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1 **Roles of macrophages in tumor development: a spatio-temporal perspective**

2

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28 **Abstract**

29 Macrophages are critical regulators of tissue homeostasis but are also abundant in the
30 tumor microenvironment (TME). In both primary tumors and metastases, such tumor-
31 associated macrophages (TAMs) seem to support tumor development. While we know
32 that TAMs are the dominant immune cell in the TME, their vast heterogeneity and
33 associated functions is only just being unraveled. In this review, we outline the various
34 known TAM populations found thus far, and delineate their specialized roles
35 associated with the main stages of cancer progression. We discuss how macrophages
36 may prime the pre-metastatic niche to enable the growth of a metastasis and then how
37 subsequent metastasis-associated macrophages can support secondary tumor
38 growth. Finally, we speculate on the challenges that remain to be overcome in TAM
39 research.

40

41 **Introduction**

42 Macrophages are tissue-resident immune cells that emerge from multiple waves of
43 hematopoiesis during embryonic development to seed their organs of residency¹. In
44 mice, primitive myeloid progenitors arise in the yolk sac around embryonic day E7-8
45 and give rise to microglia, which self-maintain locally from this initial seeding,
46 independently from circulating monocytes². A second semi-definitive wave of
47 hematopoiesis starts at E8.25 and gives rise to progenitors that transiently shelter in
48 the fetal liver^{3,4} and subsequently colonize fetal tissues. These cells are known as
49 resident tissue macrophages (RTMs). A final, third wave of hematopoietic precursors
50 emerge from the aorta-gonado-mesonephros region at E10.5 and leads to the
51 generation of hematopoietic stem cells that will later establish definitive hematopoiesis
52 in the fetal liver and then in the bone marrow. Monocytes from this third wave are
53 recruited into tissues from late embryonic stages to adulthood, thus somewhat diluting
54 the pre-existing embryonic RTMs in a tissue-dependent manner⁵. Thus, contrary to
55 early consensus⁶, many RTMs found in adult tissues are long-lived cells with
56 embryonic origins.

57

58 During organogenesis, macrophages undergo tissue imprinting whereby embryonic
59 progenitors first acquire a core macrophage differentiation program including pattern
60 recognition and cytokine receptors⁷. Then, tissue-specific programs emerge during
61 embryonic development with the differential activation of transcription factors and gene
62 networks⁷. Such tissue imprinting is not an event restricted to embryonic development:
63 as we draw on in this review, the relatively long lifespan of RTMs means that they are
64 inevitably exposed to both non-homeostatic events, such as inflammation or infection,
65 and systemic signals. The somewhat continuous imprinting that ensues as a result of
66 a dynamically altered niche can lead to RTM dysregulation, which in turn might favor
67 oncogenesis⁸.

68

69 In the most basic sense, oncogenesis occurs as a result of an accumulation of
70 mutations in oncogenes that permit normal cells to overcome restrictions on cellular
71 replication such that they can grow without restraint to form a tumor. But it is not only
72 the tumor that has pathological consequences; other components that form the tumor
73 microenvironment (TME) influence oncogenesis and cancer progression⁹. The TME

74 comprises the blood and lymphatic vessels, extracellular matrix (ECM), and distinct
75 host cells including fibroblasts and immune cells in the immediate ecosystem that
76 surrounds the tumor, in addition to the tumor cells themselves¹⁰. Among the immune
77 cellular components of the TME, macrophages have received particular attention.
78 These so-called tumor-associated macrophages (TAMs) are typically the most
79 abundant immune population within the TME and their abundance is in fact now
80 leveraged as a diagnostic marker, as it often correlates with a poor prognosis¹¹⁻¹³. In
81 this review, we discuss the recent advances made in clarifying the roles of various
82 populations of TAM at key stages of cancer progression from tumor initiation to
83 metastasis (**Figure 1**).

84

85 **Macrophage diversity in the cancer context**

86 Macrophage diversity is broadly conceptualized through the prism of two antagonistic
87 polarization states: pro-inflammatory M1 and anti-inflammatory M2 macrophages¹⁴.
88 Upon the identification of TAMs in the TME, a similar paradigm was adopted with
89 immunosuppressive TAMs clinically associated with a bad prognosis considered as
90 M2 macrophages¹⁵⁻¹⁷. A therapeutic extension of this view has culminated in attempts
91 to repolarize TAMs from M2 to M1 states by modulating key factors involved in
92 macrophage development, such as colony-stimulating factor 1^{18,19} or IFN- γ ²⁰.

