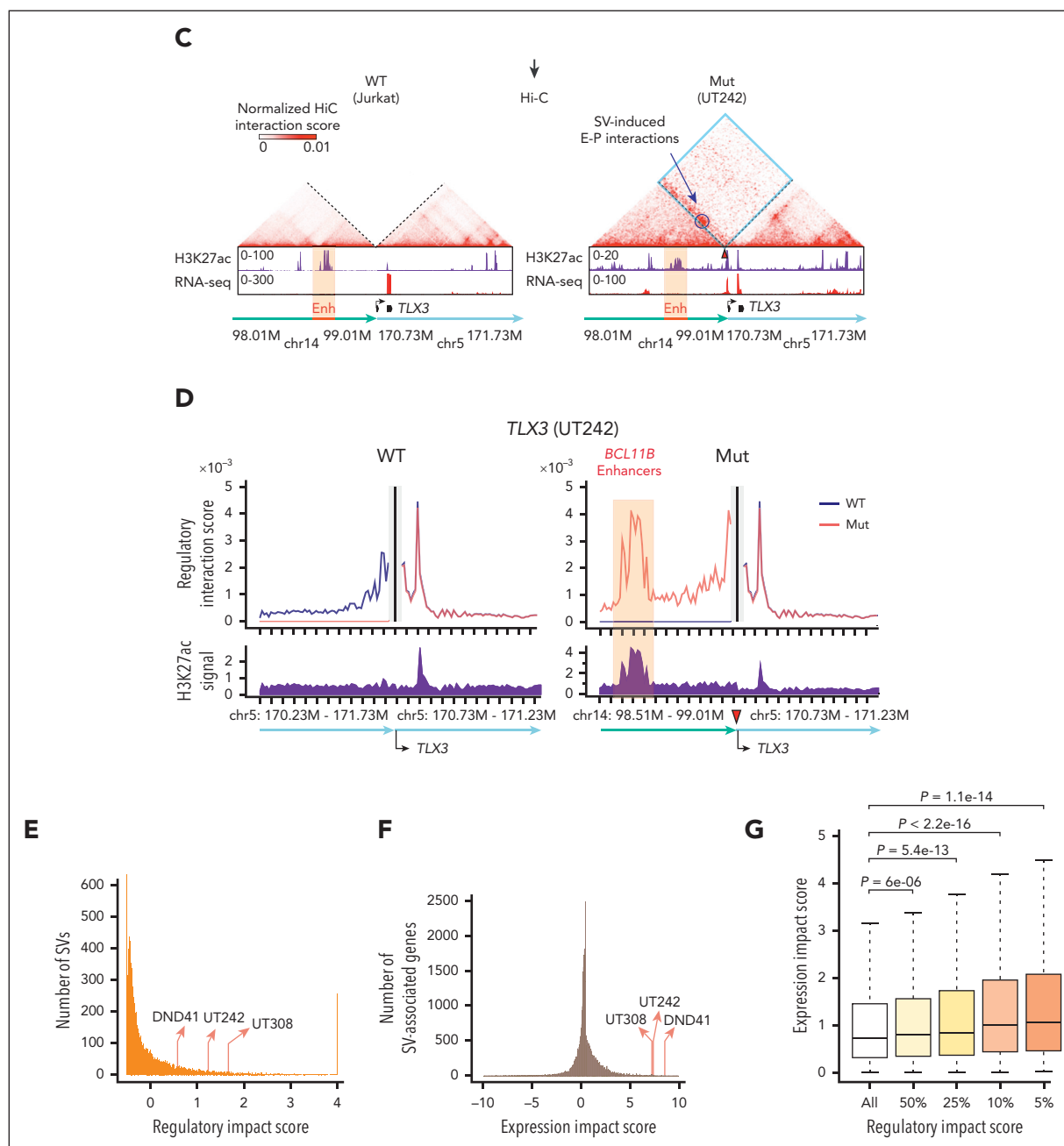


Figure 2 (continued) Identification of SVs with functional consequences. (A) Schematic overview of identifying tier II SVs with functional consequences. For each SV, the regulatory impact score was derived from modeling 3D genome interactions using Orca¹⁴ trained in 3 separate modes for AML, T-ALL, and B-ALL, whereas the gene expression impact score was calculated by comparing normalized RNA-seq expression values for genes within 500 kb of chromosomal break point and compared between samples containing an SV against other samples without the SV. (B) Predicted SV-induced 3D genome interactions for t(5;14) in UT242. Contact matrices for chr5 (WT) and t(5;14) TL alleles (Mut) at 4 to 32 Mb scale resolutions are shown. The 2 Mb t(5;14) contact matrices are centered on the chromosomal break point for UT242. Annotated enhancers on chr14 are denoted by red box. Predicted gain of E-P interactions are circled. (C) Hi-C contact matrices within 2 Mb window centered on the UT242 chromosomal break point. Hi-C contact map for Jurkat cells is shown as the WT control. H3K27ac ChIP-seq and RNA-seq signals for each sample are shown below. Hi-C contacts within dotted blue lines indicate ectopic interactions induced by t(5;14). (D) The predicted 3D regulatory interaction scores (y-axis) for the *TLX3* promoter within a 2 Mb window (x-axis) in UT242. Black vertical lines indicate the *TLX3* transcription start site. Scores for WT and SV (Mut) alleles are shown in the left and right panels, respectively. The predicted gain of interactions at the *BCL11B* enhancers on chr14 is shown by orange highlights. Arrowheads indicate chromosomal break points. Coordinates correspond to hg19 genome assembly. (E) Distribution of the regulatory impact scores for each SV. The rankings of t(5;14)-containing samples are indicated by arrows. (F) Distribution of the expression impact scores for SV-associated genes. (G) SV expression impact scores correlated with regulatory impact scores. Results are averaged expression impact scores for each group and were analyzed by a Student t test.

leukemia (T/MPAL) cases (UT242 and UT308) and DND-41 T-ALL cells (Figure 1F-G). To validate the genomic mapping, we performed optical genome mapping (OGM), which analyzes DNA molecules up to Mb in size³⁰ (supplemental Figure 2A-B).

Integrating OGM with WGS validated the known *KMT2A-MLL3* TL in MOLM-13 AML cells³¹ and *GATA2-MECOM* rearrangement^{9,32} in an inv(3) AML sample (supplemental Figure 3A-B). By combining WGS, OGM, and Sanger

