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1 **A temporal perspective for tumor-associated macrophage identities**
2 **and functions**

3

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1 **Abstract**

2 **Cancer is a progressive disease that can develop and evolve over decades, with**
3 **inflammation playing a central role at each of its stages, from tumor initiation to**
4 **metastasis. In this context, macrophages represent well-established bridges**
5 **reciprocally linking inflammation and cancer via an array of diverse functions that**
6 **have spurred efforts to classify them into subtypes. Here, we discuss the**
7 **intertwines between macrophages, inflammation, and cancer with an emphasis on**
8 **temporal dynamics of macrophage diversity and functions in pre-malignancy and**
9 **cancer. By instilling temporal dynamism into the more static classic view of tumor-**
10 **associated macrophage biology, we propose a new framework to better**
11 **contextualize their significance in the inflammatory processes that precede and**
12 **result from the onset of cancer and shape its evolution.**

1 Introduction

2 Perturbations of tissue homeostasis, such as infection or injury, induce a cascade
3 of events resulting in redness, warmth, swelling, and pain that is known as
4 inflammation^{1,2}. Physiologically, inflammation is a protective response of the immune
5 system that facilitates the elimination of the triggering insult and initiates tissue repair.
6 However, when the nature, intensity, and/or duration of this response is altered, it can
7 lead to a wide range of pathologies, including heart disease, autoimmune disorders and
8 cancer^{3,4}. Accordingly, cancer commonly arises at inflamed tissue sites, and inflammation
9 remains a primary risk factor for tumor development, independent of sexual dimorphism,
10 lifestyle, or exposure to specific mutagens^{5,6}.

11 Among the myriad of cell types involved in physiological and pathological
12 inflammatory responses, the most abundant cell type is often macrophages. In the context
13 of cancer, these are commonly referred to as tumor-associated macrophages (TAMs)^{7,8}
14 to differentiate them from **resident tissue macrophages (RTMs)** present in normal, non-
15 tumoral tissues^{9,10}. TAMs have been long known to be associated with tumor
16 development and generally exhibit pro-tumoral functions^{11,12}. However, it is becoming
17 clear that this “TAM” denomination fails to fully reflect the dynamic heterogeneity of
18 macrophage populations^{13,14}. Indeed, single-cell and lineage-tracing technologies have
19 exposed how TAM biology is inherently complex, with the co-existence of distinct
20 subpopulations with a differential ontogeny, spatial tissue localization, and cancer stage-
21 dependent functions that evolve over time¹⁵⁻²¹. Moreover, even before the onset of cancer
22 initiation, macrophages within inflamed (or previously inflamed) normal tissues can
23 modulate the susceptibility of epithelial cells to subsequent neoplastic transformation by
24 instructing them with an epigenetic memory of inflammation²².

25 Despite their significance as bridges between inflammation and cancer
26 pathogenesis, the current “TAM” nomenclature only formally refers to macrophages
27 present in *established* tumors, and currently no distinction is made between these and
28 those previously present in the tissue from *before* tumor onset. As both types of
29 macrophages can have different ontogenies and exposure to tissue signals, two well-
30 established determinants of macrophage heterogeneity discussed below, an updated
31 framework that integrates the time axis into the biology of TAMs is needed to better

1 conceptualize their roles orchestrating the inflammatory responses that often precede,
2 result from, and fuel cancer.

3 Herein, we discuss recent advances in immunology and oncology that, combined,
4 shape our current vision of TAM biology. We propose a new perspective that captures
5 temporal aspects of macrophage evolutionary histories within normal, pre-malignant and
6 malignant tissues, to better describe how their physiological functions are co-opted in
7 cancer²³⁻²⁶ and expose means to interfere with their disease-promoting roles more
8 selectively. We argue for a distinction between intra-tumor macrophages that resided in
9 the tissue *before* cancer onset (PreTAMs), which connect inflammation with cancer
10 initiation and are further reprogrammed within the TME of developing tumors, and
11 macrophages that are recruited *after* their establishment (TAMs), whose later arrival
12 imposes distinct interactions and features influencing their fate and functions (**Figure 1**).