93

94 ***Tumor-associated RTMs and monocyte-derived TAMs***

95 Although TAMs undoubtedly exhibit immunosuppressive properties, the M1/M2
96 paradigm does not fully embrace the extent of macrophage and TAM heterogeneity²¹
97 and the different states of macrophage activation uncovered as a result of recent
98 single-cell and lineage-tracing technologies²²⁻²⁷ (**Table 1**). Concerning their ontogeny,
99 most TAMs derive from circulating monocytes²⁸ and can almost completely dilute the
100 pre-existing population of embryonically-derived RTMs. A small population of native
101 embryonic macrophages can, however, persist in the TME and have distinct functions
102 from TAMs, including specific remodelling of extracellular matrix²⁹. More strikingly, a
103 differential location within the TME has been revealed for pre-existing tumor-
104 associated RTMs and monocyte-derived TAMs in various cancers, including breast
105 cancer³⁰, non-small cell lung carcinoma³¹, and glioma³². In both lung³¹ and glioma³²,
106 embryonic-derived RTMs are found preferentially at the periphery of the tumor while

107 monocyte-derived TAM are infiltrating the tumor. Nevertheless, monocyte-derived HO-
108 1⁺ TAM have also been shown to preferentially localize at the invasive margins of
109 primary tumors and metastasis in the MN-MCA1 murine model of cancer³³, therefore
110 arguing for a disease specific localisation of ontogenically distinct TAM populations.
111 Altogether, these results suggest that such distinction between tumor-associated
112 RTMs and monocyte-derived TAMs should be better taken into consideration when
113 considering TAM identities and functions.

114

115 **TAM function in primary tumors**

116 TAMs were originally considered remnants of an abortive immune response against
117 the tumor³⁴. But in 2001, the group of Jeffrey Pollard showed that mice with a recessive
118 null mutation in the Colony-Stimulating-Factor 1 gene (*Csf1^{op}*), the major macrophage
119 growth factor, and genetically modified to develop mammary cancer, had a delay in
120 the development of metastatic carcinomas, therefore showing the involvement of
121 macrophages in malignant progression of breast cancer³⁵. These seminal findings
122 have led to investigations into the mechanisms of these pro-tumoral roles of
123 macrophages. We now know that within the TME, TAMs have several supporting
124 functions that promote tumor development (**Figure 2**), which we describe below.

125

126 ***Vascularization***

127 TAMs promote vascularization to supply oxygen and nutrients to cancer cells in the
128 well-described process of angiogenesis³⁶. Numerous investigations into this process
129 have led to the detection of angiogenesis-promoting molecules produced by TAMs,
130 including Vascular Endothelial Growth Factor- (VEGF-A) in the context of non-small
131 cell lung cancer³⁷, and adrenomedullin in melanoma³⁸. Of note, this angiogenesis-
132 promoting property is also observed in macrophages across healthy embryonic
133 development³⁹; we might speculate, therefore, that this feature represents a function
134 acquired by macrophages early in evolution, which reappears in these two different
135 yet comparable contexts⁴⁰. As a consequence, VEGF/VEGF receptor targeting
136 compounds are emerging as very promising immunotherapies and start to be used
137 notably to treat non-small cell lung carcinomas⁴¹.