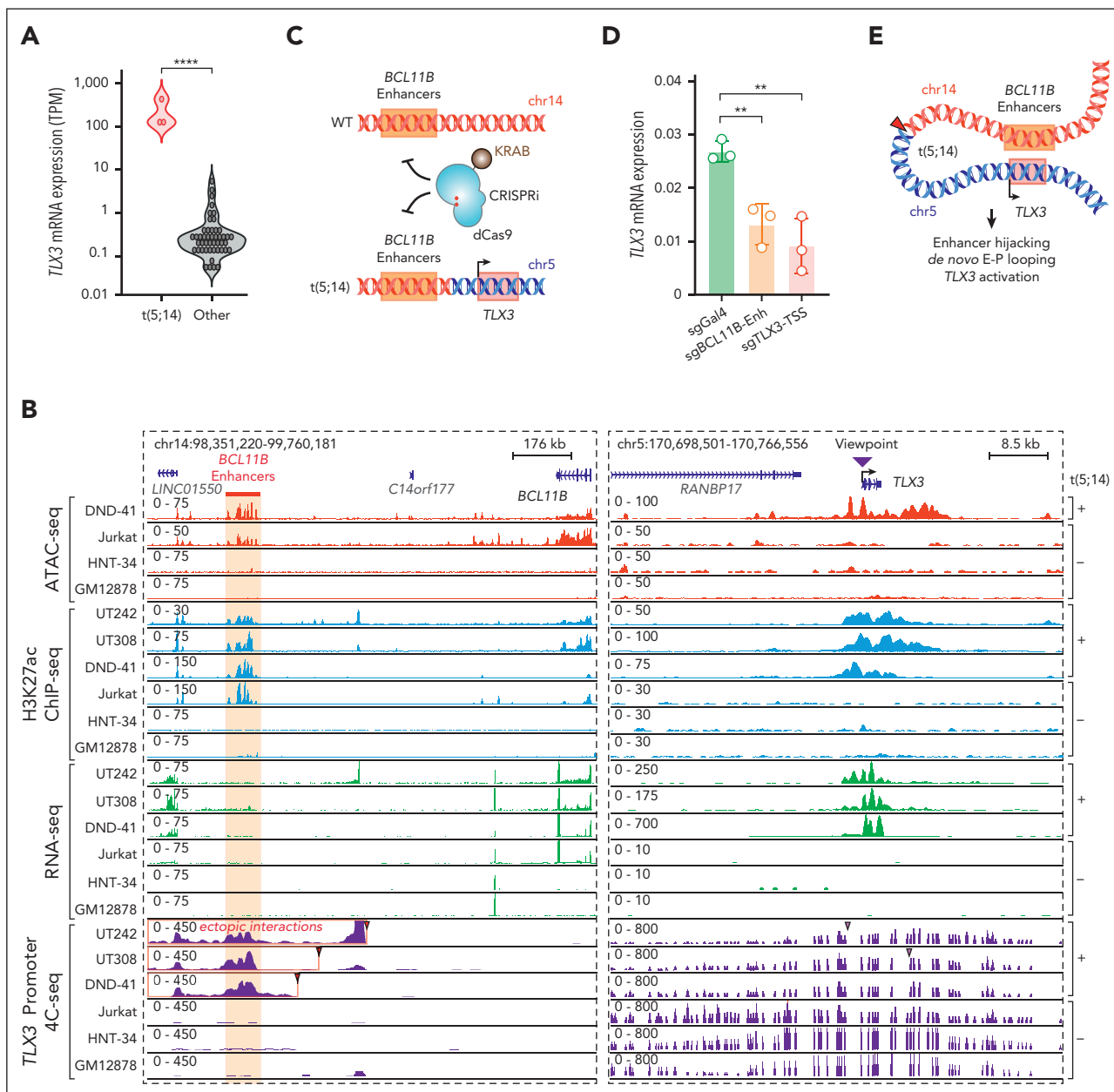


Figure 3. Enhancer hijacking activates TLX3 in t(5;14) leukemia. (A) Violin plot showing TLX3 mRNA expression in t(5;14)-containing samples vs other samples without t(5;14). (B) Genome browser tracks for ATAC-seq, H3K27ac ChIP-seq, RNA-seq, and 4C-seq for t(5;14)-positive (DND-41, UT242, and UT308) and t(5;14)-negative (Jurkat, HNT-34, and GM12878) cells. The purple arrowhead indicates the 4C-seq viewpoint. Coordinates correspond to hg19 genome assembly. Jurkat, HNT-34, and GM12878 represent negative controls for T-lymphoid, myeloid, and B-lymphoid lineages, respectively. (C) Schematic overview of CRISPRi-mediated perturbation of the BCL11B enhancers or the TLX3 transcription start site (TSS). (D) Expression of TLX3 mRNA upon CRISPRi-mediated enhancer and promoter repression in DND-41 cells normalized to GAPDH. Results are mean ± standard deviation (SD) (N = 3 biological replicates) and were analyzed by a Student t test. **P < .01, ****P < .0001. (E) Schematic representation of enhancer hijacking for TLX3 activation in t(5;14) leukemia.

sequencing of break points, we identified the t(5;14) TLs as balanced, unbalanced, and complex rearrangements in UT242, DND-41, and UT308, respectively (supplemental Figure 4A-C). Together, these studies uncover candidate SVs (tier I) for further investigation (supplemental Table 2).

Identifying SVs with pathogenic potential in silico

To identify candidate pathogenic SVs, we generated multi-modal measures based on the likelihood of a given SV to alter 3D genome structure and/or expression of proximal genes

(Figure 2A). Specifically, to determine how SVs affect genome configuration, we adapted a deep learning approach, Orca, which predicts 3D architecture from kilobase to whole-chromosome scale in silico using only genomic sequences.¹⁴ Orca uses Hi-C-based data to model the sequence dependencies of diverse types of chromatin interactions.¹⁴ Unlike approaches requiring chromatin interaction data from SV-containing samples,^{7,33} Orca predicts 3D genome impact of any SVs directly from DNA sequence and thus does not require data from samples carrying those SVs.¹⁴

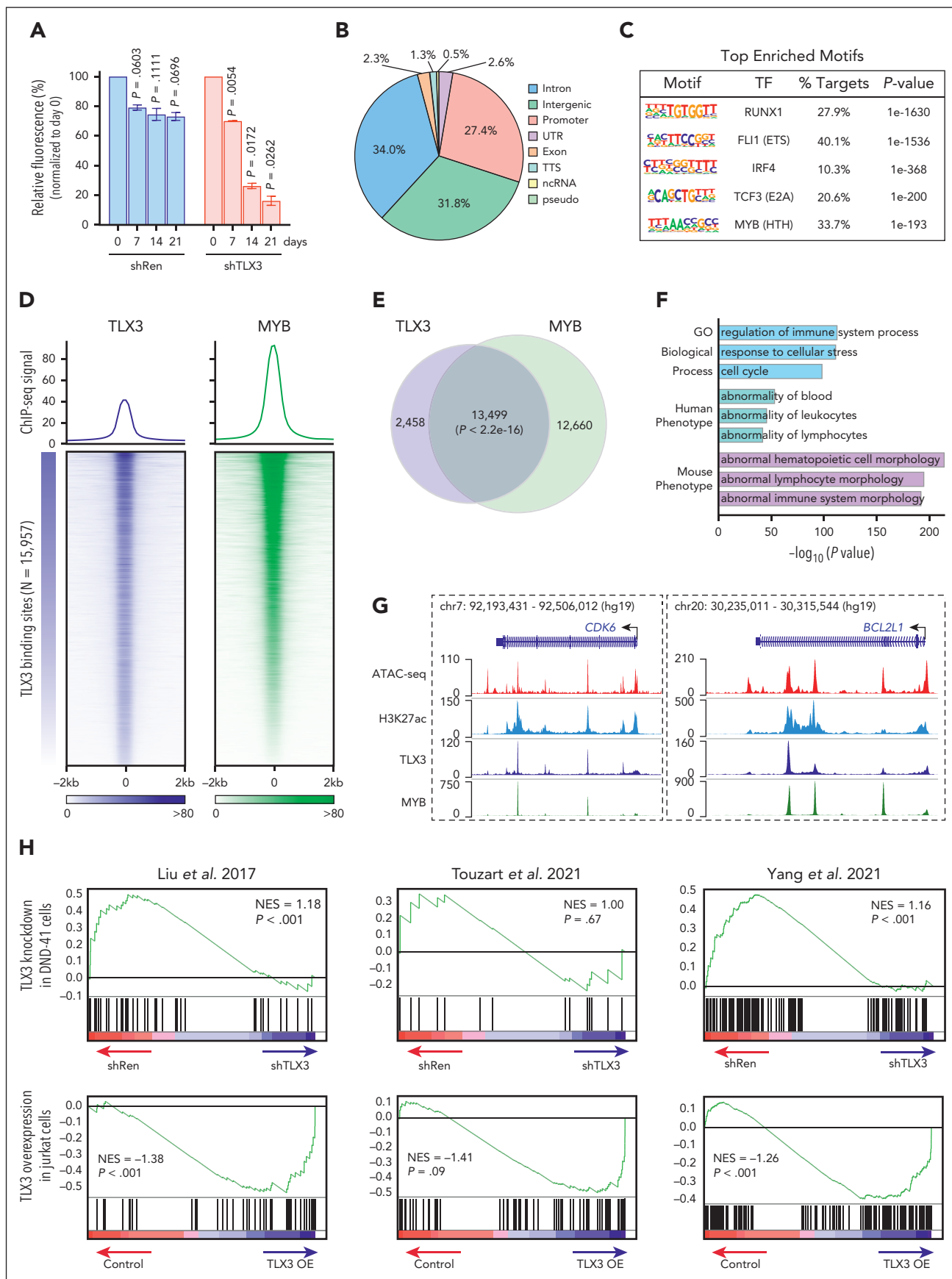


Figure 4.

To capture sequence-based features in leukemic genomes, we trained a new model, "Orca-Leukemia," in 3 separate modes for AML, T-ALL, and B-ALL using publicly available Hi-C and H3K27ac ChIP-seq data sets (THP1, K562, and KBM7 for AML¹⁵; Jurkat, ETP and non-ETP for T-ALL^{14,15}; and NALM6 and GM12878 for B-ALL),¹⁸ with chromosomes 8, 9, and 10 held out for validation studies (Figure 2A; supplemental Figure 5). We next generated a "3D regulatory impact score" derived from the sum of SV-induced gain- or loss-of-interactions and the regulatory potential of corresponding interactions in each leukemia subtype (Figure 2A). By integrating leukemia-associated chromatin features, the Orca-Leukemia-computed regulatory impact scores capture SV-induced effects on genome organization. In parallel, to determine SV-induced effects on gene expression, we performed RNA-seq of all samples and derived an "expression impact score" to compute the difference in log₂-transformed expression value for genes within 500kb of a break point in SV-containing (yes) vs other (no) samples (Figure 2A).

Next, we validated the Orca-Leukemia predictions using holdout chromosomes by comparing with Hi-C experimental data. We obtained Pearson's correlation 0.949 to 0.963 for log-predicted interaction scores across holdout chromosomes (supplemental Figure 6A), indicating strong correlation between predicted and experimental interactions. Orca-Leukemia-predicted interactions displayed consistent patterns as Hi-C at representative 2 Mb genomic regions on holdout chromosomes (supplemental Figure 6B), indicating the faithful capture of cell type-specific features required for genome configuration. Finally, the leukemia-trained Orca modes validated known pathogenic SVs in AML,^{9,34} T-ALL,¹⁷ and B-ALL,³⁵ respectively (supplemental Figure 7A-F). Hence, by integrating genomic sequence, conformation, H3K27ac-associated chromatin state, and transcriptomics, our multimodal approach enables quantitation and prioritization of SVs with pathogenic potential in leukemia.³⁶

Recurrent t(5;14) causes enhancer hijacking and TLX3 activation

Among the identified SVs, recurrent t(5;14) displayed persistent alterations of genomic configuration and target gene expression. Specifically, Orca-predicted contact matrices were generated for UT242-associated wild-type (WT) and mutant (Mut) t(5;14) alleles at multiple resolution scales (Figure 2B). We observed loss of chromatin interactions for WT alleles, but significant gain of ectopic interactions between *TLX3* on chr5 and H3K27ac-enriched enhancers 1.0 to 1.2 Mb downstream of *BCL11B* on chr14 at the t(5;14) allele (Figure 2B). By *in situ* Hi-C, we validated the SV-induced ectopic loops between *TLX3* and H3K27ac-enriched enhancers in UT242, whereas no ectopic

interaction was detected in t(5;14)-negative Jurkat cells (Figure 2C). Moreover, the samples harboring t(5;14) consistently displayed high regulatory impact scores in 2 T-lymphoid/myeloid mixed-phenotype acute leukemia (UT242 and UT308) and DND-41 cells (Figure 2D-E; supplemental Figure 8A-B).