13

14 **Heterogeneity of macrophages in normalcy, premalignancy, and cancer**

15 Historically, macrophage identities within normal and tumor contexts have been
16 reduced to two antagonist types of ‘activation’ or polarization states: M1 considered as
17 pro-inflammatory, and M2 as anti-inflammatory^{27,28}. While such classification was
18 convenient for explaining distinct transcriptional and functional states identified *in vitro*, it
19 has become evident that this dichotomy is insufficient to reflect the many levels of
20 macrophage heterogeneity observed *in vivo*, across distinct anatomical sites, tissue
21 niches, and disease contexts^{9,29,30}.

22 A well-established determinant of macrophage heterogeneity is ontogeny, which
23 largely varies according to tissues²⁹. During embryogenesis, fetal macrophage precursors
24 seed different tissues and give rise to RTMs, for example, microglia in the brain or Kupffer
25 cells in the liver³¹⁻³⁴. Although RTMs can be maintained by local self-renewal during
26 adulthood^{35,36}, tissues such as the gut lamina propria or the dermis can postnatally recruit
27 bone marrow-derived monocytes which also give rise to RTM populations from a different
28 ontogeny^{37,38}. During physiological and pathological inflammation, such as during tissue
29 damage or ageing, macrophage tissue diversity is further reprogrammed, with an
30 increase in monocyte-derived macrophages, which can be short-lived or long-lived and

1 have context-dependent effects that deserve deeper investigation (see³⁹ for an extended
2 review on macrophage dynamics in normal tissues).

3 Beyond ontogeny, macrophages are highly plastic cells and their identity is further
4 shaped by tissue signals from their niche of residence or arrival that can largely remodel
5 their phenotype, gene expression, and chromatin architecture⁴⁰. For example, within the
6 liver, studies suggest that macrophage identity is influenced by local, sub-tissular
7 interactions with fibroblasts, endothelial cells, or lymphocytes⁴¹⁻⁴³. In the lung, alveolar
8 macrophages differ from interstitial macrophages, and even among these lung interstitial
9 macrophages, perivascular and perineural subpopulations have been distinguished with
10 different proximities to endothelial cells and smooth muscle cells, or nerve bundles and
11 endings^{44,45}, respectively

12 As in healthy tissues, ontogeny and tissue environment also contribute to the
13 remarkable intra- and inter-tumor heterogeneity of macrophages observed in cancer.
14 Initially, the assumption was that TAMs derived from circulating monocytes that are
15 continuously recruited in response to tumor-induced inflammation⁴⁶. While subsequent
16 research has confirmed that monocyte-derived cells often represent the majority of
17 TAMs⁴⁷⁻⁴⁹; these cells co-inhabit the TME with macrophages that resided in the tissue
18 before tumor onset. Importantly, embryonic-derived RTMs and adult monocyte-derived
19 TAMs exhibit distinct functions. For example, in a murine model of pancreatitis and
20 pancreatic cancer, embryonic RTMs are potent promoters of fibrotic responses⁵⁰ whereas
21 adult monocyte-derived counterparts regulate adaptive immune responses⁵¹.
22 Regardless, neither ontogeny nor intra-tumoral location of macrophages explain when
23 and how they came to be “TAMs”, as monocyte-derived macrophages with TAM-like and
24 tumor-promoting phenotypes can be recruited to tissues *before* the formation of
25 neoplastic lesions, such as during injury-induced pancreatic metaplasia^{52 53}. State-of-the-
26 art fate-mapping⁵⁴, temporally-resolved single-cell profiling⁵⁵⁻⁵⁷ and innovative strategies
27 are needed to better define the temporal histories and “birthdates” of distinct types of
28 macrophages within normal, inflamed, pre-malignant and malignant tumor lesions.

29 Once within a tumor microenvironment, TAMs are influenced by interactions with
30 their neighboring cells that are largely dependent on their precise spatial location within
31 the tumor lesion. For example, specific TAM subpopulations defined by a set of markers

1 and locations have been identified, such as FOLR2⁺ macrophages in perivascular areas
2 of primary breast tumors⁵⁸ or TREM2⁺ macrophages in tumor nests of liver, lung, and
3 lymph node metastases⁵⁹. Nevertheless, these identities are not definitive, as TAMs
4 display a high level of plasticity and are influenced by interactions with tumor and non-
5 tumor cells that evolve during disease progression (see⁶⁰ for a recent perspective on this
6 topic). As an example, pre-clinical models of hepatocellular carcinomas show that TAMs
7 undergo a shift to states reminiscent of embryonic development, termed “oncofetal
8 reprogramming⁶¹⁻⁶³; yet when tumor cells undergo senescence, they can acquire anti-
9 tumor states that facilitate CD8 T cell-mediated immunity⁶⁴.

