



HAL
open science

CALR-mutated patients with low allele burden represent a specific subtype of essential thrombocythemia: A study on behalf of FIM and GBMHM.

Laura Aubin, Rúben Vilas Boas, Rafael Daltro de Oliveira, Valoris Le Brun, Marion Divoux, Jérôme Rey, Olivier Mansier, Jean-Christophe Ianotto, Cédric Pastoret, Anne Desmares, et al.

► To cite this version:

Laura Aubin, Rúben Vilas Boas, Rafael Daltro de Oliveira, Valoris Le Brun, Marion Divoux, et al.. CALR-mutated patients with low allele burden represent a specific subtype of essential thrombocythemia: A study on behalf of FIM and GBMHM.. American Journal of Hematology, In press, 10.1002/ajh.27265 . hal-04520328

HAL Id: hal-04520328

<https://hal.science/hal-04520328>

Submitted on 25 Mar 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License

CORRESPONDENCE

CALR-mutated patients with low allele burden represent a specific subtype of essential thrombocythemia: A study on behalf of FIM and GBMHHM

To the Editor:

BCR::ABL1-negative myeloproliferative neoplasms (MPNs) are clonal disorders characterized by an overproduction of myeloid mature cells, including essential thrombocythemia (ET), primary myelofibrosis (PMF), and polycythemia vera (PV). Somatic gain-of-function mutations in one of the three driver genes (*JAK2*, *CALR* or *MPL*) are the causative events leading to the constitutive activation of the JAK/STAT pathway and are found in almost 80% of MPN cases.¹ The quantification of the *JAK2V617F* allele burden in blood leukocytes, reflecting the size of the mutant clone, is highly variable from 1% to 100% in MPN patients and correlates with an increased risk of thrombosis and myelofibrotic evolution in PV and ET patients with an allele burden >50%. Some MPN patients display a low *JAK2V617F* allele burden in blood leukocytes (i.e. ≤20%). A recent study demonstrated that this is caused by a bias in clonal hematopoiesis with a late expansion of the mutated clone restricted to the erythroid and/or megakaryocytic lineages.² Conversely, the allele burden of *CALR* mutations appeared to be less variable, with values around 40%–50% associated with clonal dominance in hematopoietic stem cells and progenitors compartments.³ In the literature, *CALR*-mutated MPNs with a low allele burden appear to be present in a small number of patients, but scarce data are available concerning the profile of these patients.

We aimed to characterize *CALR*-mutated MPN with a low allele burden (i.e. <20%) by screening our local cohort and subsequently 11 French hospitals for patients with low allele burden. This study was registered by the French authority CNIL (Commission nationale de l'informatique et des libertés, French Data Protection Authority, authorization ar22-0016v0) and a non-opposition of patients was obtained. *CALR* allele burden quantifications were performed at the time of diagnosis and prior to any cytoreductive treatment (details in Data S1). In our local cohort, a low allele burden was encountered in 15.4% of *CALR*-mutated MPN (22/143) (Figure S1A), and a global frequency of 10.4% in the multicentric screening involving a total of 1479 *CALR*-mutated MPNs was found (Figure S1B). Among low allele burden patients, the diagnostic of ET was found in the vast majority of cases (127 of 135 patients, 94%).

We therefore aimed to compare the 127 low allele burden ET with a multicentric control cohort of 207 *CALR*-mutated ET with an

allele burden ≥20% at the time of diagnosis (details in Data S1). All comparisons are shown in Table S1. The median and range of *CALR* allele burden were of 14% [0.4–19.8] and 38% [20.3–64.6] in the low and high allele burden groups, respectively (Figure S2). The majority of patients (54%) with a low allele burden had an allele burden between 5% and 15%, whereas 4.5% of patients had an allele burden below 5%. The sex ratio was reverted, with a female predominance in low allele burden patients compared with a male predominance in patients with *CALR* allele burden ≥20% (M/F: 0.63 vs. 1.49, $p = 0.0002$). No difference was observed for age at the time of diagnosis ($p = 0.473$). ET patients with *CALR* low allele burden had lower platelets and neutrophils counts, and lower LDH levels than *CALR*-mutated ET patients with a *CALR* allele burden ≥20% ($p = 0.0005$, $p = 0.0008$ and $p = 0.009$, respectively; Figure S3). Among *CALR*-mutated ET, the subtype of *CALR* mutation influenced the phenotype with higher platelet counts in type 2/2-like patients.⁴ However, the phenotype observed in low allele burden *CALR*-mutated ET is not linked to the subtype of *CALR* mutation because the distribution of mutation subtypes was similar between the two groups, with a majority of type 1/1-like mutations (53% and 57% of patients for low and high allele burden, respectively).