We next ranked SVs based on the expression changes of SV-associated genes in SV-containing samples vs all other samples (Figure 2A). Strikingly, the expression impact scores for *TLX3* in UT242, UT308, and DND-41 were ranked at the top 0.05%, 0.08%, and 0.01%, respectively (Figure 2F). A significant and positive correlation was observed between 3D regulatory impact scores and gene expression impact scores (Figure 2G). Together, we uncovered 164 SVs that are within the top 10% by the regulatory impact scores and associated with significant expression impact scores ($P < .05$; tier II, supplemental Table 3). This list includes several known and new SVs with altered genomic configuration and upregulation or downregulation of SV-associated genes (supplemental Figure 9), thus establishing a repertoire of pathogenic SVs for further interrogation.

TLX3 is activated by t(5;14)-induced enhancer hijacking

The ectopic interactions involving *TLX3*- and H3K27ac-enriched enhancers support a model of enhancer hijacking.⁵ Consistent with this notion, *TLX3* is markedly upregulated in t(5;14)-containing leukemia samples relative to other samples (Figure 3A); however, the chromatin features associated with t(5;14) enhancer hijacking and the underlying mechanisms remain incompletely understood.

To this end, we performed 4C-seq using the *TLX3* promoter as the viewpoint in UT242, UT308, and DND-41 and compared them with the t(5;14)-negative Jurkat T-ALL, HNT-34 AML, and B-lymphoid GM12878 cells. We further characterized chromatin landscapes by H3K27ac ChIP-seq and ATAC-seq. Consistent with Orca-Leukemia modeling and Hi-C (Figure 2B-D), *TLX3* acquired ectopic interactions with H3K27ac-marked enhancers in t(5;14)-containing samples (Figure 3B). These enhancers interact with *BCL11B* in Jurkat and DND-41 cells by HiChIP,⁶ and thus are annotated as "*BCL11B* enhancers" in this study. To validate whether the *BCL11B* enhancers contribute to *TLX3* activation, we performed CRISPRi using inducible dCas9-KRAB³⁷ (Figure 3C). Compared with the nontargeting sgGal4, DND-41 cells expressing sgRNAs targeting the *BCL11B* enhancers (sg*BCL11B*-Enh) down-regulated *TLX3* to similar levels as cells expressing sgRNAs against the *TLX3* promoter (sg*TLX3*-transcription start site) (Figure 3D). These results provide direct evidence that t(5;14)-induced enhancer hijacking is responsible for *TLX3* activation (Figure 3E).

Figure 4. Analysis of TLX3-dependent transcriptional programs. (A) Bar plot representation of BFP+ cells relative to starting cell culture (day 0) in DND-41 cells expressing shRNA against *TLX3* (sh*TLX3*) or *Renilla* luciferase (shRen). Results are mean \pm SD and were analyzed by two-way analysis of variance (ANOVA). (B) Genome-wide distribution of *TLX3* ChIP-seq peaks in DND-41 cells. (C) Top enriched motifs in *TLX3* ChIP-seq binding sites by HOMER. (D) Heatmaps of *TLX3* (blue) and *MYB* (green) ChIP-seq signals centered on *TLX3*-binding sites in DND-41 cells. Each row represents a single *TLX3*-binding region. (E) Venn diagram of the overlap between *TLX3*- and *MYB*-binding sites in DND-41 cells. P value was calculated by the Fisher exact test. (F) Top enriched gene ontology (GO) biological process, human phenotype, and mouse phenotype terms associated with the nearest-neighbor genes within 100 kb of *TLX3* and *MYB* co-occupied genomic regions. The $-\log_{10}(P$ value) was calculated using binomial test with the GREAT software. (G) Genome browser tracks for ATAC-seq and H3K27ac, *TLX3*, and *MYB* ChIP-seq in DND-41 cells for *CDK6* and *BCL2L1*. (H) Gene set enrichment analysis of gene sets enriched in *TLX3*+ leukemia samples from 3 independent studies^{17,43,44} comparing differentially expressed genes upon *TLX3*-depletion in DND-41 cells ($N = 2$ replicate RNA-seq) or *TLX3* overexpression (*TLX3*-OE) in Jurkat cells ($N = 3$ replicate RNA-seq).

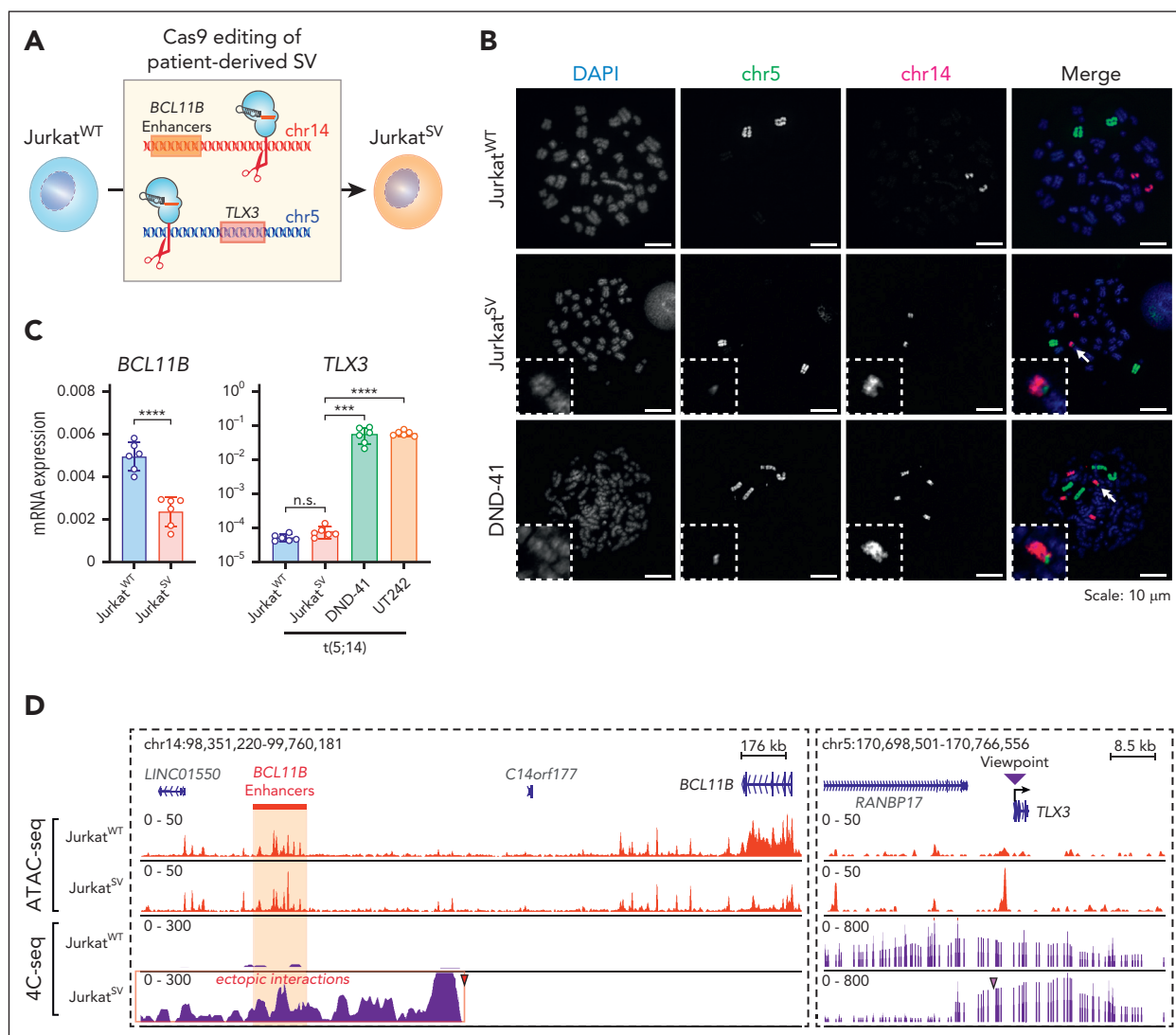


Figure 5. Engineered t(5;14) is not sufficient for TLX3 activation. (A) Schematic overview of the experimental strategy for CRISPR-mediated genome editing to generate t(5;14) isogenic Jurkat cells using UT242-associated chromosomal break points. Jurkat^{WT} indicates parental cell line, whereas Jurkat^{SV} indicates t(5;14)-edited clonal cell line. (B) Detection of t(5;14) in DND-41 and Jurkat cells by DNA FISH on metaphase chromosomes. Dotted boxes and arrows mark t(5;14) alleles. (C) Expression of *BCL11B* and *TLX3* mRNA in parental and edited Jurkat cells normalized to *GAPDH*. Results are mean ± SD ($N = 6$ biological replicates) and analyzed by a Student *t* test. **** $P < .001$, **** $P < .0001$, n.s. not significant. (D) Genome browser tracks for chr5 and chr14 showing ATAC-seq and 4C-seq in parental and edited Jurkat cells. Coordinates correspond to hg19 genome assembly. The purple arrowhead indicates the 4C-seq viewpoint.