10 Therefore, origin and environment influence TAM biology, and the relative
11 contribution of each of these determinants is an age-old debate between nature and
12 nurture⁶⁵⁻⁶⁷. We argue that, in addition to these two determinants, time should be
13 integrated into the “TAM” classification framework to reflect the continuum between
14 inflammation and cancer (which starts well *before bona fide* tumor establishment), and
15 better apprehend how temporal aspects shaping their diversity impact pre-malignant and
16 malignant evolution. Of note, this paradigm shift has begun with the RTM characterization
17 over the last decade and we envision such evolution will also be fruitful in cancer studies.

18

19 **A new proposal for defining TAM heterogeneity: time as the protagonist**

20 The initiation of cancer can be relatively slow and silent, with genetic mutations,
21 epigenetic aberrations, and/or chronic inflammation being present in tissues even years
22 before the emergence of a full-blown cancer⁶⁸⁻⁷⁰. Yet, the current TAM denomination is
23 limited to macrophages present in the tissue *after* the establishment of tumor lesions,
24 which is arguably a late event in the pathogenesis of the disease and obliterates the early
25 steps of cancer development. Indeed, macrophages play key roles in early transitions
26 from tissue normalcy to metaplasia, its reprogramming into pre-malignant neoplastic
27 lesions, and subsequent malignant and metastatic evolution. Moreover, macrophages
28 lifespan can vary from days to months, and even years – with long-lived subpopulations,
29 such as microglial cells, lasting on average 4.2 years old⁷¹. Therefore, considering only
30 TAMs as tumor-inhabiting cells without considering their lifespan, history of prior

1 exposures, and functions prior to tumor onset provides an incomplete picture of their
2 relevance interconnecting inflammation and cancer.

3 To emphasize such early roles, we propose to incorporate a prefix to distinguish
4 macrophages pre-existing in the tissue *before* tumor onset (PreTAMs), with “*Pre*” to
5 emphasize they were previously present in the tissue (irrespective of their specific
6 ontogeny) and “*TAM*” to underline their significance as cellular constituents and drivers
7 of the resulting tumors. *As such, PreTAMs would link pre-existing inflammation to cancer
8 pathogenesis, whereas remaining TAMs recruited from circulating monocytes after tumor
9 formation play the reciprocal role linking back this process to tumor-elicited inflammation,*
10 in collaboration with PreTAMs that are further reprogrammed within the TME. Of note,
11 this denomination is only based on the time dimension and agnostic of ontogeny, as
12 PreTAMs can be either of embryonic or adult origin, the fundamental point being their
13 presence from before tumor onset.

14 The PreTAM distinction sheds light on an essential yet ill-defined component of
15 macrophage biology: their memory of previous challenges. Pioneering studies in the
16 infectiology field have revealed that macrophages, as other innate immune cells, can
17 keep a “memory” of previous immunogenic challenges and mount a more efficient
18 response to subsequent ones, even from unrelated pathogens⁷²⁻⁷⁴, a phenomenon coined
19 as trained immunity⁷⁵. At the cellular level, this process involves enhancer
20 reprogramming⁷⁶, 3D chromatin topology reorganization⁷⁷, and metabolic rewiring⁷⁸. At a
21 population level, it may also involve changes in the cellular origin of macrophages within
22 a given tissue. For example, embryonic alveolar macrophages can be replaced by adult
23 monocyte-derived ones during lung infection and acquire similar phenotypes yet with
24 enhanced protective capacities⁷⁹. Interestingly, while in the context of an infection, such
25 “memory” can increase the resistance of the tissue to new challenges and, as such, be
26 harnessed to enhance trained immunity, increasing evidence suggests this can be
27 associated with the induction of altered inflammatory responses that can both promote or
28 restrain tumor progression⁸⁰⁻⁸².

29 The underlying mechanisms and implications of time-dependent macrophage
30 features as determinants of cancer susceptibility and evolution are, however, still far from
31 being completely understood. It is tempting to speculate that PreTAMs will be influenced

1 by ontogeny-related and environment-induced scars of “tissue history” by insults that can
2 be recent or transient or long-established and chronic, depending on the temporal scales
3 linking inflammation and tumorigenesis. Regardless, we envision that time-dependent
4 features will influence macrophage diversity within tissues and disease evolution by
5 various mechanisms that represent open questions to address in future studies.