138

139 ***Inflammation***

140 Another prominent function of TAMs in primary tumors is their role in establishing and
141 maintaining an inflammatory environment. Examples of such TAM-derived
142 inflammatory factors favoring tumor development are multiple but include CXCL8 in
143 endometrial cancer⁴², IL-6 in breast cancer⁴³, and IL-1 β in pancreatic cancer⁴⁴. While
144 this pro-inflammatory profile is supposed to support an active immune response
145 against tumors, the remarkable plasticity of TAMs makes them more often associated
146 with immunosuppression. As such, TAMs have the capacity to promote regulatory T-
147 cell (T_{reg}) recruitment. This phenomenon has been highlighted in ovarian cancer⁴⁵,
148 nasopharyngeal carcinoma⁴⁶, and liver cancer⁴⁷ where these T_{regs} can then deactivate
149 cytotoxic T cells directed against tumor cells⁴⁸. TAMs can also directly promote
150 cytotoxic T cell exhaustion^{30,49,50} and actually, many current immunotherapies aim to
151 reactivate anti-tumoral cytotoxic T cells by inhibiting the immune checkpoint PD-1/PD-
152 L1 pathway⁵¹. It is worth noting, however, that TAMs also express PD-1⁵² or PD-L1²²
153 and could therefore be considered as off-targets/second targets of current protocols
154 using pembrolizumab or nivolumab. The impact of such indirect TAM targeting on
155 patient responses to treatment is largely unknown, but should be taken into
156 consideration in future studies, particularly as the effectiveness of current
157 immunotherapies is variable. Nevertheless, the apparent heterogeneity in patient
158 responses to treatment is likely mediated, in part, by TAMs. For example, macrophage
159 recruitment is enhanced in patients with prostate cancer treated with androgen
160 blockade therapy, and this recruitment subsequently contributes to tumor
161 development. Those administered anti-CSF-1 in parallel, however, showed an
162 improved response to treatment⁵³. In a similar manner, macrophage depletion with an
163 anti-CSF-1 antibody reduces tumor growth in a mouse model of mammary gland
164 tumors treated with radiotherapy⁵⁴.

165

166 **Epithelial to mesenchymal transition**

167 TAMs also promote epithelial to mesenchymal transition (EMT), a process during
168 which epithelial-like, early proliferating cancer cells lose the capacity for cell–cell
169 adhesion and adopt a fibroblast-like phenotype with invasive and migratory
170 properties^{55,56}. EMT ultimately later permits metastatic cell dissemination. At the
171 molecular level, EMT is orchestrated by the transcription factors Zinc-fingers E-box
172 Binding homeobox factor 1 ZEB1^{57,58}, Snail^{59,60} and Twist⁶¹ (reviewed in⁶²). TAMs can
173 regulate these EMT-modulating factors through their secretome^{55,63}. For example,

174 TAM-produced tumor necrosis factor (TNF)- α stabilizes Snail through NF- κ B
175 signaling⁶⁴ while TAM-produced TGF- β induces Snail and ZEB1 expression by
176 activating the β -catenin pathways⁶⁵⁻⁶⁷. Moreover, mesenchymal cell production of GM-
177 CSF induces TAM activation and CCL18 production, and further promoting EMT in a
178 positive feedback loop⁶⁸.

179

180 ***ECM remodeling***

181 TAM are also involved in active ECM remodeling, collaborating notably with cancer
182 associated fibroblasts (CAF) to promote tumor cell intravasation⁶⁹. Indeed, tumors
183 often display a dense ECM that notably impairs drug penetration limiting treatment
184 efficacy and resulting later in more metastases^{70,71}. TAMs express and secrete various
185 membrane-associated proteases that degrade ECM collagen fibers, such as matrix
186 metalloproteinases (MMPs)^{72,73}, secreted protein acidic and rich in cysteine
187 complex⁷⁴, and cathepsins^{69,73}. Once degraded, TAMs mediate collagen fragment
188 turnover via phagocytosis and degradation in the lysosome by cathepsins⁶⁹. TAMs by
189 producing cross-linking enzymes from the lysyl hydroxylase (LH) family, such as LH2
190 in triple negative breast cancer⁷⁵, also increase ECM stiffness that promote tumor
191 progression and metastasis by mechanical forces⁷⁶. In addition, in models of lung
192 adenocarcinoma and breast cancer, a subset of TAMs expressing Fibroblast
193 Activating Protein (FAP)- α , that acts both as a signaling protein for CAFs and as a
194 collagenase, and Heme Oxygenase (HO)-1, was found associated with ECM
195 remodelling^{77,78}. Altogether, these observations suggest that similar mechanisms are
196 involved in both wound healing and tumor formation, following the famous statement
197 that tumors are “wounds that do not heal”⁷⁹.