TLX3 activates oncogenic gene transcription in leukemia

TLX3 expression in T-lineage leukemia is associated with decreased survival and increased risk for relapse.^{38,39} Of note, TLX3 knockdown significantly impaired DND-41 cell growth relative to control (shRen) in competition-based proliferation assays (Figure 4A; supplemental Figure 10A). Despite its known association with T-ALL,^{40,41} the underlying mechanisms for TLX3-mediated transcription remain elusive. To this end, we performed ChIP-seq to identify TLX3-bound genomic regions in DND-41 (supplemental Figure 10B-C). Among the identified TLX3-binding sites ($N = 15\,957$), 27.4% were located at gene promoters and 65.8% in promoter-distal noncoding regions (Figure 4B). RUNX1 and ETS were the top enriched motifs (Figure 4C), consistent with previous findings that TLX3 interacts with ETS1 and colocalizes with RUNX1 binding by

ChIP-chip.^{40,42} Interestingly, the MYB motif was detected in 33.7% of TLX3-binding sites (Figure 4C).

The oncogenic function of MYB has been implicated in multiple leukemia subtypes,^{32,45} but its association with TLX3 in the context of TLX3+ leukemia has not been explored. We performed MYB ChIP-seq and observed pervasive TLX3 and MYB co-occupancy (84.6% of TLX3-binding sites) in DND-41 (Figure 4D-E). Genes associated with TLX3 and MYB binding were associated with the regulation of immune system processes, response to stress, cell cycle, and abnormalities of blood and lymphocytes (Figure 4F). We validated TLX3 and MYB enrichment at individual loci, including *CDK6* and *BCL2L1* (Figure 4G). Interestingly, we also noted TLX3 and MYB co-occupancy at *BCL11B*, *TLX3*, and *MYB* loci (supplemental Figure 10D-G), indicating a possible feed-forward regulatory circuit imposed by oncogenic TLX3 in t(5;14) leukemia.

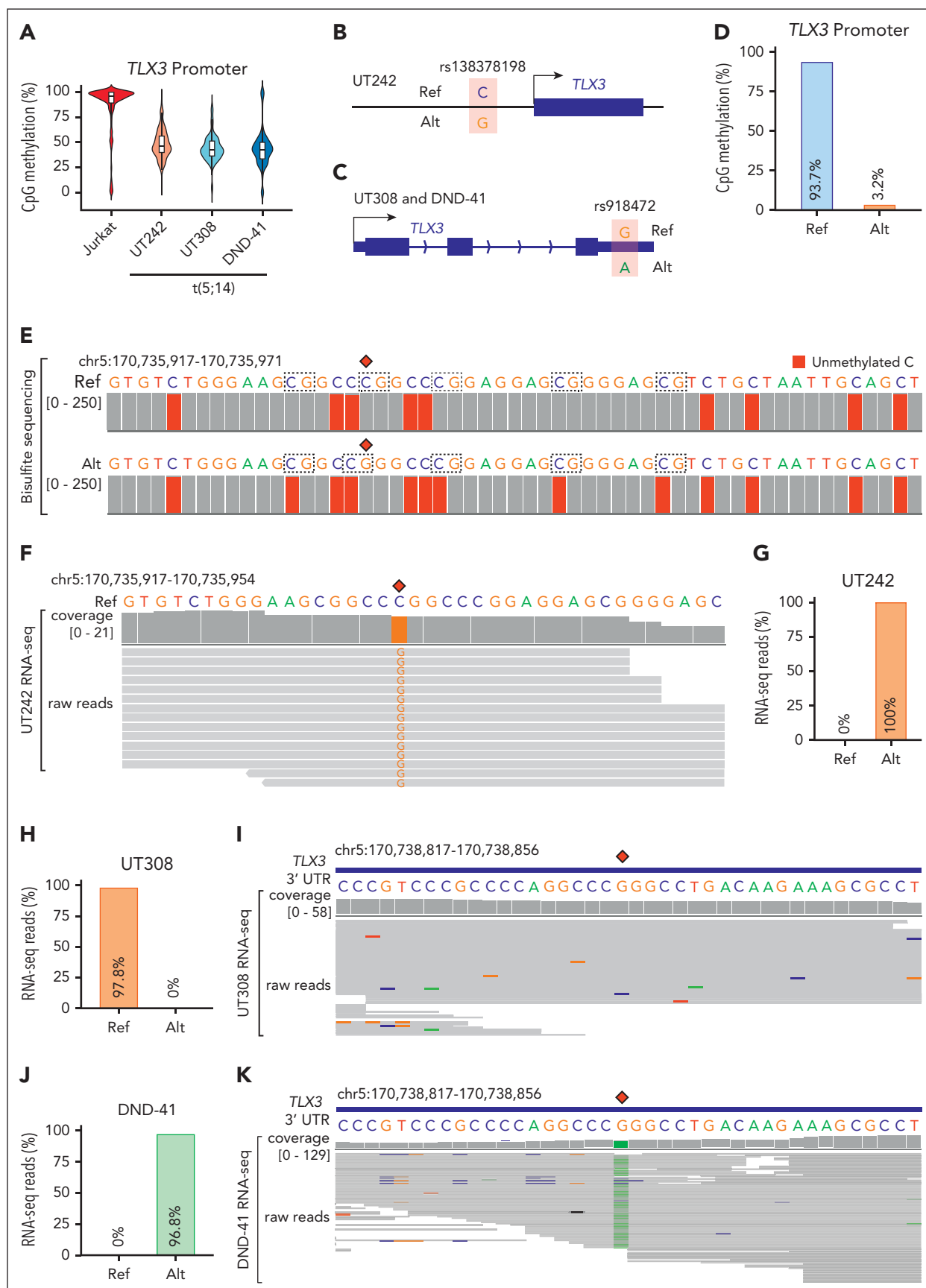


Figure 6. Allele-specific regulation of *TLX3* by enhancer hijacking. (A) Violin plot with median and quartiles showing CpG methylation at the *TLX3* promoter in t(5;14)-negative Jurkat and t(5;14)-positive (UT242, UT308, and DND-41) cells. (B) Schematic of single nucleotide polymorphism (SNP) rs138378198 identified by WGS in UT242,

To further elucidate TLX3-dependent programs, we performed RNA-seq with TLX3 depletion in DND-41 and TLX3 overexpression in TLX3-negative Jurkat cells (supplemental Figure 10H; supplemental Table 4). By gene set enrichment analysis comparing TLX3 depletion or overexpression-induced changes with TLX3-associated gene signatures from T-ALL cohorts,^{17,43,44} we observed reciprocal profiles such that genes enriched in TLX3+ leukemia were depleted in TLX3 knockdown DND-41 cells but enriched in TLX3-overexpressing Jurkat cells (Figure 4H). Altogether, these findings demonstrate that TLX3 is required and sufficient for oncogenic transcription in TLX3+ leukemia.

Engineered t(5;14) induces enhancer-promoter looping but not TLX3 activation

To determine whether t(5;14) is responsible for enhancer hijacking and TLX3 activation, we established isogenic cells. Jurkat cells do not express TLX3 (Figure 3B), but display comparable *BCL11B* expression and enhancer features as DND-41 cells (supplemental Figure 11A-B). Given the balanced TL in UT242, we engineered t(5;14) in Jurkat cells by sgRNAs targeting the UT242-associated break points on chr5 and chr14, respectively (Figure 5A). We generated a single-cell-derived clonal line harboring t(5;14), Jurkat^{SV}, which displayed a similar growth rate and karyotype as Jurkat^{WT} cells (supplemental Figure 11C-H). By DNA fluorescence in situ hybridization with probes targeting chr5 and chr14 in Jurkat^{WT} and Jurkat^{SV} cells, together with DND-41 as the positive control, we validated a single copy t(5;14) in Jurkat^{SV} but not Jurkat^{WT} cells (Figure 5B).

BCL11B expression was reduced by 2.1-fold in Jurkat^{SV} relative to Jurkat^{WT} cells, together with decreased chromatin accessibility at the *BCL11B* locus (Figure 5C-D). Surprisingly, TLX3 expression remained unchanged in Jurkat^{SV} cells in contrast to DND-41 and UT242 harboring endogenous t(5;14) (Figure 5C). Of note, significant gain of enhancer-promoter interactions between the TLX3 promoter and *BCL11B* enhancers was detected by 4C-seq in Jurkat^{SV} but not Jurkat^{WT} cells (Figure 5D). The pattern of ectopic enhancer-promoter looping resembles that of UT242 (Figure 3B). Hence, engineering of patient-derived t(5;14) enables de novo formation of enhancer-promoter looping but is not sufficient for TLX3 activation in Jurkat cells.