In terms of prognosis, *CALR* low allele burden ET exhibited a better overall survival than ET patients with a *CALR* allele burden ≥20% (Figure 1A). Multivariable analysis confirmed the better overall survival of *CALR* low allele burden and showed that age >60 years and a previous history of thrombosis were also independently associated with a decreased overall survival (Figure 1B). No differences were found regarding hematological transformations (Figure 1C) and thrombosis-free survival, although *CALR* low allele burden ET patients may have a lower long-term risk of thrombosis (Figure 1D). Nevertheless, the follow-up period for this cohort is limited (with a median duration of 51 months for low allele burden), and a longer follow-up would provide more conclusions regarding the impact of *CALR* allele burden on hematological transformations and thrombosis. Compared with *JAK2V617F* driver mutation, *CALR* mutations are associated with a better prognostic in PMF, but no difference was found in ET in terms of overall survival, thrombotic events, or fibrotic transformation. Thus, *CALR*-mutated ET patients with a low allele burden seemed to represent a subtype of ET with a more favorable prognosis.

This is an open access article under the terms of the [Creative Commons Attribution](https://creativecommons.org/licenses/by/4.0/) License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2024 The Authors. *American Journal of Hematology* published by Wiley Periodicals LLC.

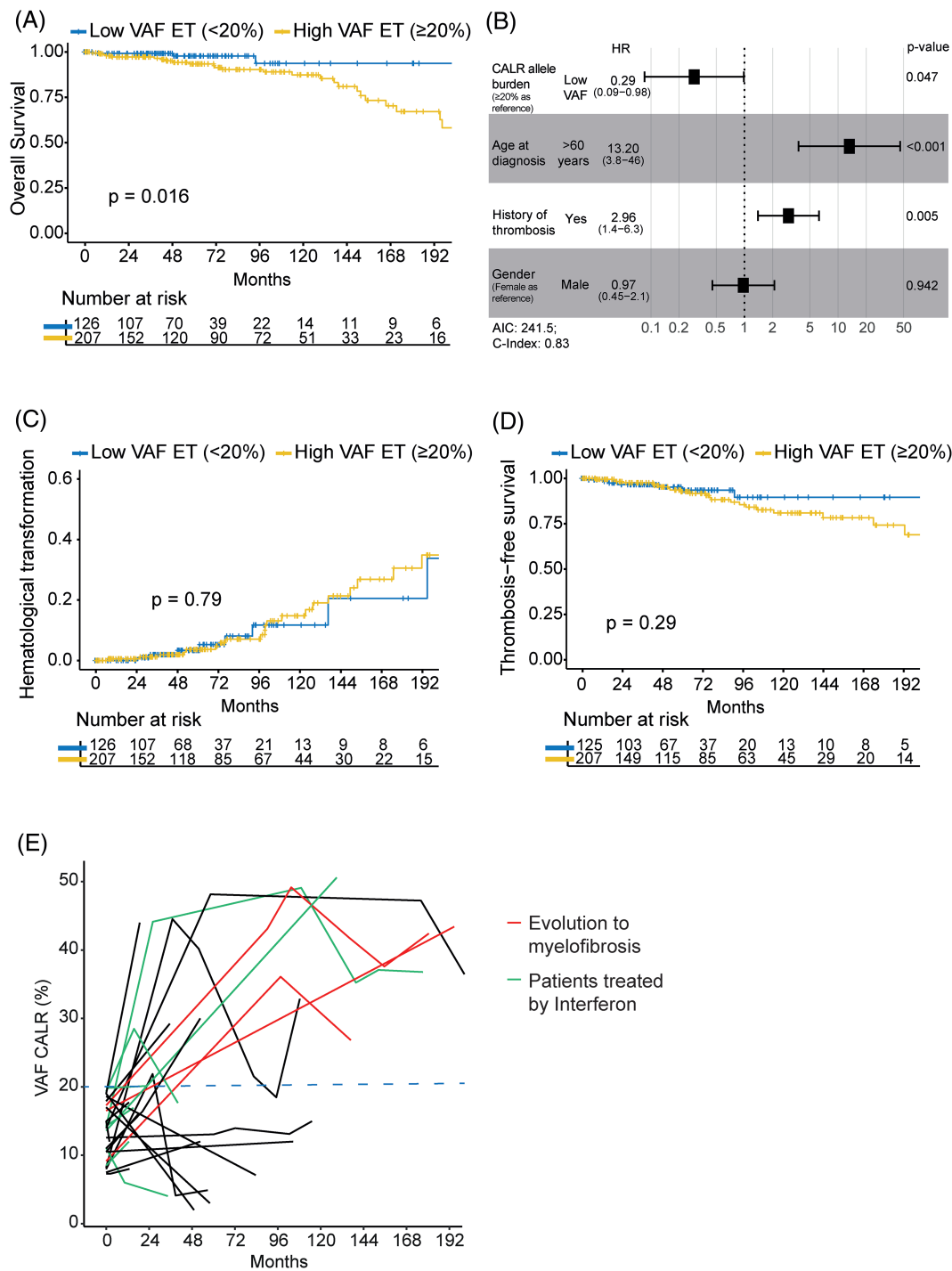


FIGURE 1 Kaplan-Meier curve representing overall survival (A) according to the allele burden of CALR-mutated patients at the time of diagnosis. Low and high allele burden groups have been defined using the cutoff of 20%. Forest plot (B) showing the multivariable analysis for overall survival in CALR-mutated ET. Kaplan-Meier curves representing the incidence of hematological transformation (C) (either secondary myelofibrosis, myelodysplastic syndrome, or acute leukemia) and thrombosis-free survival (D) according to the allele burden of CALR-mutated patients at the time of diagnosis. Trajectories of the allele burden of CALR mutations were represented for 26 low allele burden patients (E).

We then aimed to study the evolution of CALR allele burden in low allele burden patients. Follow-up samples were available for 26 CALR low allele burden ET patients, providing a total of 80 CALR measurements with a mean follow-up of 6 years between diagnosis and the last CALR quantification. Fifteen of the 26 patients (58%)

remained with a low CALR allele burden below 20%, while 11 patients (42%) had an increase of CALR allele burden during their follow-up (Figure 1E). Three of the 26 patients progressed to secondary myelofibrosis, and all of them had an increase of their allele burden. This increase was observed at the time of myelofibrotic transformation for

