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Tumor-Associated Macrophages as Treatment Targets in Oncology

Alberto Mantovani[1,2], **Federica Marchesi**[1,3], **Alberto Malesci**[1,3], **Luigi Laghi**[1], and **Paola Allavena**[1,2]

[1]Istituto Clinico Humanitas IRCCS, via Manzoni 113, 20089 Rozzano, Italy

[2]Humanitas University, via Manzoni 113, 20089 Rozzano, Italy

[3]Dept. Medical Biotechnology and Translational Medicine, University of Milan, Milan, Italy

Abstract

Macrophages are crucial drivers of tumor-promoting inflammation. Tumor-associated macrophages (TAM) contribute to tumor progression at different levels, including promoting genetic instability, nurturing cancer stem cells, paving the way to metastasis, taming protective adaptive immunity. TAM can exert a dual, yin yang influence on the effectiveness of cytoreductive therapies (chemotherapy and radiotherapy), antagonizing antitumor activity by orchestrating a tumor-promoting, tissue repair response or, instead, contributing to their ultimate antineoplastic efficacy. TAM express triggers of checkpoints of T cell activation and are targets of checkpoint blockade immunotherapy. Macrophage-centered therapeutic approaches include: strategies to block recruitment and survival in tumors; functional reeducation to an antitumor, M1-like mode; tumor-directed monoclonal antibodies which elicit extracellular killing or phagocytosis of cancer cells. We surmise that TAM can provide tools to tailor cytoreductive therapies and immunotherapy and that TAM-centered therapeutic strategies have the potential to complement and synergize with chemotherapy and immunotherapy.

1 Introduction

Inflammatory cells are a key component of the ecological niche of cancer 1–4. The formation of an inflammatory microenvironment in tumors is driven by genetic events which cause cancer (oncogenes and oncosuppressor genes) or by chronic non-resolving inflammatory conditions such as inflammatory bowel disease, which increase the risk of developing cancer 1. In general, cancer-associated inflammation is characterized by being non-resolving 5.

Macrophages are a major component of the leukocyte infiltrate present in widely different amounts in all tumors 6. Tumor-associated macrophages (TAM) have served as a paradigm for leukocytes and inflammatory mediators present in the tumor context and play a dominant role as orchestrators of cancer-related inflammation (CRI). CRI is considerably diverse in

Correspondence to: Alberto Mantovani, MD, Istituto Clinico Humanitas IRCCS, via Manzoni 113, 20089 Rozzano, Italy; alberto.mantovani@humanitasresearch.it.
AM, FM and PA equally contributed to this review.

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tumors arising in different tissues 2, 7. However, though cellular components of CRI differ in quality and quantity and mediators which orchestrate macrophage function can differ considerably in different cancers, TAM represent a final common pathway driving CRI 8.

In the ’70 it was realized that macrophages activated by bacterial products and cytokines acquired the capacity to kill tumor cells 9–11. On the other hand it was soon realized that TAM from malignant metastatic tumors promoted tumor growth and metastasis 12. Thus, early on evidence suggested that macrophages could engage in a dual yin yang relationship with cancer.

Here we review current understanding of the role of TAM in different cancer treatment modalities as well as emerging macrophage targeting therapeutic strategies. As a premise, a concise overview of the role of macrophages in tumor initiation and progression will be provided. Previous reviews on CRI and specifically on myeloid cells in tumors provide the background of the present essay 1–3, 6, 13–15.