Allele-specific regulation of TLX3 in t(5;14)

The lack of TLX3 activation despite the de novo formation of SV-induced enhancer-promoter looping raises questions about the underlying mechanisms. We first noted strong correlations between DNA methylation and TLX3 expression by surveying reduced representation bisulfite sequencing (RRBS) profiles⁴⁶ in TLX3-expressing and nonexpressing cells (supplemental Figure 12A). The TLX3 locus also harbors a 4693 bp CpG island containing its promoter. DNA methylation serves as a repressive epigenetic modification^{47,48}; however, whether DNA methylation at TLX3 contributes to t(5;14)-mediated oncogenic transcription has not been investigated. By RRBS, we noted that the TLX3 promoter was hypermethylated in Jurkat cells with an average methylation level of 86.4%, whereas the TLX3-expressing UT242, UT308, and DND-41 cells displayed lower methylation levels of 48.4%, 44.1%, and 43.9%, respectively (Figure 6A).

We then examined whether allele-specific regulation accounts for the intermediate levels of DNA methylation at TLX3 in t(5;14) samples. By WGS, we detected a single nucleotide polymorphism located 318 bp upstream of the TLX3 transcription start site in UT242 cells (Figure 6B). UT308 and DND-41 instead harbor a single nucleotide polymorphism at the 3'UTR (Figure 6C). By bisulfite sequencing of UT242 genomic DNA, we observed an allelic imbalance such that the reference C allele displayed 93.7% CpG methylation, whereas the alternative G allele displayed 3.2% CpG methylation at the TLX3 promoter (Figure 6D-E). Moreover, RNA-seq revealed reads harboring only the unmethylated G allele in UT242, suggesting that allele-specific DNA methylation contributes to allele-specific TLX3 expression (Figure 6F-G). Similar analyses also revealed allele-specific TLX3 expression in UT308 and DND-41 (Figure 6H-K). These results demonstrate a strong association between t(5;14)-mediated enhancer hijacking and allele-specific TLX3 activation.

Enhancer hijacking cooperates with loss of DNA methylation for TLX3 activation

DNA methylation is regulated by DNA methyltransferases, whose activity can be disrupted by hypomethylating agents, including 5-azaD.⁴⁹ To determine whether the functional output of engineered t(5;14) is dependent on the epigenetic state of TLX3, we treated Jurkat^{WT} and Jurkat^{SV} cells with 5-azaD (Figure 7A; supplemental Tables 5 and 6). Strikingly, TLX3 was markedly

Figure 6 (continued) located 318 bp upstream of the TLX3 transcription start site. The reference (Ref) C and alternative (Alt) G allele sequences are indicated, allowing for allelic interrogation of DNA methylation and gene expression. (C) Schematic of SNP rs918472 identified by WGS in patient UT308 and DND-41 at the 3'UTR of TLX3. The reference (Ref) G and alternative (Alt) A allele sequences are indicated, allowing for interrogation of allele-specific TLX3 expression. (D) Bar plot representation of composite CpG methylation calculated from bisulfite sequencing reads mapped to Ref or Alt allele. (E) Genome browser track with nucleotide alignment coverage scores of bisulfite sequencing data in UT242. The nucleotide sequences within the 56 bp region of the TLX3 promoter are shown with SNP rs138378198 indicated as red diamonds. Chromosomal coordinates correspond to hg19 genome assembly. Nucleotide sequence color represents IGV genome browser viewer track color scheme. A, green; C, blue; G, brown; and T, red. Dashed boxes indicate CpG dinucleotides. Gray coverage bars underlying a given nucleotide denote correct alignment matches. The presence and relative proportion of coloration within these bars indicate which mismatched nucleotide is detected for a given position and the depth of sequencing reads carrying the mismatch, respectively. For bisulfite sequencing, an unmethylated C (blue) converted to T (red) is represented as red coloration underlying C bases, whereas methylated C are not converted. (F) Genome browser track with nucleotide alignment coverage scores and underlying raw reads of RNA-seq data in UT242. The nucleotide sequences within a 38 bp region of the TLX3 promoter are shown with SNP rs138378198 indicated as a red diamond. Chromosomal coordinates correspond to hg19 genome assembly. (G) Bar plot representation of the proportion of UT242 RNA-seq reads aligning to Ref or Alt allele. (H) Bar plot representation of the proportion of UT308 RNA-seq reads aligning to Ref or Alt allele. (I) Genome browser track with nucleotide alignment coverage scores and underlying raw reads of RNA-seq data in UT308. The nucleotide sequences within a 40 bp region of the TLX3 3'UTR are shown with SNP rs918472 indicated as a red diamond. Chromosomal coordinates correspond to hg19 genome assembly. (J) Bar plot representation of the proportion of DND-41 RNA-seq reads aligning to Ref or Alt allele. (K) Genome browser track with nucleotide alignment coverage scores and underlying raw reads of RNA-seq data in DND-41. The nucleotide sequences within a 40 bp region of the TLX3 3'UTR are shown with SNP rs918472 indicated as a red diamond.

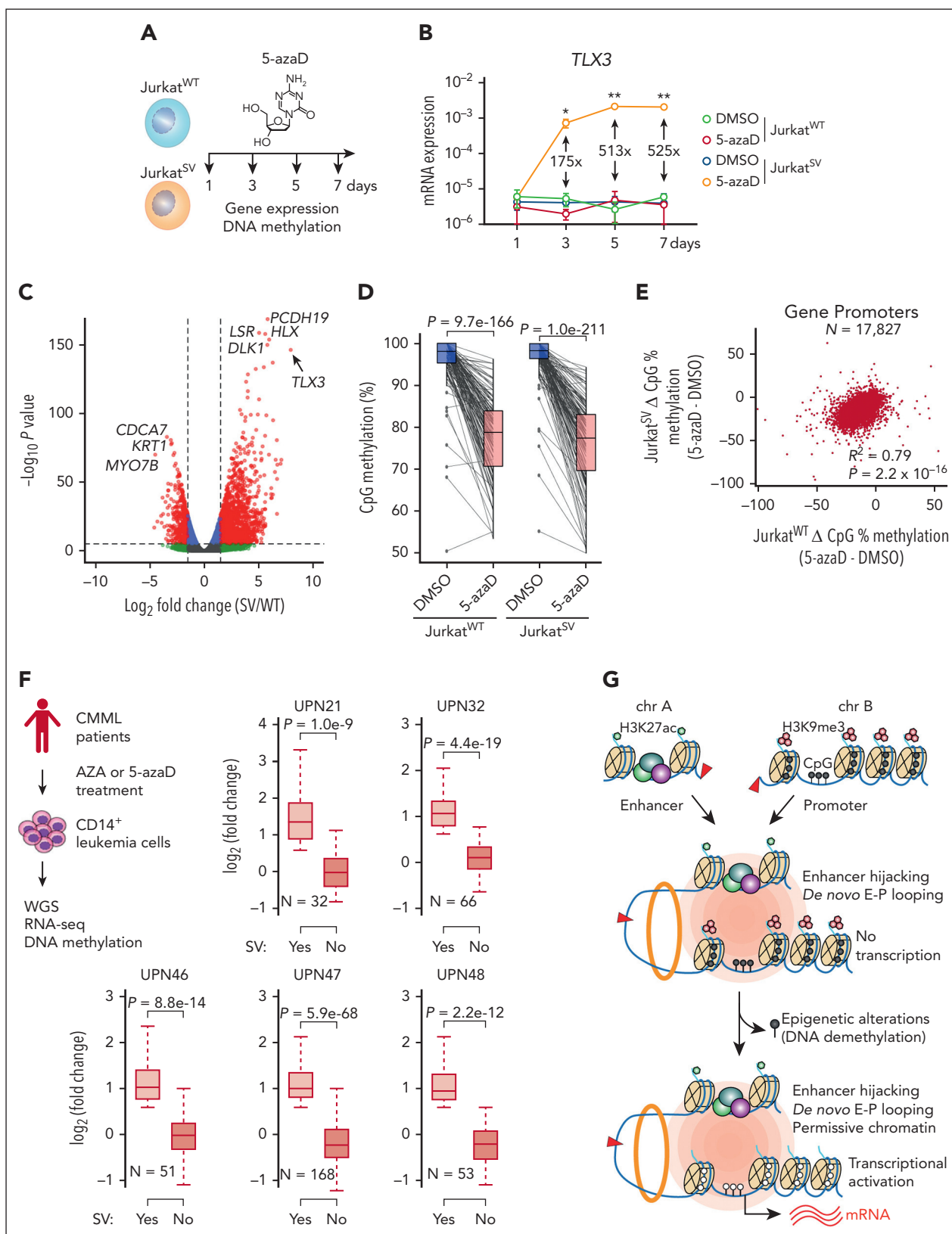


Figure 7. DNA methylation is an epigenetic barrier to enhancer hijacking. (A) Experimental strategy for 5-azaD-mediated DNA demethylation. (B) Expression of TLX3 mRNA following 5-azaD treatment in Jurkat^{WT} and Jurkat^{SV} cells normalized to GAPDH. Results are mean \pm SD (N = 3 biological replicates) and analyzed by two-way ANOVA with Dunnett multiple comparison test. Fold changes and P values represent comparisons between dimethyl sulfoxide (DMSO) and 5-azaD treatment in Jurkat^{SV} cells. *P < .05, **P < .01. (C) Volcano plot of gene expression changes upon 5-azaD treatment in Jurkat^{WT} and Jurkat^{SV} cells (N = 3 biological replicates). Red dots indicate genes displaying log₂ (fold change) ≥ 1.5 and an adjusted P value $< 1 \times 10^{-6}$. (D) CpG methylation changes at the TLX3 promoter upon 5-azaD treatment in Jurkat^{WT} and Jurkat^{SV} cells. Average methylation levels were derived from 2 independent biological replicates. Differential methylation analysis was performed by the Fisher exact test. (E) Scatterplot of global methylation changes for all gene promoters in Jurkat^{WT} and Jurkat^{SV} cells and analyzed by Pearson correlation. Results are averaged methylation differences between 5-azaD and DMSO