two patients and 40 months before for the third patient. An increase of CALR allele burden could be related to an increase of the clone size and/or to a transition to homozygosity by a loss of heterozygosity (LOH) at chromosome 19. Such homozygosity is a rare event but associated with disease progression.⁵ A previous study also reported the prognostic impact of an increase of CALR allele burden in a cohort of ET patients.⁶ Sixteen of the 26 patients were treated by cytoreductive therapy during follow-up: 6 by hydroxycarbamide, 7 by interferon- α , and 3 by anagrelide. Treatment by interferon- α was not associated with a decrease of CALR allele burden, as 4 out of 7 patients showed an increase during follow-up.

Finally, we aimed to investigate whether a clonal expansion restricted to a lineage exists in CALR MPN, similarly to what has been described in MPN with low allele burden JAK2 mutation.² We quantified CALR mutations in platelets, granulocytes, monocytes, B cells, T cells, NK cells, hematopoietic stem cells (CD34+CD38-, HSC), and hematopoietic progenitor cells (CD34+CD38+, HPC) for 19 CALR-mutated patients, including 13 patients undergoing treatment (Table S2 and Figure S4A). Median time between diagnosis and cell sorting was 6 years [2.76;13.48] (Figure S4A). The median allele burden of CALR mutation in total leukocytes was of 38%, and 6 of the 19 patients had low allele burden (i.e., <20%). The allelic burden of CALR mutation was relatively stable among HSC, HPC, and myeloid lineages (Figure S4B). For some patients, we found a lower CALR allele burden in platelets. Among lymphoid lineages, NK cells had the higher level of CALR allele burden, followed by B cells and lastly T cells, which were mostly non-clonal. These results indicate that a skewed expansion of mutated hematopoiesis is not present in CALR-mutated MPN. Future research may help elucidate the mechanisms by which a small clone in all compartments contributes to the observed phenotype.

Overall, our results indicate that CALR-mutated ET patients with a low allele burden exhibit a milder phenotype compared with CALR-mutated ET patients with an allele burden $\geq 20\%$. A low allele burden of CALR mutation at the time of diagnosis also influences the disease course with a better overall survival independently of classical prognostic markers such as age and previous history of thrombosis. In this retrospective real-world study, the diagnoses of ET were determined locally by a multidisciplinary team; however, we cannot exclude the possibility that certain cases may actually represent pre-fibrotic forms. Future large-scale studies with long-term follow-up and systematic monitoring of allele burden are essential for a more comprehensive understanding of the dynamics of evolution of these patients.