198

199 ***Intravasation***

200 EMT and ECM remodeling precede the intravasation of tumor cells into the circulation
201 and their subsequent dissemination to distal organs. This key event in metastasis
202 formation occurs at sites known as “tumor microenvironment metastasis (TMEM)
203 doorways”, characterized by the dynamic association between one endothelial cell,
204 one TAM and one cancer cell⁸⁰⁻⁸². TAMs from the TMEM doorway arise from recruited
205 monocytes that become CXCR4⁺ TAMs upon TGF- β stimulation in the TME. Attracted
206 by fibroblast-derived CXCL12, these TAMs migrate towards the vascular niche, where
207 they adopt a perivascular TAM phenotype and disrupt the junctions between

208 endothelial cells which allow tumor cells to intravasate into the circulation⁸³⁻⁸⁵. Of note,
209 TMEM density in tumors has been linked with increased metastatic burden and could
210 be used as a predictive tool for the occurrence of metastasis^{80,86}.

211

212 In addition, activation of a paracrine loop also allows cancer cells that produce CSF-
213 1, and TAMs that produce EGF, to migrate together toward TMEM doorways. Thus,
214 blocking CSF-1 or EGF receptors reduced cancer cell migration and invasiveness in
215 breast cancer rodent models⁸⁷. Furthermore, IL-4-producing TH₂-CD4⁺ T cells
216 stimulate EGF production by TAMs and depletion of CD4⁺T cells or IL-4 neutralizing
217 antibody treatment reduced the metastatic burden⁸⁸. Collectively, these examples
218 demonstrate the crucial role of macrophages to initiate the metastatic process by
219 favoring the migration and intravasation of cancer cells to the blood circulation.

220

221 **RTM roles in shaping the pre-metastatic niche**

222 In the late 19th century, Paget proposed the “seed and soil” theory of metastasis⁸⁹ in
223 which tumor cells (the “seeds”) can only grow in a hospitable environment (the “soil”).
224 While the nature of the “hospitable” environment remains to be defined, this theory
225 suggests that changes occur in distant tissues before the arrival of cancer cells to
226 ensure the environment favors metastatic growth. These changes constitute the
227 development of a “pre-metastatic niche” (**Figure 3**). As key mediators of inflammation,
228 macrophages produce various cytokines which directly prime naive tissue to welcome
229 disseminated tumor cells⁹⁰.

230

231 ***Influence of macrophage origins***

232 Investigations are ongoing to understand how macrophages shape the pre-metastatic
233 niche and whether their origins have a differential impact. This latter question has been
234 tackled using the *Cx3cr1*^{CreERT2}-based fate mapping mouse model challenged with
235 ovarian cancer cells with the capacity to form metastases in the omenta, a visceral
236 adipose tissue located in the abdomen⁹¹. In this context, a specific subset of embryonic
237 TIM4⁺ CD163⁺ omentum macrophages were shown to favor metastatic dissemination
238 from ovaries to omenta, their depletion resulting in reduced ascitic volume and
239 metastatic invasion to this organ⁹¹. For now, the molecular mechanisms by which

240 embryonic macrophages preferentially shape the pre-metastatic niche remains to be
241 clarified but their documented very long half-life could represent a key parameter.

242

243 ***Macrophage imprinting and extracellular vesicles***

244 Mechanistically, pre-metastatic niche development and macrophage imprinting within
245 that niche have been shown to be dependent on extracellular vesicles (EVs) that
246 originate from the primary tumor and circulate within the blood. Of note, EVs have
247 been classified based on their specific size and biogenesis and encompass
248 microvesicles (150 – 1,000 nm) arising from membrane budding which are involve in
249 local communication⁹² and smaller exosomes (30 – 150 nm) which derive from late
250 endosome and circulate over longer distances between tissues^{92,93}. EVs transport
251 various cargos such as RNAs, lipids, metabolites, or proteins that they can transfer to
252 other cell types to modulate their phenotype and functions. Through their distinct
253 cargos, EVs impact the pre-metastatic niche through immune cell modulation, ECM
254 remodeling and angiogenesis⁹⁴. Findings derived from a seminal study from the group
255 of David Lyden showed that integrins at the surface of tumor exosomes drive
256 metastatic organotropism, as their patterns correlated with metastatic sites⁹³. In the
257 liver, for example, ITGαvβ5⁺ exosomes bind specifically to liver resident macrophages
258 (known as Kupffer cells) whereas ITGα6β4⁺ and ITGα6β1⁺ exosomes recognize lung
259 fibroblasts and epithelial cells.