upregulated in Jurkat^{SV} but not Jurkat^{WT} cells (Figure 7B). By RNA-seq, *TLX3* was the top significantly induced gene in 5-azaD-treated Jurkat^{SV} cells relative to Jurkat^{WT} cells (Figure 7C). Although 5-azaD led to similar levels of demethylation at *TLX3* and promoter hypomethylation in Jurkat^{WT} and Jurkat^{SV} (Figure 7D-E), *TLX3* is only induced in Jurkat^{SV} cells harboring the engineered t(5;14). 5-azaD treatment had no effect on enhancer-promoter interactions between the hijacked enhancers and the *TLX3* promoter (supplemental Figure 12B-C).

To examine whether these effects are constrained only to Jurkat T-ALL cells, we engineered t(5;14) in 2 non-T-ALL cell types (supplemental Figure 13A). HuT78, a CD4+ cutaneous T-cell lymphoma line derived from a patient with Sézary syndrome, contains DNA hypermethylation at the *TLX3* promoter and active *BCL11B* enhancers similar to Jurkat cells (supplemental Figure 13B-C). Engineering t(5;14) (supplemental Figure 13D) resulted in a 1.6-fold increase of *TLX3* expression in HuT78^{SV} cells (supplemental Figure 14A). To determine the effects of DNA demethylation in this context, HuT78^{WT} and HuT78^{SV} cells were treated with 5-azaD, which led to marked *TLX3* upregulation (21.6-fold) in HuT78^{SV} but not HuT78^{WT} cells (supplemental Figure 14B-C). Therefore, t(5;14)-mediated enhancer hijacking cooperates with loss of DNA methylation to activate *TLX3* in both Jurkat^{SV} and HuT78^{SV} cells.

We performed similar experiments in HEK293T cells, which displayed low DNA methylation at *TLX3* but inactive *BCL11B* enhancers (supplemental Figure 13B-C). Engineering t(5;14) had no effect on *TLX3* expression in single-cell-derived HET293T^{SV} cells (supplemental Figure 14D). CRISPRa³⁷ using *BCL11B* enhancer-targeting sgRNA (sgBCL11B-Enh) also did not activate *TLX3* in contrast to *TLX3* promoter-targeting sgRNA (sgTLX3-transcription start site, positive control) in HEK293T^{SV} cells (supplemental Figure 14E-F). Importantly, the *BCL11B* locus, including its enhancers, harbors extensive H3K27me₃, a PRC2-catalyzed repressive mark, in HEK293T cells (supplemental Figure 14G). Treating HEK293T^{SV} cells with EZH1/2 inhibitor UNC1999 for 1 week before *BCL11B* enhancer-targeting CRISPRa significantly induced *TLX3* expression (6.9-fold) relative to the nontargeting sgGal4 (supplemental Figure 14H). These findings further demonstrate the cooperation between enhancer hijacking and permissive chromatin at the hijacked enhancers and/or target gene(s) for oncogene activation.

To evaluate the relevance of the interdependency of enhancer hijacking on permissive chromatin for *TLX3* activation in T-ALL pathogenesis, we surveyed the chromatin landscapes of *BCL11B* and *TLX3* in CD34+ hematopoietic stem/progenitor cells (HSPCs) and T-cell differentiation stages (supplemental Figure 15A).⁵⁰ *BCL11B* enhancers displayed chromatin accessibility and H3K27ac from immature CD34+CD1- T-cell precursors to mature αβ T cells with concomitant *BCL11B* expression (supplemental Figure 15B-C). By contrast, *TLX3* expression was undetectable during T-cell development but displayed chromatin accessibility from HSPCs to ISP CD28+ post-β selection thymocytes.⁵¹ Thus,

both *BCL11B* and *TLX3* are associated with permissive chromatin during early T-cell differentiation, which may facilitate t(5;14)-mediated *TLX3* activation for oncogenic transformation. Consistent with this notion, T-ALLs arrested at the early cortical stage of thymocyte development frequently harbor *TLX3* activation caused by t(5;14).^{52,53} Disease modeling using engineered t(5;14) in primary HSPCs, although technically challenging, will be necessary to test this possibility in T-cell leukemogenesis.

Epigenetic therapies affect SV-associated gene expression in leukemia

Hypomethylating agents are commonly used to treat hematologic malignancies, including AML, myelodysplastic syndromes, and chronic myelomonocytic leukemia (CMML),⁵⁴ and exert antitumor effects through altered DNA methylation.^{55,56} Given our findings that SVs cooperate with epigenetics to modulate oncogene transcription, we reasoned that altered DNA methylation may contribute to SV-induced transcription in patients after epigenetic therapies.

As a proof-of-principle test, we performed WGS of CD14+ monocytic leukemia cells from 5 patients with CMML after 6 to 42 cycles of AZA or 5-azaD therapy^{56,57} (Figure 7F; supplemental Table 7). We identified candidate SVs and SV-associated genes in each sample following the same pipelines (supplemental Table 8). We further analyzed RNA-seq and DNA methylation in leukemia cells before and after treatment⁵⁶ and identified SV-associated genes displaying loss of DNA methylation (>25%) at promoter regions. Importantly, we noted significantly increased expression of SV-associated genes in SV-containing samples (yes) compared to other samples (no) without the same SVs (Figure 7F). SV-induced gene activation after AZA/5-azaD treatment was consistently observed in all CMML cases, suggesting that SVs cooperate with altered DNA methylation to affect gene expression after epigenetic therapies in patients with leukemia.

Taken together, by developing a multimodal approach for identifying pathogenic SVs and modeling SV-induced enhancer hijacking in isogenic cells, our findings uncover a new mechanism whereby the cooperation between the rewired chromatin configuration and the epigenetic state of target genes controls SV-mediated oncogenic transcription. In particular, the formation of de novo enhancer-promoter looping is required but not sufficient for SV-mediated gene activation, whereas permissive chromatin at the juxtaposed target genes is indispensable for productive enhancer hijacking (Figure 7G). This mechanism helps explain aberrant gene expression caused by epigenetic drugs in patients with leukemia, with clinical implications for understanding the pathogenic roles of noncoding SVs in human disease and therapy outcome.

Discussion

SVs are hallmarks of cancer,⁴ yet the impact of SVs remains difficult to predict and/or interpret. By integrating genome sequencing, sequence-based structure modeling, epigenetic

Figure 7 (continued) treatment from 2 replicate RRBS experiments. (F) Boxplots showing the mRNA expression changes of SV-associated genes (N) in SV-containing sample (yes) vs all other samples that do not contain the corresponding SVs (No) for 5 patients with CMML. The overview of treatment and experimental scheme is shown on the left. Boxes show median of the data and quartiles, and whiskers extend up to 1.5× of the interquartile range. *P* values were calculated by a one-sided paired *t* test. (G) Model for the cooperation between 3D genome organization and the epigenetic state of target genes as the molecular determinant of enhancer hijacking-mediated oncogenic transcription.

landscapes, and transcriptomics, we developed a multimodal framework and identified a repertoire of SVs associated with gene dysregulation, thus enabling future investigations of the oncogenic potential of SVs in leukemogenesis.

As the proof-of-principle validation, we focused on recurrent t(5;14) that reconfigures enhancer-promoter looping to activate the *TLX3* proto-oncogene. Although t(5;14) was previously described in a subset of T-ALL, it has remained elusive how these SVs cause *TLX3* activation.^{39,58-62} In particular, enhancer hijacking was speculated to cause *TLX3* or *NKX2-5* activation based on the proximity to accessible chromatin and histone marks at the *BCL11B*-distal sites⁶⁰ or the chromatin conformation of the *TLX3* locus.¹⁷ Here, we establish direct evidence that t(5;14) causes *TLX3* activation through hijacking *BCL11B* enhancers by CRISPRi perturbation. Moreover, although *BCL11B* is a common SV-associated locus, prior studies focused on SVs affecting *BCL11B* function or expression in lineage-ambiguous stem cell leukemias.^{6,63} Our studies provide evidence that the hijacking of *BCL11B* enhancers can deregulate other proto-oncogenes, including *TLX3*, causing activation of oncogenic programs distinct from those of *BCL11B*-driven leukemias.^{6,63} With emerging technologies such as long-read sequencing and improved computational pipelines, we speculate that additional oncogenic drivers involving enhancer hijacking will be identified as important entities in leukemia pathophysiology.