AUTHOR CONTRIBUTIONS

V.U., L.C., and D.L.P. conceived the research; L.A., R.V.B., V.L.B., L.C., and D.L.P. performed and analyzed the experiments; R.V.B., R.D.D.O., M.D., J.R., O.M., J.C.I., C.P., A.D., A.M., V.d.M., S.T., F.G., J.S.D., N.B., V.G.M., F.B., C.O., D.R., E.C., L.L.C., C.N., C.R., T.B.L., B.C., P.R., G.B., C.B., K.L., L.B., and E.L. provided clinical and biological data; and L.C. and D.L.P. supervised the project. L.A., L.C. and D.L.P. wrote the original draft and all authors reviewed and approved the manuscript. This paper was written on behalf of the French Intergroup of Myeloproliferative Neoplasms (FIM) and of the 'Groupe des Biologistes Moléculaires des Hémopathies Malignes' (GBMHM).

ACKNOWLEDGMENTS

The authors are indebted to Catherine Guillet from the PACeM facility, who helped with FACS sorting experiments and Rébecca Jouanneau-Courville, who performed CALR quantifications. The authors thank physicians and biologists from the FIM and GBMHM for helpful scientific discussions. The authors also thank all participating biobanks (Centres de Ressources Biologiques) for providing high-quality DNA samples. The authors thank patients, biologists, pathologists, hematologists, and data managers who participated in the FIMBANK project, especially Lea Sureau for data coordination and Elouan Rouméas-Noël for data collection. Finally, the authors thank Willy Vaillant, Marc Muller, Pascale Cornillet-Lefebvre, Tasnim Fethi, Eric Delabesse, Jean Claude Chomel, Nathalie Sorel, Aurélie Chauveau, Vincent Rebière, Françoise Jeanne, and Corinne Souyris for collecting data. This study was supported by grants from the French National Institute of Cancer (INCa BCB 2013 & 2022).

FUNDING INFORMATION

Institut National Du Cancer, Grant/Award Number: FIMBANK-BCB-2013&2022.

CONFLICT OF INTEREST STATEMENT

All authors declare no competing financial interests related to this work.

DATA AVAILABILITY STATEMENT

Data available on request due to privacy/ethical restrictions (individual data cannot be shared without a new regulatory approval).

Laura Aubin^{1,2,3}, Rúben Vilas Boas⁴, Rafael Daltro De Oliveira⁵, Valoris Le Brun⁶, Marion Divoux^{7,8}, Jérôme Rey⁹, Olivier Mansier^{10,11}, Jean-Christophe Ianotto¹², Cédric Pastoret³ , Anne Desmares³, Anne Murati¹³, Véronique de Mas¹⁴, Suzanne Tavitian¹⁵, François Girodon⁴, Juliette Soret Dulphy⁵, Nabih Maslah¹⁶, Valérie Goncalves Monteiro¹⁷, Françoise Boyer¹⁸, Corentin Orvain¹⁸ , Dana Ranta⁷, Émilie Cayssials¹⁹, Lenaig Le Clech²⁰, Christophe Nicol²¹, Camille Rottier²², Teresa Botin Lopez²³, Brice Castel²⁴, Patrick Rispal²⁵, Guillaume Beziat²⁶, Charles Bescond²⁷, Kamel Laribi²⁸, Lina Benajiba⁵, Valérie Ugo¹, Eric Lippert^{6,29} , Laurane Cottin¹, Damien Luque Paz¹ 

¹Univ Angers, Nantes Université, CHU Angers, Inserm, CNRS, CRCI2NA, Angers, France

²Laboratoire d'Hématologie, CH St Malo, St Malo, France

³Laboratoire d'Hématologie, CHU Rennes, Rennes, France

⁴Laboratoire d'Hématologie, CHU Dijon, Dijon, France

⁵INSERM U944/CNRS UMR7212, Hôpital Saint Louis APHP, Université de Paris, Paris, France

⁶Laboratoire d'Hématologie, CHRU Brest, Brest, France

⁷Hématologie Clinique, CHU Nancy, Nancy, France

⁸Laboratoire d'Hématologie, CHU Nancy, Nancy, France

⁹Centre de Recherche en Cancérologie de Marseille (CRCM), Département d'Hématologie, Institut Paoli-Calmettes, CRCM, Inserm, Marseille, France