6 Finally, in addition to the concepts of TME dynamism and macrophages as
7 “guardians of tissue memory”, the time parameter is inexorably linked to aging, the top
8 risk factor for cancer development⁸³. As one mechanism, chronic inflammation is
9 considered a hallmark of aging⁸⁴. In parallel, aged epithelial cells accumulate (epi)genetic
10 alterations that prime them for clonal expansions⁸⁵ that appear to be unleashed when
11 exposed to inflammatory insults^{86,87}. In parallel, long-lived RTMs or hematopoietic stem
12 cell precursors of TAMs can, themselves acquire age-associated alterations that can be
13 directly pathogenic⁸⁸⁻⁹⁰ as observed in neurodegeneration phenotypes driven by
14 BRAF^{V600E} mutation in microglia⁹¹.

15 Therefore, both long-standing and more recent observations coincide in how
16 macrophage biology is highly time-dependent, a notion which should be considered when
17 approaching their relevance as bridges between inflammation and cancer. This
18 constitutes the basis of our proposal of incorporating a temporal mark that distinguishes
19 macrophages that participated in inflammatory processes that preceded (PreTAM) and
20 not only resulted from the tumor development process (TAMs) (**Figure 2**).

21 **PreTAM and TAM heterogeneity in cancer initiation, progression and metastasis**

22 *Pre-existing and tumorigenesis-induced macrophage heterogeneity modulates cancer* 23 *initiation*

24
25 Cancer initiation is driven by cooperation between genetic and environmental
26 inflammatory insults⁹²⁻⁹⁶. As limiting the exposure to such insults may be challenging,
27 gaining insights into how they impact PreTAMs may expose strategies to prevent or
28 intercept links between inflammation and cancer pathogenesis. In this section, we will
29 present how diverse cancer-initiating insults, such as exposure to environmental
30 pollutants, tissue damage, infection, and genetic mutations, shape PreTAM diversity and
31 functions. **Indeed, while appropriately activated, macrophages can recognize and**

1 eliminate damaged and mutant cells and protect the host from tumor development⁹⁷, but
2 these physiological functions can be reprogrammed by cancer-initiating insults to trigger
3 tumor-promoting inflammation and cancer development.

4 Recent perspectives highlight the contribution of non-mutagenic mechanisms of
5 environmental carcinogens to cancer pathogenesis^{93,98}. For example, pollutant
6 particulates can promote cancer through inducing IL-1 β production by macrophages⁹², a
7 well-established macrophage pro-inflammatory mechanism targetable by anti-
8 inflammatory therapy showing protection against lung cancer incidence and mortality in
9 a randomized clinical trial^{99,100}. Similarly, cigarette smoke triggers a basal level of lung
10 inflammation by modulating the inflammatory state of lung alveolar macrophages¹⁰¹,
11 including IL-1 β release¹⁰², further supporting IL-1 β targeting as a means to prevent
12 and/or intercept lung carcinogenesis⁹⁹. Also in the lung, exposure to asbestos triggers
13 apoptosis of otherwise long-lived alveolar macrophages, resulting in the infiltration of
14 monocytes and their differentiation into alveolar macrophage-like cells¹⁰³. Recruited
15 macrophages persist and further enhance inflammation within the fibrotic lesions of
16 asbestos-treated mice¹⁰⁴. These responses can also be activated by toxins such as
17 aflatoxin B1, which can activate macrophages directly¹⁰⁵, as well as by triggering
18 apoptosis of parenchymal cells and their phagocytosis by macrophages¹⁰⁶. When these
19 inflammatory responses lead to tumors, these ontogenically-distinct populations
20 “educated” within tissues exposed to carcinogens would be considered PreTAMs.