Prior studies of enhancer hijacking have relied largely on correlative evidence.^{6-9,64} To establish causality, we engineered isogenic cells harboring patient-derived t(5;14) but observed no effect on *TLX3* expression despite the de novo formation of enhancer-promoter looping. This finding contradicts the notion that repositioning hijacked enhancers to the proximity of gene target(s) would be sufficient for gene activation. Rather, loss of epigenetic repression at the enhancer-targeted gene promoter is indispensable for enhancer hijacking-induced transcription. Given the observed permissive chromatin at both *BCL11B* and *TLX3* in early T-cell development, our findings support a model whereby the cooperation between genetic alterations and permissive chromatin of target genes, which may reflect the epigenetic state of tumor cell-of-origin, serves as a critical determinant of SV-mediated oncogene activation.

The relationship between DNA methylation and SVs has significant clinical implications, given the use of hypomethylating agents as cancer therapies.⁵⁴ Altered epigenetics caused by hypomethylating treatments can cooperate with pre-existing or acquired SVs, some of which may involve enhancer hijacking,⁶⁵ to induce more profound gene expression changes. Thus, our findings support the possibility that the pathogenic potential of SVs can be modulated by therapeutic regimens affecting epigenetics. Given the recent discovery of the demethylation and reactivation of an oncogene after hypomethylating therapy,⁶⁶ it is plausible that the mechanism described herein is applicable to other contexts. Hence, it is important to investigate in future studies whether the affected genes and cellular pathways contribute to therapy outcome and/or serve as prognostic biomarkers for better stratification of patients for epigenetic therapies.

Our studies illustrate a new layer of SV-mediated gene dysregulation and highlight the importance of integrating

genetic alterations with chromatin states for a more accurate assessment of the pathogenic potential of SVs. Computational approaches modeling SV-mediated chromatin reorganization may also benefit from integrating epigenetic information to predict SV pathogenicity with increased precision. Furthermore, leveraging the interdependency of genetic alteration on chromatin variation through epigenetics-modulating agents or epigenomic perturbations^{58,59} may compromise SV-mediated oncogenic programs, thus providing opportunities to reprogram gene regulation as targeted therapies.

Acknowledgments

The authors thank the Children's Research Institute Sequencing Core for assistance with next-generation sequencing and other Xu laboratory members for discussion and technical support.

This work was supported by the American Heart Association Predoctoral Fellowship (827324) (G.A.B.); and grants from the Institut National du Cancer (PLBIO 2021-1-PLBIO-02-IGR-1) (E.S.), the National Cancer Institute, National Institutes of Health (NIH) (R01CA230631 and R01CA259581) (J.X.), the National Institute of Allergy and Infectious Diseases, NIH (R21AI158240) (J.X.), the National Institute of Diabetes and Digestive and Kidney Diseases, NIH (R01DK111430) (J.X.), the National Institute of General Medical Sciences, NIH (DP2GM146336) (J.Z.), CPRIT grants (RP190417, RP220337, and RP220375) (J.X.) and RR190071 (J.Z.), and Welch Foundation grant (I-1942) (J.X.). J.X. is a Scholar of the Leukemia & Lymphoma Society and an American Society of Hematology Scholar.

Authorship

Contribution: G.A.B., Y.Z., K.D., J.Z., and J.X. conceptualized the study; G.A.B., Y.Z., K.D., Y.J.K., X.L., J.T.S., A.I., N.D., H.C., P.K., K.E.D., K.R.K., M.C., W.C., E.S., P.L., and J.X. performed the methods; G.A.B., K.D., Y.J.K., X.L., J.T.S., A.I., N.D., H.C., and P.K. investigated the data; G.A.B., Y.Z., and J.X. wrote the original draft; G.A.B., Y.Z., K.D., J.Z., X.L., J.T.S., P.L., E.S., and J.X. reviewed and edited the manuscript; E.S., P.L., J.Z., and J.X. acquired funding; and J.Z. and J.X. supervised the project.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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Footnotes

Submitted 28 June 2022; accepted 10 March 2023; prepublished online on *Blood* First Edition 22 March 2023. <https://doi.org/10.1182/blood.2022017555>.

All raw and processed RNA-seq, ATAC-seq, ChIP-seq, 4C-seq, Hi-C, and RRBS data are available in GEO under accession code GSE199701. WGS data are available through European Genome-Phenome Archive under accession code EGAS00001006140. The information of the

genomic datasets, key reagents, and resources is listed in supplemental Tables 10 and 11.

Data are available on request from the corresponding authors, Yuannyu Zhang (yuannyu.zhang@utsouthwestern.edu), Jian Zhou (jian.zhou@utsouthwestern.edu), and Jian Xu (jian.xu@stjude.org).

The online version of this article contains a data supplement.

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REFERENCES

- Hanahan D. Hallmarks of cancer: new dimensions. *Cancer Discov.* 2022;12(1):31-46.
- Alonso-Curbelo D, Ho YJ, Burdziak C, et al. A gene-environment-induced epigenetic program initiates tumorigenesis. *Nature.* 2021;590(7847):642-648.
- Flavahan WA, Gaskell E, Bernstein BE. Epigenetic plasticity and the hallmarks of cancer. *Science.* 2017;357(6348):eaal2380.
- Weischenfeldt J, Symmons O, Spitz F, Korbel JO. Phenotypic impact of genomic structural variation: insights from and for human disease. *Nat Rev Genet.* 2013;14(2):125-138.
- Spielmann M, Lupianez DG, Mundlos S. Structural variation in the 3D genome. *Nat Rev Genet.* 2018;19(7):453-467.
- Montefiori LE, Bendig S, Gu Z, et al. Enhancer hijacking drives oncogenic BCL11B expression in lineage-ambiguous stem cell leukemia. *Cancer Discov.* 2021;11(11):2846-2867.
- Wang X, Xu J, Zhang B, et al. Genome-wide detection of enhancer-hijacking events from chromatin interaction data in rearranged genomes. *Nat Med.* 2021;18(6):661-668.
- Northcott PA, Lee C, Zichner T, et al. Enhancer hijacking activates GF11 family oncogenes in medulloblastoma. *Nature.* 2014;511(7510):428-434.
- Groschel S, Sanders MA, Hoogenboezem R, et al. A single oncogenic enhancer rearrangement causes concomitant EVI1 and GATA2 deregulation in leukemia. *Cell.* 2014;157(2):369-381.
- Hnisz D, Weintraub AS, Day DS, et al. Activation of proto-oncogenes by disruption of chromosome neighborhoods. *Science.* 2016;351(6280):1454-1458.
- Layer RM, Chiang C, Quinlan AR, Hall IM. LUMPY: a probabilistic framework for structural variant discovery. *Genome Biol.* 2014;15(6):R84.
- Rausch T, Zichner T, Schlattl A, Stütz AM, Benes V, Korbel JO. DELLY: structural variant discovery by integrated paired-end and split-read analysis. *Bioinformatics.* 2012;28(18):i333-i339.
- Wala JA, Bandopadhyay P, Greenwald NF, et al. SvABA: genome-wide detection of structural variants and indels by local assembly. *Genome Res.* 2018;28(4):581-591.
- Zhou J. Sequence-based modeling of three-dimensional genome architecture from kilobase to chromosome scale. *Nat Genet.* 2022;54(5):725-734.
- Phanstiel DH, Van Bortle K, Spacek D, et al. Static and dynamic DNA loops form AP-1-bound activation hubs during macrophage development. *Mol Cell.* 2017;67(6):1037-1048.e6.
- Kloetgen A, Thandapani P, Ntziachristos P, et al. Three-dimensional chromatin landscapes in T cell acute lymphoblastic leukemia. *Nat Genet.* 2020;52(4):388-400.
- Yang L, Chen F, Zhu H, et al. 3D genome alterations associated with dysregulated HOXA13 expression in high-risk T-lineage acute lymphoblastic leukemia. *Nat Commun.* 2021;12(1):3708.
- Rao SSP, Huntley MH, Durand NC, et al. A 3D map of the human genome at kilobase resolution reveals principles of chromatin looping. *Cell.* 2014;159(7):1665-1680.
- Servant N, Varoquaux N, Lajoie BR, et al. HiC-Pro: an optimized and flexible pipeline for Hi-C data processing. *Genome Biol.* 2015;16:259.
- Gu Z, Liu Y, Zhang Y, et al. Silencing of LINE-1 retrotransposons is a selective dependency of myeloid leukemia. *Nat Genet.* 2021;53(5):672-682.
- Liu X, Chen Y, Zhang Y, et al. Multiplexed capture of spatial configuration and temporal dynamics of locus-specific 3D chromatin by biotinylated dCas9. *Genome Biol.* 2020;21(1):59.
- Dobin A, Davis CA, Schlesinger F, et al. STAR: ultrafast universal RNA-seq aligner. *Bioinformatics.* 2013;29(1):15-21.
- Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* 2014;15(12):550.
- Subramanian A, Tamayo P, Mootha VK, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci U S A.* 2005;102(43):15545-15550.
- Heinz S, Benner C, Spann N, et al. Simple combinations of lineage-determining transcription factors prime cis-regulatory elements required for macrophage and B cell identities. *Mol Cell.* 2010;38(4):576-589.
- McLean CY, Bristor D, Hiller M, et al. GREAT improves functional interpretation of cis-regulatory regions. *Nat Biotechnol.* 2010;28(5):495-501.
- Krijger PHL, Geeven G, Bianchi V, Hilvering CRE, de Laat W. 4C-seq from beginning to end: a detailed protocol for sample preparation and data analysis. *Methods.* 2020;170:17-32.
- Li K, Liu Y, Cao H, et al. Interrogation of enhancer function by enhancer-targeting CRISPR epigenetic editing. *Nat Commun.* 2020;11(1):485.
- MacDonald JR, Ziman R, Yuen RKC, Feuk L, Scherer SW. The Database of Genomic Variants: a curated collection of structural variation in the human genome. *Nucleic Acids Res.* 2014;42(database issue):D986-D992.
- Barseghyan H, Tang W, Wang RT, et al. Next-generation mapping: a novel approach for detection of pathogenic structural variants with a potential utility in clinical diagnosis. *Genome Med.* 2017;9(1):90.
- Matsuo Y, MacLeod RA, Uphoff CC, et al. Two acute monocytic leukemia (AML-M5a) cell lines (MOLM-13 and MOLM-14) with interclonal phenotypic heterogeneity showing MLL-AF9 fusion resulting from an occult chromosome insertion, ins(11;9)(q23;p22p23). *Leukemia.* 1997;11(9):1469-1477.
- Smeenk L, Ottema S, Mulet-Lazaro R, et al. Selective requirement of MYB for oncogenic hyperactivation of a translocated enhancer in leukemia. *Cancer Discov.* 2021;11(11):2868-2883.
- Wang X, Luan Y, Yue F. EagleC: a deep-learning framework for detecting a full range of structural variations from bulk and single-cell contact maps. *Sci Adv.* 2022;8(24):eabn9215.
- Ottema S, Mulet-Lazaro R, Erpelinck-Verschueren C, et al. The leukemic oncogene EVI1 hijacks a MYC super-enhancer by CTCF-facilitated loops. *Nat Commun.* 2021;12(1):5679.
- He B, Gao P, Ding YY, et al. Diverse noncoding mutations contribute to deregulation of cis-regulatory landscape in pediatric cancers. *Sci Adv.* 2020;6(30):eaba3064.
- Zhou J. 2021. Orca. Accessed 15 November 2021. <https://github.com/jzhoulab/orca>
- Gilbert LA, Horlbeck MA, Adamson B, et al. Genome-scale CRISPR-mediated control of gene repression and activation. *Cell.* 2014;159(3):647-661.
- Ferrando AA, Neuberg DS, Staunton J, et al. Gene expression signatures define novel oncogenic pathways in T cell acute lymphoblastic leukemia. *Cancer Cell.* 2002;1(1):75-87.
- Ballerini P, Landman-Parker J, Cayuela JM, et al. Impact of genotype on survival of children with T-cell acute lymphoblastic leukemia treated according to the French protocol FRALLE-93: the effect of TLX3/