¹⁰Laboratoire d'Hématologie, CHU Bordeaux, Bordeaux, France

¹¹Inserm U1034, Université de Bordeaux, Bordeaux, France

¹²Service d'Hématologie Clinique, CHU Brest, Brest, France

¹³Centre de Recherche en Cancérologie de Marseille (CRCM),

Département de Biopathologie et Département d'Oncologie Prédictive, Institut Paoli-Calmettes, Inserm, Marseille, France

¹⁴Laboratoire d'Hématologie, Institut Universitaire du Cancer de Toulouse Oncopole, CHU Toulouse, Toulouse, France

¹⁵Service d'Hématologie, Institut Universitaire du Cancer de Toulouse Oncopole, CHU Toulouse, Toulouse, France

¹⁶Laboratoire de Biologie Cellulaire, Université Paris Cité, APHP, Hôpital Saint-Louis, Paris, France

¹⁷Laboratoire d'Hématologie, CHU Reims, Reims, France

¹⁸Service des Maladies du Sang, CHU Angers, Angers, France

¹⁹Service d'Oncologie Hématologique et Thérapie Cellulaire, CHU de Poitiers, Poitiers, France

²⁰Hématologie Clinique, CH de Cornouaille, Quimper, France

²¹Hématologie Clinique, CH des Pays de Morlaix, Morlaix, France

²²Laboratoire d'oncobiologie moléculaire, CHU Amiens-Picardie, Amiens, France

²³Hématologie Clinique, CH Castres, Castres, France

²⁴Service de Médecine Interne, CH de Bigorre, Tarbes, France

²⁵Service de Médecine Interne, CH Agen, Agen, France

²⁶Hématologie Clinique, CH Albi, Albi, France

²⁷Hématologie Clinique, CH Cholet, Cholet, France

²⁸Hématologie Clinique, CH Le Mans, Le Mans, France

²⁹INSERM, U1078, Université de Brest, Brest, France

Correspondence

Laurane Cottin and Damien Luque Paz, Laboratoire d'Hématologie, Institut de Biologie en Santé, CHU ANGERS, 4 Rue Larrey, 49933 ANGERS Cedex 9, France.

Email: laurane.cottin@chu-angers.fr; damien.luquepaz@chu-angers.fr

Laurane Cottin and Damien Luque Paz contributed equally to this work.

ORCID

Cédric Pastoret  <https://orcid.org/0000-0002-5675-3154>

Corentin Orvain  <https://orcid.org/0000-0002-8717-0824>

Eric Lippert  <https://orcid.org/0000-0002-2312-6584>

Damien Luque Paz  <https://orcid.org/0000-0002-4523-675X>

REFERENCES

1. Luque Paz D, Kralovics R, Skoda RC. Genetic basis and molecular profiling in myeloproliferative neoplasms. *Blood*. 2023;141:1909-1921. doi:[10.1182/blood.2022017578](https://doi.org/10.1182/blood.2022017578)
2. Nienhold R, Ashcroft P, Zmajkovic J, et al. MPN patients with low mutant JAK2 allele burden show late expansion restricted to erythroid and megakaryocytic lineages. *Blood*. 2020;136:2591-2595. doi:[10.1182/blood.2019002943](https://doi.org/10.1182/blood.2019002943)
3. El-Khoury M, Cabagnols X, Mosca M, et al. Different impact of calreticulin mutations on human hematopoiesis in myeloproliferative neoplasms. *Oncogene*. 2020;39:5323-5337. doi:[10.1038/s41388-020-1368-3](https://doi.org/10.1038/s41388-020-1368-3)
4. Pietra D, Rumi E, Ferretti VV, et al. Differential clinical effects of different mutation subtypes in CALR-mutant myeloproliferative neoplasms. *Leukemia*. 2016;30:431-438. doi:[10.1038/leu.2015.277](https://doi.org/10.1038/leu.2015.277)
5. Guglielmelli P, Maccari C, Sordi B, et al. Phenotypic correlations of CALR mutation variant allele frequency in patients with myelofibrosis. *Blood Cancer J*. 2023;13:21. doi:[10.1038/s41408-023-00786-x](https://doi.org/10.1038/s41408-023-00786-x)
6. Cottin L, Riou J, Orvain C, et al. Sequential mutational evaluation of CALR-mutated myeloproliferative neoplasms with thrombocytosis reveals an association between CALR allele burden evolution and disease progression. *Br J Haematol*. 2020;188:935-944. doi:[10.1111/bjh.16276](https://doi.org/10.1111/bjh.16276)

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.