21 Additional non-mutagenic insults such as tissue damage can also unleash tumor-
22 promoting inflammation through the rewiring of both RTMs and monocyte-derived
23 macrophages, or their relative abundance. For example, in the pancreas, inflammation
24 triggered by tissue damage, usually referred to as pancreatitis, induces metaplasia and
25 markedly accelerates the development of pancreatic ductal adenocarcinoma in mice¹⁰⁷ in
26 a manner dependent on the presence and functions of macrophages^{52,108,109}. Tumor-
27 initiating inflammation can also be triggered by chronic viral infections such as Human
28 Papilloma Virus (HPV) or Human Immunodeficiency Virus (HIV)⁵⁷. Despite the lifelong
29 treatment of HIV and preventing patients from progressing to acquired immunodeficiency
30 syndrome (AIDS), systemic inflammation is sustained throughout the life of an HIV
31 patient¹¹⁰. Even in antiretroviral therapy-treated patients, viral reservoirs exist in various

1 tissues including the gastrointestinal tract¹¹¹. As a consequence of these persistent viral
2 reservoirs, macrophages are constitutively engaged in inflammatory responses, which
3 impair intestinal barrier function causing bacterial product translocation into the blood¹¹².
4 Beyond such systemic immune activation, the immunosuppression of HIV patients can
5 lead to Kaposi sarcomas driven by co-infection with human herpesvirus 8 (HHV8)¹¹³; and
6 which progress by complex mechanisms involving chemokine homologues encoded by
7 the virus and downregulation of the macrophage chemokine scavenger ACKR2 in tumor
8 cells, resulting in cancer-related inflammation by TAMs and monocyte recruitment¹¹⁴. The
9 relative contribution of PreTAMs and TAMs remains to be fully deciphered.

10 Beyond environmental cues, genetic alterations known to predispose to cancer
11 can also create tumor-permissive and -promoting niches by modulating macrophage
12 states directly or indirectly. As an example, TAMs from *BRCA1*-deficient breast tumors
13 are reprogrammed and can directly mediate immune suppression¹¹⁵. In addition, highly
14 metastatic inflammatory breast cancers (IBC) with *BRCA1/2* mutations have a
15 characteristic inflammatory milieu suggested to alter TAM states in favor of cancer
16 dissemination^{116,117}. In a different disease context, a phenotypically distinct TME has been
17 noted in *BRCA1/2* mutated high-grade serous ovarian cancers as compared to *BRCA1/2*
18 wild-type counterparts, which exhibit a lower abundance of macrophages¹¹⁸. Finally, in
19 thyroid carcinomas, *PTEN*-inactivating mutations in tumor cells have been associated
20 with a specific macrophage phenotype with high IL-6, TNF- α , IL-8 and MCP-1 expression
21 downstream of hyperactivated mTOR signalling¹¹⁹. It is also tempting to speculate that
22 other cancer-predisposing inflammatory disorders associated with Human Leukocyte
23 Antigen (HLA) polymorphisms¹²⁰ involve TAMs, given the specific high expression of HLA
24 antigens by macrophages. Indeed, HLA encodes Major Histocompatibility Complex
25 (MHC) molecules that are responsible notably for the communication of the innate
26 immune system such as macrophages and dendritic cells with T cells through antigen
27 presentation. The HLA-B27 expression has been notably associated with an increased
28 risk of inflammatory bowel disease and colorectal cancer development^{121,122}.

29 Overall, it is becoming clear that PreTAMs and TAMs act as key cellular effectors
30 of environmental and genetic cancer-initiating insults. While precise mechanisms, as well
31 as the lifespan and fate of PreTAMs and TAMs within metaplastic, pre-malignant and

1 malignant tissues need to be further clarified, these examples underline the importance
2 of PreTAMs as critical players in cancer pathogenesis.

3 4 *Tumor-induced rewiring of PreTAMs and TAMs promotes disease progression*

5 Once tumors are formed, malignant cells require access to nutrients and complex
6 adaptations to meet their metabolic growth demands. One such adaptation is the
7 promotion of angiogenesis, with TAMs playing a pivotal role by secreting effector
8 molecules such as Vascular Endothelial Growth factor (VEGF) or matrix
9 metalloproteinases^{123,124}. Failure to induce a sufficient blood supply will result in nutrient
10 deprivation and hypoxia in the tumor core which will turn necrotic¹²⁵, a phenomenon that
11 can also lead to inflammation and disease progression¹²⁶⁻¹²⁸. In this context of tumor
12 necrosis, macrophages perform one of their most well-described functions: phagocytosis
13 of debris and dying cells, with CD206⁺ macrophages being recognized as the most
14 actively phagocytic populations¹²⁹. Interestingly, CD206⁺ TAMs have also been shown to
15 cross-present tumor antigens to CD8⁺ T cells in tumors^{130,131}, highlighting a complex
16 interplay between dynamic inflammatory responses within evolving tumors,
17 macrophages, and adaptive immunity.