260

261 The mechanisms of action of tumor-derived EVs are only beginning to be elucidated.

262 For example, macrophage migratory inhibiting factor (MIF) produced by primary
263 pancreatic tumors and conveyed by EVs remotely induces liver Kupffer cell
264 production of TGF-β⁹⁵. This process subsequently activates hepatic stellate cells

265 (HSCs), which initiate liver fibrosis through fibronectin production, inducing the
266 recruitment of inflammatory cells such as neutrophils and monocytes. Furthermore,
267 activated HSCs also express CXCL12, which induces the quiescence of natural killer
268 (NK) cells⁹⁶ and excludes CD8⁺ cytotoxic T cells from the pre-metastatic niche^{97,98}.

269 The overall effect of this pathway is to reduce immunosurveillance of the pre-
270 metastatic niche. Interestingly, monocyte-derived macrophages but not embryonically
271 derived macrophages in the hepatic pre-metastatic niche secrete granulin, which
272 serves to maintain HSC activation and liver fibrosis⁹⁹. Further studies are needed to

273 understand this observation, but as addressed earlier, it seems that macrophage origin
274 affects the roles these cells play in determining metastasis.

275

276 Still in the liver, EV lipid cargo is handled by a specific subset of CD206⁺ Kupffer
277 cells^{100,101}, leading to an upregulation of their expression of the fatty acid transporter
278 CD36 and a polarization towards an anti-inflammatory phenotype¹⁰². This phenotype
279 favors immunosuppressive CD8⁺ T cells and improve growth potential of disseminated
280 tumor cells. Others have shown that EVs from lung adenocarcinoma induce notably
281 the upregulation of CD206, PD-L1 and GLUT1 by lymph node CD68⁺ macrophages.
282 GLUT1 expression by macrophages increase their glucose uptake, this glycolytic shift
283 favoring the establishment of the pre-metastatic niche¹⁰³. In line with this, myeloid cells
284 including TAM have been shown to have the greatest capacity to take up glucose in
285 the TME, as compared to cancer cells notably¹⁰⁴, redefining the well-described
286 Warburg effect. Coupled with the notion of the heterogeneity of TAM metabolic
287 features¹⁰⁵, these findings definitely argue that pre-metastatic niche priming relies on
288 metabolic capabilities of macrophages and promise groundbreaking discoveries with
289 the rise of immunometabolism-related research.

290

291 These emerging findings which place macrophages on the frontstage when referring
292 to pre-metastatic niche establishment can also be envisaged in the context of the
293 macrophage network between distal tissues which has been demonstrated to play a
294 role notably in the context of myocardial infraction¹⁰⁶. Indeed, it has been shown that
295 macrophages from unrelated tissues such as lungs are activated after a heart-
296 restricted challenge. Molecular mechanisms are still not deciphered but further studies
297 would precise the actors involved in this phenomenon and assess its relevance in the
298 context of cancer.

299

300 Finally, it should be noticed that while EVs are scrutinized for their role in the priming
301 of pre-metastatic niches, tumor cells can also prime distal macrophages via their
302 release of free enzymes such as lysyl oxidase (LOX)¹⁰⁷.

303

304 **Roles of macrophages in maintaining metastases**

305 Macrophages continue to support metastasis development after tumor cell migration
306 has occurred. This is evident based on the finding that inhibiting TAM recruitment to a
307 metastatic site results in a lower metastatic burden, as shown for lung¹⁰⁸⁻¹¹⁰ and liver¹¹¹
308 metastases murine models. Specifically in the liver, macrophages produce hepatic
309 growth factor that binds to c-Met at the surface of migrating tumor cells¹¹², stopping
310 their circulation and promoting their extravasation within the liver. In the lungs, a similar
311 phenomenon occurs but is mediated by interactions between VCAM-1 at the surface
312 of migrating tumor cells and integrin $\alpha 4$ at the surface of lung macrophages¹⁰⁹. In
313 addition, this interaction triggers the Ezrin-PI3K/Akt pathway in tumor cells that confers
314 some protection against pro-apoptotic cytokines¹⁰⁸.

315

316 ***Metastasis-associated macrophages (MAMs)***

317 Once the secondary tumor is established, macrophages qualified as metastasis-
318 associated macrophages (MAM) in literature¹¹³ maintain immunosuppression by
319 impairing cytotoxic T-cell activation. Specifically, and as mentioned earlier, EV-
320 mediated priming of lung macrophages leads to a metabolic switch in these cells
321 towards glycolytic respiration that produces lactate as a by-product¹⁰³. Lactate
322 subsequently upregulates PD-L1 expression, blocking T-cell activation due to PD-1
323 engagement. Meanwhile in the liver, macrophages induce the systemic loss of T cells
324 by triggering their apoptosis through the FAS-L pathway¹¹⁴.