2 Role in Tumor Progression

Fig. 1 provides a schematic representation of the origin and function of TAM and a general framework for subsequent sections focused on therapy (see also Box 1). It has long been held that TAM originate from the blood compartment and that chemotactic signals originating from tumor cells or from normal cells present in the cancer microenvironment recruit monocytic precursors at the primary and metastatic tumor sites 11, 15–19. However, recent evidence raises questions as to this long held view. In the mouse, resident macrophages in some tissues (e.g microglia in the brain) originate from precursors seeding there during fetal and embryonal life rather than from circulating monocytes (Box 1) 20,21. In gliomas, tumor-associated macrophages constitute a mixed population that includes resident brain microglia, infiltrating blood monocytes, and macrophages. The relative contribution of these cells has been investigated in a genetically engineered mouse model: accumulation of Ly-6C^{hi} circulating “inflammatory” monocytes into tumor tissue was responsible for the increased tumor incidence and shorter survival times, with no contribution of microglial cells 22. In the perspective of macrophage function in the tumor microenvironment, it is noteworthy that recent results support that in the mouse the ontogenetic origin does not have an appreciable impact on the macrophage phenotype in response to tissue-derived cues 23. Whether embryo-derived tissue macrophages contribute to the number, location and diversity of TAM remains an open question 24. TAM proliferation has been observed in murine and human sarcomas and murine breast carcinomas but this does not appear to be a general mechanism sustaining TAM numbers in the face of growing tumors 25, 26, 27. Circulating precursors that are recruited into tumor tissues and there differentiate into TAM include conventional inflammatory monocytes and Mo-MDSC (see Box 1). Down regulation of the transcription factor STAT3 plays a key role in the differentiation of Mo-MDSCs into mature TAM28. Inflammatory monocytes, defined in the mouse as Ly6C^{+}/CCR2^{+} cells have been shown to contribute to TAM accumulation and maintenance in a mouse mammary tumor model 27 and pulmonary metastases of murine and human breast cancer cells 19. The process of differentiation of mouse inflammatory monocytes into TAM was dependent on the transcriptional regulator RBPJ and

its genetic deletion in TAM resulted into reduced tumor burden, confirming a non-redundant function of monocyte-derived TAM in tumor growth 27. In contrast, protective function has been shown for $CX3CR1^{+}/Ly6C^{-}$ "nonclassical" patrolling mouse monocytes, which depend on the N4a1 transcription factor and patrol the lung microvasculature in steady state conditions. These cells, which rarely extravasate into tissues and differentiate into macrophages, rapidly accumulate into lung metastases and inhibit tumor cell seeding and growth in mouse models 29. Their antitumor function includes scavenging of tumor debris and recruitment and activation of natural killer cells 29.

Chemoattractants involved in monocyte recruitment include chemokines (e.g. CCL2, CCL5), colony-stimulating factor-1 (CSF-1), and members of the VEGF family. TAM themselves can be a source of CCL2 in cancer. Recently, genetic evidence in the mouse suggested that Complement components, C5a in particular, play an important role in recruitment and functional polarization of TAM 30. Chemotactic factors are more than attractants in that they activate transcriptional programs in macrophages and contribute to functional skewing (e.g. 31). CSF-1 in particular is a monocyte attractant as well as a macrophage survival and polarization signal 6, 32. Unlike M-CSF, GM-CSF activates macrophage functions related to anti-tumor activity 33.

Signals originating from tumor cells, T and B lymphocytes and stroma orchestrate TAM function and their diversity. Classically activated macrophages (M1, Box 1) can kill tumor cells extracellularly and mediate tissue destructive reactions centered on the vessel wall (hemorrhagic necrosis) 15,9,10,11. Accordingly, there is evidence that macrophages contribute to the early elimination phase of nascent tumors orchestrated by T cells and interferons 34 . Tumor progression is associated with skewing and subversion of macrophage function. In established progressing tumors such as murine and human breast and pancreatic cancer, IL-4 and IL-13 derived from TH2 cells 35–37, eosinophils 38 or basophils 39 elicit alternative M2 activation of TAM (Box 1, Fig. 1). In addition, signals originating from tumor cells (e.g. chemokines, CSFs, TGFβ), B cells (immune complexes) and stromal cells (e.g. IL-1) can act as drivers to skew macrophage function to diverse phenotypes (for a recent summary, 8) which do not fit with classic M1/M2 polarized cells (Box 1). In general, in spite of intra and inter-tumor diversity, TAM in progressing neoplasms have surface molecules (e.g. the scavenger receptor CD163; mannose receptor CD206) and properties related to angiogenesis, suppression of specific immunity and promotion of cancer growth and metastasis. For convenience, we and others refer to these diverse populations as M*2*-like. In line with a consensus recommendation 33 (Box 1) we use M2 when IL-4 or IL-13 are major drivers of polarization.

There is evidence that the relative importance of distinct pathways varies in different tumors, resulting in heterogeneous phenotypes 2, 7. However as diverse as CRI can be in different cancers, TAM polarization appears to represent a final common pathway. Within a given human or mouse tumor, TAM exhibit different phenotypes, influenced for instance by access to oxygen and activation of the HIF pathway 40, 41 42. Dissecting TAM diversity at the single cell level and integrating information represent current challenges.