18 Our understanding of the crosstalk between myeloid and lymphoid cells in the TME
19 has significantly increased since the appreciation of the process of cancer immunoediting
20 two decades ago⁹⁷. It is well appreciated that interferon gamma produced by innate (NK,
21 ILC1s) and adaptive (CD8⁺ T) immune cells induces a pro-inflammatory phenotype of
22 macrophages, which is associated with anti-tumor activities^{132,133}. Such knowledge has
23 led to the design cellular backpacks filled with interferon gamma as a means to prime and
24 maintain anti-tumor responses in TAMs¹³⁴. However, as tumors evolve, macrophage–T
25 cell direct interactions are dynamically reprogrammed and most often ultimately dampen
26 anti-tumor immunity. Consistently, PreTAMs and TAMs can have differential capacities
27 to instruct adaptive responses^{58,61,135}, with the latter promoting recruitment of regulatory
28 T-cells (Tregs), for example in ovarian¹³⁶ or liver cancer¹³⁷, and/or promoting CD8 T-cell
29 exhaustion^{138,139}. Therefore, while immunotherapy protocols based on reactivating
30 cytotoxic activities of T cells have shown some impressive success in several cancer
31 types¹⁴⁰, a better understanding of when and how PreTAMs and tumor-recruited TAMs

1 enhance vs suppress adaptive immunity is warranted to better understand their dual
2 potential as igniters of tumor-promoting inflammation and anti-tumor immunity, and tilt the
3 balance towards the latter.

4 Beyond their immunomodulatory roles, another targetable trait of macrophages
5 that is reprogrammed in established tumors is their metabolism¹⁴¹⁻¹⁴⁴. While Otto Warburg
6 had described a century ago the higher consumption of glucose by tumor cells¹⁴⁵, the
7 metabolic rewiring of macrophages within TME has been more recently reported^{146,147},
8 yet its specific impact on the course of the disease remains still poorly characterized. For
9 example, an upregulation of REDD1 in hypoxic TAMs has been shown to promote
10 angiogenesis¹⁴⁸ while enhanced tryptophan degradation in interleukin-4-induced-1
11 (IL4I1)⁺ macrophages promotes tumor progression¹⁴⁹. Understanding whether different
12 macrophage subpopulations differing in their ontogeny, specific tissue contexts, and/or
13 time of pre-existence in the tumor tissue, are differentially “primed” for such diverse tumor-
14 promoting functions will be an important step for predicting and interfering with tumor
15 progression. This will require the use of precise preclinical cancer models allowing for
16 differential targeting of PreTAMs and TAMs in a spatiotemporally-controlled manner.

17 18 Macrophage diversity in primary tumors and secondary localizations shape metastasis

19 As the ultimate expression of tumor evolution, metastasis defines the process by
20 which tumor cells disseminate away from the originating tumor to ultimately produce a
21 new one in nearby or distant site^{150,151}. While metastasis requires cancer cells to acquire
22 cell-intrinsic properties necessary for cell migration, invasion, immune evasion, survival
23 in circulation and colonization of the new site, it also involves a complex interplay with
24 TME cells that can favor or restrain each of these processes in a non-cell-autonomous
25 manner. Heterogeneous functions of TAMs participate in all these different steps.

26 In many solid tumors, migration and dissemination of cancer cells involve cellular
27 plasticity, such as full or hybrid epithelial to mesenchymal cell (EMT) transitions, a
28 process which is facilitated by TAMs acquiring transcriptional states that drive secretion
29 of EMT inducers, such as TGF- β ¹⁵². Interestingly, EMT transitions can happen very early
30 during tumor development, are exacerbated by inflammation¹⁵³, and involve TAMs^{153,154}.
31 Accordingly, spatial single-cell analyses reveal inflammatory cells in proximity with

1 transitioning mesenchymal-like cells¹⁵⁵. Considering that different subpopulations of
2 TAMs (such as PreTAMs) organize differentially within the TME of primary tumors^{58,153}
3 and that EMT can be restricted to the leading edge or inflamed areas, functional studies
4 involving targeting of specific TAM subpopulations will provide valuable insights into
5 microenvironmental determinants of cancer dissemination, and the influence of
6 macrophage ontogeny, tissue location, and time as regulators of this process (**Figure 3**).