325

326 Many studies have described the recruitment of CCR2-expressing monocytes to the
327 metastatic niche upon CCL2 production by stromal cells, that give rise to MAMs^{115,116}.
328 These monocytes might have different roles compared to RTMs present from the
329 inception of the pre-metastatic niche. In MMTV-PyMT breast tumor-bearing mice, for
330 example, monocyte-derived MAMs have a crucial role in cancer cell extravasation in
331 the lung by producing VEGF-A¹¹⁶ that can bind to VEGF receptor on endothelial cells
332 thus inducing the remodeling of blood vessel at the metastatic site¹¹⁷. Monocyte-
333 derived MAMs also seem to impact tumor-infiltrating lymphocytes in colorectal
334 carcinoma metastasis to the liver. Specifically, a study in which colorectal cancer cells
335 (MC38) were injected into the spleen of wild type or CCR2 knockout mice showed that
336 the knockout mice had a higher abundance of CD8⁺ and CD4⁺ lymphocytes and a
337 reduced metastatic burden¹¹⁵.

338

339 ***Kupffer cells promote liver metastases***

340 The liver is the most common site for metastasis, likely due to its dense blood vessel
341 architecture, with the portal vein supplying a large amount of blood and hepatic
342 sinusoids offering a secondary network with lower pressure and thus more time for
343 potential migrating tumor cells to attach to the organ¹¹⁸. As shown in rats, Kupffer cells
344 within the sinusoids limit these events through phagocytosis, clearing 90% of
345 circulating tumor cells¹¹⁹. When Kupffer cells are overloaded, however, tumor cells can
346 extravasate into the liver¹²⁰. As mentioned earlier, Kupffer cells can also favor
347 metastasis by activating HSCs and creating a fibrotic and inflammatory pre-metastatic
348 niche that sustains tumor cell invasion^{95,96}. Kupffer cells also act as key drivers of liver
349 metastatic tropism through their specific engulfment of tumor-derived exosomes⁹³.
350 Accordingly, depletion of Kupffer cells before the induction of liver metastasis resulted
351 in an increased metastatic burden while depletion of KC after metastatic establishment
352 was reducing metastatic growth¹²⁰. Of note, other populations of macrophages also
353 populate the liver, such as capsular or lipid-associated macrophages (LAMs)¹²¹⁻¹²³.
354 These cells have only recently been described and their role in cancer has not yet
355 been fully characterized, though LAM accumulation in metastases has been
356 reported¹²⁴.

357

358 ***Lung macrophages modulate lung metastases***

359 After the liver, the lungs constitute the second most frequent site of metastases.
360 Exosomal priming of lung macrophages promotes the pre-metastatic niche by inducing
361 T-cell suppression¹⁰³ and neutrophil recruitment¹²⁵. Lung macrophages also promote
362 metastatic invasion by serving as anchors for circulating tumor cells allowing their
363 extravasation¹⁰⁸⁻¹¹⁰. Again, heterogenous macrophage populations with different
364 features inhabit the lungs¹²⁶ and could have various roles in the metastasis of different
365 primary tumors to this organ. For example, interstitial macrophages evolve over time
366 in the metastatic niche, first exhibiting an anti-tumoral phenotype and later a pro-
367 tumoral phenotype likely due to signals received from the stroma¹⁰³. Alveolar
368 macrophages also play a role in metastasis development, and notably a subpopulation
369 of lipid-laden Trem2⁺ ones displaying metabolic, immunosuppressive and matrix
370 remodeling features that accumulate in metastases¹²⁷.

371

372 **Limits and future perspectives in TAM research**

373 In this review, we have highlighted various facets of tumor-associated macrophage
374 biology that influence cancer development through its different steps. The versatility
375 of TAM functions is evident, thus also is the difficulty to identify one unified target that
376 might be of clinical benefit¹²⁸⁻¹³⁰. The very limited efficacy of global approaches such
377 as ones targeting CSF1/CSF1R or CCL2/CCR2 pathways illustrates well the challenge
378 we face. So refinement of our strategies is needed, and is on-going as exemplified by
379 the efficacy of a combination of a TREM2-specific antibody with the widely-used anti
380 PD-1 in different cancer models¹³¹.