- HOX11L2 gene expression on outcome. *Haematologica*. 2008;93(11):1658-1665.
40. Dadi S, Le Noir S, Payet-Bornet D, et al. TLX homeodomain oncogenes mediate T cell maturation arrest in T-ALL via interaction with ETS1 and suppression of TCRalpha gene expression. *Cancer Cell*. 2012;21(4):563-576.
 41. Renou L, Boelle PY, Deswarte C, et al. Homeobox protein TLX3 activates miR-125b expression to promote T-cell acute lymphoblastic leukemia. *Blood Adv*. 2017;1(12):733-747.
 42. Della Gatta G, Palomero T, Perez-Garcia A, et al. Reverse engineering of TLX oncogenic transcriptional networks identifies RUNX1 as tumor suppressor in T-ALL. *Nat Med*. 2012;18(3):436-440.
 43. Liu Y, Easton J, Shao Y, et al. The genomic landscape of pediatric and young adult T-lineage acute lymphoblastic leukemia. *Nat Genet*. 2017;49(8):1211-1218.
 44. Touzart A, Mayakonda A, Smith C, et al. Epigenetic analysis of patients with T-ALL identifies poor outcomes and a hypomethylating agent-responsive subgroup. *Sci Transl Med*. 2021;13(595):eabc4834.
 45. Mansour MR, Abraham BJ, Anders L, et al. Oncogene regulation. An oncogenic super-enhancer formed through somatic mutation of a noncoding intergenic element. *Science*. 2014;346(6215):1373-1377.
 46. Gu H, Smith ZD, Bock C, Boyle P, Gnirke A, Meissner A. Preparation of reduced representation bisulfite sequencing libraries for genome-scale DNA methylation profiling. *Nat Protoc*. 2011;6(4):468-481.
 47. Bird A. DNA methylation patterns and epigenetic memory. *Genes Dev*. 2002;16(1):6-21.
 48. Deaton AM, Bird A. CpG islands and the regulation of transcription. *Genes Dev*. 2011;25(10):1010-1022.
 49. Patel K, Dickson J, Din S, Macleod K, Jodrell D, Ramsahoye B. Targeting of 5-aza-2'-deoxycytidine residues by chromatin-associated DNMT1 induces proteasomal degradation of the free enzyme. *Nucleic Acids Res*. 2010;38(13):4313-4324.
 50. Roels J, Kuchmiy A, De Decker M, et al. Distinct and temporary-restricted epigenetic mechanisms regulate human $\alpha\beta$ and $\gamma\delta$ T cell development. *Nat Immunol*. 2020;21(10):1280-1292.
 51. Wakabayashi Y, Watanabe H, Inoue J, et al. Bcl11b is required for differentiation and survival of alphabeta T lymphocytes. *Nat Immunol*. 2003;4(6):533-539.
 52. Belver L, Ferrando A. The genetics and mechanisms of T cell acute lymphoblastic leukaemia. *Nat Rev Cancer*. 2016;16(8):494-507.
 53. Cordo V, van der Zwet JCG, Canté-Barrett K, Pieters R, Meijerink JPP. T-cell acute lymphoblastic leukemia: a roadmap to targeted therapies. *Blood Cancer Discov*. 2021;2(1):19-31.
 54. Navada SC, Steinmann J, Lübbert M, Silverman LR. Clinical development of demethylating agents in hematology. *J Clin Invest*. 2014;124(1):40-46.
 55. Tsai HC, Li H, Van Neste L, et al. Transient low doses of DNA-demethylating agents exert durable antitumor effects on hematological and epithelial tumor cells. *Cancer Cell*. 2012;21(3):430-446.
 56. Merlevede J, Droin N, Qin T, et al. Mutation allele burden remains unchanged in chronic myelomonocytic leukaemia responding to hypomethylating agents. *Nat Commun*. 2016;7:10767.
 57. Braun T, Itzykson R, Renneville A, et al. Molecular predictors of response to decitabine in advanced chronic myelomonocytic leukemia: a phase 2 trial. *Blood*. 2011;118(14):3824-3831.
 58. Bernard OA, Busson-LeConiat M, Ballerini P, et al. A new recurrent and specific cryptic translocation, t(5;14)(q35;q32), is associated with expression of the Hox11L2 gene in T acute lymphoblastic leukemia. *Leukemia*. 2001;15(10):1495-1504.
 59. Ballerini P, Blaise A, Busson-Le Coniat M, et al. HOX11L2 expression defines a clinical subtype of pediatric T-ALL associated with poor prognosis. *Blood*. 2002;100(3):991-997.
 60. Nagel S, Scherr M, Kel A, et al. Activation of TLX3 and NKX2-5 in t(5;14)(q35;q32) T-cell acute lymphoblastic leukemia by remote 3'-BCL11B enhancers and coregulation by PU.1 and HMGA1. *Cancer Res*. 2007;67(4):1461-1471.
 61. Van Vlierberghe P, Homminga I, Zuurbier L, et al. Cooperative genetic defects in TLX3 rearranged pediatric T-ALL. *Leukemia*. 2008;22(4):762-770.
 62. Baak U, Gokbuget N, Orawa H, et al. Thymic adult T-cell acute lymphoblastic leukemia stratified in standard- and high-risk group by aberrant HOX11L2 expression: experience of the German Multicenter ALL Study group. *Leukemia*. 2008;22(6):1154-1160.
 63. Di Giacomo D, La Starza R, Gorello P, et al. 14q32 rearrangements deregulating BCL11B mark a distinct subgroup of T-lymphoid and myeloid immature acute leukemia. *Blood*. 2021;138(9):773-784.
 64. Lausch M, Bartusel M, Rehimi R, et al. Modeling the pathological long-range regulatory effects of human structural variation with patient-specific hiPSCs. *Cell Stem Cell*. 2019;24(5):736-752.e12.
 65. Ahmed M, Soares F, Xia JH, et al. CRISPRi screens reveal a DNA methylation-mediated 3D genome dependent causal mechanism in prostate cancer. *Nat Commun*. 2021;12(1):1781.
 66. Liu YC, Kwon J, Fabiani E, et al. Demethylation and up-regulation of an oncogene after hypomethylating therapy. *N Engl J Med*. 2022;386(21):1998-2010.
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