7 Beyond changes in the primary tumor ecosystem, the “seed and soil” theory
8 highlights how a given tumor will preferentially metastasize to predefined tissues
9 “permissive” to this metastasis outgrowth¹⁵⁶. Interestingly, this theory emerged
10 concomitantly with the first description of macrophages, and these two seminal
11 discoveries have evolved in parallel for decades but without conceptual relationship^{157,158}.
12 Today, immune cells are recognized as key building components of what has been
13 defined as the pre-metastatic niche¹⁵⁹. Indeed, a seminal study has highlighted that
14 specific bone marrow progenitors were homing to pre-metastatic sites and were
15 facilitating arrival of disseminating cancer cells¹⁶⁰. Furthermore, RTMs present in distant
16 tissues before metastatic dissemination should also be considered as a specific subset
17 of PreTAMs, considering that they populate the tissue before disseminating cancer cells,
18 but with the noticeable difference that they likely have already been subjected to the
19 systemic effects of the primary tumor. In this regard, pre-metastatic niche PreTAMs can
20 be already reprogrammed, at least partially, and contribute to the priming of the tissue
21 “soil” for metastatic outgrowth. Expanding the concept of “PreTAMs” beyond the primary
22 tumor site, this phenomenon is highly tissue-specific, being orchestrated by both RTMs
23 and bone marrow-derived macrophages present in, or recruited to, the secondary site
24 before the arrival of any disseminating cancer cell. Mechanistically, such remote priming
25 of macrophages at the pre-metastatic niche can involve both extracellular vesicles¹⁶¹⁻¹⁶⁴
26 and secreted factors¹⁶⁵ produced by primary tumors, as well as, potentially, by tumor-
27 unrelated cues (eg prior environmental or mutagenic insults) that modulate macrophage
28 identities and functions at distal sites.

29 After successful metastatic seeding by tumor cells, pre-metastatic niche PreTAMs
30 and TAMs subsequently recruited by seeded tumor cells coexist and favor secondary
31 tumor outgrowth by establishing a reciprocal interplay with cancer cells and other

1 stromal/immune cells in the environment that provides growth factors and facilitates
2 escape from immune surveillance¹⁶⁶. For example, seminal work shows that inhibition of
3 CCL2–CCR2-recruitment of monocyte-derived macrophages reduces metastasis burden
4 in the lung⁴⁹. Such dynamic heterogeneity of macrophages at metastatic sites continues
5 to impact (and can help predict) disease progression in highly tissue-specific manner. As
6 a few examples, in the liver, single-cell analyses of metastasis-associated macrophages
7 have revealed subpopulations with distinct morphologies and transcriptional programs¹⁶⁷
8 and identified clinically relevant markers (eg GPNMB¹⁶⁸, C1Q¹⁶⁹, SPP1¹⁷⁰) of poor
9 prognosis. In this line, mouse models of colorectal carcinoma metastasis to liver associate
10 specific subpopulations of recruited macrophages with T cell exclusion and transition from
11 micro- to macro-metastasis¹⁷¹. In the omentum, RTMs play a pivotal role in the
12 establishment and development of metastasis in ovarian cancer¹⁷². Similarly, in the bone,
13 metastasis-infiltrating macrophages instruct resistance to androgen deprivation therapies
14 to prostate cancer cell¹⁷³, mediating a highly-tissue specific interplay between
15 inflammation and hormones. In the brain, TAM heterogeneity of brain metastasis is
16 distinct from that of primary tumors (gliomas), with an increased abundance of tumor-
17 educated microglia (PreTAMs) with differential immunomodulatory capacities^{174,175}. Thus,
18 just like at the primary tumor, tissue contexture specificities and temporal aspects
19 influence macrophage diversity at metastatic sites.

20 Whether organismal-level effects of macrophages dictate metastasis risk and
21 evolutionary patterns deserves further investigation. In a murine model of myocardial
22 infarction, local inflammation/injury can trigger changes in macrophage numbers and
23 states at uninjured distant sites, underscoring a remarkable network of remote, highly
24 tissue-specific, macrophage responses spanning different organs¹⁷⁶. Taking this concept
25 into the context of cancer, it is conceivable that pre-existing or tumor-induced systemic
26 inflammatory responses influence metastatic disease progression by impacting the
27 heterogeneity and function of [pre-metastatic and/or metastasis-recruited TAMs](#) in a
28 tissue-specific manner.