TAM influence tumor cell intrinsic properties as well as the tumor microenvironment (Fig. 1). TAM can stimulate tumor cell proliferation, migration and genetic instability. Acting at the primary tumor site or at sites of secondary localization they promote invasion and metastasis (Fig. 1). TAM promote angiogenesis and lymphoangiogenesis as well as tissue remodeling with fibrous tissue deposition.

Myelomonocytic cell contribute to suppression of effective adaptive immunity, a key feature of cancer 3, at various levels and through multiple mechanisms. MDSC, and in particular MoDSCs, suppress immunity in lymphoid organs. MoMDSC are recruited in tumors contributing to an immunosuppressive microenvironment and here they undergo differentiation into TAM 28 (Box 1). TAM can promote T regulatory cell (Treg) in a bidirectional interaction 15. Immunosuppressive cytokines (IL-10 and TGFβ) are produced by macrophages in the tumor context. Aminoacid metabolism in polarized macrophages and TAM results in metabolic starving of T cells and production of immunosuppressive metabolites via the indoleamine 2,3 dioxygenase (IDO) pathway 33. Prostaglandins produced from the arachidonic acid metabolism have suppressive functions. Finally, TAM express PD-L1 and PD-L2 which trigger checkpoint blockade in T cells, as well as $B7H_4$ 43 and VISTA 44 which may exert similar functions.

Progress has been made defining the molecular pathways responsible for the orchestration of macrophage function in tumors, including members of the STAT and NFkB family 8. Among these, Myc is interesting in that it acts both in cancer cells and macrophages. The Myc oncogene orchestrates approximately 40% of the transcriptional fingerprint of human M2 activation and is overexpressed in human TAM 45. On the cancer cell side, the *Myc* oncogene was found to induce expression of CD47 and PD-L1 46. CD47 is a “don’t eat me” signal (see below). Thus, the oncogene Myc appears to tame innate immunity in the form of macrophage-mediated phagocytosis through CD47 as well as activation of effective adaptive antitumor immunity through induction of PD-L1.

3 Prognostic or Predictive Biomarkers

Studies on the prognostic significance of TAM have relied on a variety of methodological approaches, ranging from morphological identification in early efforts 11 to gene expression profiling 47. The most extensively used human macrophage marker is CD68, a pan macrophage marker. However, CD68 can occasionally be expressed in stromal cells as well as in cancer cells themselves. Therefore its use requires careful assessment 48. In many studies, CD163, a scavenger receptor associated with M2-like polarization, CD204 and CD206 (the mannose receptor, induced by IL-4) were used, with overall results comparable to usage of CD68. In addition a range of molecules have been used to characterize TAM. These include membrane molecules (e.g. stabilin-1 49, expressed in M2 polarized macrophages and TAM). Chemokines and chemokine receptors (e.g. CCL17), cytokines and cytokine receptors (e.g. IL-10 and IL-12 50). M1-like macrophages polarized with IFNγ and with antitumor activity usually present with high levels of HLA-DR 51, although this marker is widely expressed in other leukocyte populations. Different approaches are used for identification of macrophage precursor monocytes and monocyte-myeloid-derived suppressor cells (MoMDSCs) in cancer, as these circulating cells are commonly investigated

by multicolor flow cytometry. Monocytes are referred to as “inflammatory”, when $CD14^{+}$/$CD16^{-}$ 20, or “patrolling” when $CD14^{dim}$/$CD16^{+}$ 20. As to MDSCs, a recent consensus has been reached on MoMSCs as $CD11b^{+}$/$CD14^{+}$/HLA-DR^{low}//$CD15^{-}$/$CD33^{+}$, with CD33 being highly expressed only on MoMDSC (compared to NeuMDSC) 52. Macrophages infiltrating mouse and human tumors show considerable diversity within a given cancer depending on their microanatomical localization 41, 49. Hypoxia is a major driver of macrophage diversity within tumors13, 53. Therefore, an inherent limitation of available information is that it does not take into account intratumor diversity of TAM.