381 To argue for such a better consideration of TAM heterogeneity, we have discussed
382 the extent of TAM denomination which actually encompasses spatiotemporally-
383 unrelated macrophage populations within primary tumors, distal healthy tissues and
384 metastasis sites. It remains to be fully deciphered how fundamental determinants of
385 macrophage biology such as their origin, their local environment and the time spent in
386 the tissues differentially influence tumor progression in these three different
387 contexts¹³².

388

389 To tackle these fundamental questions, our methodology needs to evolve. Many
390 studies have relied on mouse models of cancer so far, but we should acknowledge the
391 inherent limitations of these systems. Orthotopic models, such as the widely used
392 canonical B16 melanoma model, are convenient as easily combinable with knock-in
393 or knock-out animals; unfortunately, this type of model is quite different from the
394 natural disease course of cancer. Indeed, while the primary TME can be more-or-less
395 recapitulated depending on the models, these systems completely bypass the key step
396 of pre-metastatic niche priming due to their fast-developing nature. This feature
397 disconnects these models from patient contexts in which, as previously stated,
398 metastasis remain the main cause of death. Genetic models closer to what is observed
399 in patients do exist but are usually less convenient, due to their lower penetrance and
400 the often asynchronous tumor emergence, impairing reproducibility and the
401 establishment of robust conclusions. By contrast, patient biopsies represent
402 unvaluable samples and are extensively used; however, disease genesis is difficult to
403 determine from the often one end-point sample in one location either primary tumor or
404 metastasis, and only limited information can be extracted from their analysis.

405

406 In view of these issues, meaningful alternatives are needed to better understand the
407 roles of macrophages in every step of the disease process. There are many avenues
408 to be explored and the recent rise of single cell-omics offering snapshots of tissue
409 activity at an unprecedented resolution will no doubt enable the precise identification
410 of targets along disease development. These approaches now need to be coupled
411 with models that consider disease dynamics, from the initial acquisition of oncogenic
412 mutations to metastasis and multi-organ failure. The most recent spatial transcriptomic
413 allows for the identification of pathways that are activated in TAMs but also from all
414 the other cells from the TME while conserving its architecture. These technologies can
415 even be applied to fixed samples, allowing the retrospective analysis of hundreds of
416 thousands samples from cancer patients stored in hospitals worldwide. The rise of
417 immunometabolism should also offers a novel vision of macrophage activity within
418 TME, which could lead to the development of a new generation of metabolite-targeted
419 therapies to reprogram TAM into anti-tumor cells. It is up to us to make fruitful use of
420 this wealth of information to generate knowledge that will inform the precise design of
421 innovative TAM-related immunotherapies.

422

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Table 1: Major TAM populations and their specificities. Adapted from ¹³³.

TAM populations <i>As defined in ²²</i>	Species	Surface Markers	Cancer	Functions	References
IL4I1⁺	Human	PD-L1, PD-L2, IDO1	.Colorectal .Hepatocellular .Glioblastoma	.Antigen presentation .Phagosome maturation .Treg recruitment	22,134-137
	Mouse	<i>To be defined</i>	.Melanoma	.T cell suppression	
TREM2⁺	Human	TREM2, APOE, CD63, CD9	.Colorectal .Breast .Glioblastoma .Melanoma	.Lipid metabolism .Matrix remodelling .Immunosuppression .Cancer cell proliferation	22,30,136-140
	Mouse	CADM1, CX3CR1, CD63, CD36	.Squamous cell carcinoma .Skin carcinoma		
FOLR2⁺	Human	FOLR2, CD163, LYVE1, CD206	.Breast .Hepatocellular	.CD8 ⁺ T cell infiltration	22,30,141
	Mouse	FOLR2, CD206, TIM4, LYVE1			
FTL⁺	Human	CD52, CD206, CXCR4, CD163	.Colorectal .Glioblastoma	.Angiogenesis	22,134,136,137,139
	Mouse	TIE2, CXCR4, CD206			
Proliferating	Human	MKI67, TOP2A	.Colorectal .Glioblastoma	.Cell cycle .Proliferation	22,134,136,137,139
	Mouse	MKI67, TOP2A			

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