29
30
31

1 **Concluding remarks**

2 While macrophages can physiologically exert tissue reparative and anti-tumor
3 immune activities¹⁷⁷, most clinical studies have established a clear correlation between
4 macrophage abundance in tumors and the severity of the prognosis^{7,178-180}, supporting
5 the notion of a subversion of these cells in pre-malignancy and cancer. Clinical
6 observations and experimental systems support that their heterogeneity, plasticity, and
7 pleiotropic functions are influenced by multiple parameters, including ontogeny, spatial
8 context, and several temporal determinants, from macrophage individual experience and
9 lifespan to the organism's age and exposome. Herein, we propose to integrate the
10 dimension of time into the current framework to better understand the roles of
11 macrophages *inter*-connecting inflammation and cancer, and their dynamism within
12 developing and evolving tumoral niches. Such a paradigm shift is occurring in non-cancer-
13 related fields, such as in neurodegenerative and metabolic diseases, where a specific
14 subpopulation of TREM2⁺ macrophages termed DAM^{181,182} or LAM^{183,184} respectively has
15 been shown to act as pathological drivers.

16 Recent innovations in single-cell sequencing technologies, lineage-tracing
17 technologies, and model systems now provide the tools and knowledge base to deeply
18 characterize TAMs at spatial and *temporal* resolution. To parallel these advances, we
19 propose to similarly refine macrophage nomenclature by distinguishing between
20 PreTAMs vs *TAMs*, depending on whether macrophages (irrespective of ontogeny or
21 spatial location) pre-existed in the tissue vs were recruited after tumor **or metastasis**
22 formation. Of note, distinguishing time and ontogeny-related features are not mutually
23 exclusive choices. We rather envision that combining both nomenclatures will be a
24 powerful and simple way to conceptualize (and refer to) the multi-factorial biology
25 underlying macrophage identity and functions in cancer. Developing and consolidating a
26 framework that considers the impact of developmental origin, tissue contexture, and time
27 in TAM identity will revolutionize our capacity to explore, chart, and therapeutically target
28 their roles in the development, establishment, and progression of cancers.

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7

1 **Figure legends**

2

3 **Figure 1: Time is a key determinant of TAM biology**

4 Macrophages can be found in healthy tissues where they are known as Resident Tissue
5 Macrophages (RTMs). They contribute to tissue homeostasis but can also be involved at
6 the stage of premalignancy influencing and being influenced by tumor onset. In this
7 context, they should be considered as PreTAMs. Once the tumor is formed, resulting
8 inflammation will promote monocyte recruitment which will differentiate into TAMs, the
9 specificity of these cells being to experience only the tumor microenvironment. Of note,
10 respective half-life and fate of these two populations need to be refined, especially during
11 the three different phases of tumor development, elimination, equilibrium and escape.

12

13 **Figure 2: PreTAMs and TAMs are different populations of macrophages**

14 As pre-existing in the healthy tissue, PreTAMs can have a tissue-dependent mixed origin,
15 with the co-existence of embryonic and monocyte-derived cells, a unique feature not
16 shared with the later recruited TAMs deriving from monocytes in response to tumor-
17 elicited inflammation. PreTAMs have their own history, inhabiting the tissue before tumor
18 onset and can therefore be impacted by prior insults. This is in contrast with monocyte-
19 derived TAMs which are only exposed to the tumor niche and have therefore a different
20 imprinting. In light of this, crosstalk with other cells from the tumor microenvironment has
21 to be considered separately.

22

23 **Figure 3: Spatial distribution of RTMs and TAMs**

24 PreTAMs and TAMs coexist in established primary tumors and metastasis. But
25 macrophages are sessile cells and their location is different. Later recruited TAMs are
26 mostly found within the core of the tumor whereas PreTAMs are mostly excluded to the
27 periphery, resulting in different interactions with other cells from the TME and different
28 functions. Of note, macrophages from the pre-metastatic niche constitute an example of
29 PreTAMs which prepare the seeding of disseminating tumor cells before their arrival.