It has long been known that in many human solid tumors high macrophage infiltration is associated with poor prognosis 54. These observations represented a pillar for the now largely accepted view that TAM promote tumor progression as discussed above. In breast carcinoma macrophage infiltration was associated with grade, lack of hormone receptors, basal like type and outcome 26. TAM were correlated with more advanced stage in breast and bladder cancer 55, 56 but the reverse was true in ovarian and gastric cancer 57, 58. The negative prognostic significance of TAM infiltration has been confirmed in a recent meta-analysis including all available data 59.

In apparent contrast with the above results in selected human tumors (non-small cell lung cancer, prostate and colorectal cancer, CRC) high macrophage infiltration has been associated with better prognosis. This observation in CRC has stood up in the meta-analysis conducted by Zhang et al 59 and were confirmed in an analysis of 209 CRC patients in our Institution (*Malesci et al., unpublished data*). Interestingly, in CRC also neutrophil infiltration was found to be associated with better survival 60. As discussed below, the favorable prognostic significance of macrophage infiltration is related to the impact of TAM on response to chemotherapy.

As discussed above, it has long been held that TAM originate from blood monocytes. However, in selected murine tumors 25 TAM proliferation was observed though this was not clearly shown in human tumors. In a recent study, proliferating macrophages were identified as PCNA+ cells in human breast carcinoma. Proliferating TAM were associated with hormone receptor negativity, basal-like phenotype and worse outcome 26. It will be important to assess the presence of PCNA+ TAM in other tumors and assess their significance.

In classic Hodgkin’s lymphoma (CHL), a gene signature of TAM and an increased number of CD68+ cells were associated with shortened survival in patients treated with chemotherapy regimens, so that TAMs have been proposed as a biomarker for risk stratification 61 (Table 1). High CD68 or CD163 expression were later confirmed to be significant independent predictors of worse survival in a multicenter randomized controlled clinical trial, reinforcing the prognostic significance of TAMs in chemotherapy-treated patients with locally extensive and advanced-stage CHL 62 (Table 1).

Previously, high TAM (CD68+) content had been found associated with unfavorable outcome also in patients with follicular lymphoma (FL) treated with multiagent chemotherapy 63, 64, a prognostic association reversed in CHOP (cyclophosphamide,

doxorubicin, vincristine, and prednisone)-treated patients receiving an anti-CD20 mAb (rituximab) 65. An independent study confirmed that high TAMs (CD163+) were predictive of a favorable outcome in FL patients receiving rituximab plus CHOP, while being oppositely associated with an adverse outcome in patients treated with rituximab, cyclophosphamide, vincristine, and prednisone (CVP) 66. The latter data not only confirm that TAM predict outcome in FL, but also underscore that their prognostic impact is dependent on treatment. As nowadays neoplastic conditions as a rule receive pharmacological treatment, differently from the past, the prognostic value of a variable is meaningless if not related to the administered treatment (i.e., prognostic *vs* predictive value). Doxorubicin is the drug differentiating CHOP and CVP, so that a striking parallelism occurs between these data and early pre-clinical studies supporting a role of TAMs in determining the antitumor efficacy of this drug in a mouse lymphoma model 67. At any event, considering that lymphoma patients are heavily treated, it should be concluded that TAMs may serve as predictive biomarker in this neoplastic setting, whose positive or negative value is determined by the type of chemotherapy.

Data in solid tumors on the predictive potential of TAM are limited. Most studies assessing the prognostic impact of TAMs do not report adjuvant therapy regimens, even when these are considered an international standard 49, 68, 69. The only published study comparing TAMs as to the outcome of patients receiving or not chemotherapy after surgery for solid tumor concerns pancreatic cancer, and supports their dual effect depending upon post-surgical chemotherapy 50. High TAMs appear to be critical determinants of responsiveness to gemcitabine following surgery for pancreatic cancer. In parallel, high TAMs density in patients with stage III colorectal cancer were associated with better outcome only in patients receiving post-surgical adjuvant 5-fluorouracil based chemotherapy, but not in untreated patients (Malesci, submitted). These studies point to macrophages as predictive factors of responsiveness to post-surgical chemotherapy, rather than being only prognostic indicators.

4 The Yin Yang of Chemotherapy and Radiotherapy

Chemotherapy can affect macrophage function directly or indirectly, the latter by modulating the function of adaptive T cell mediated immune responses (Fig. 2). Indeed, early on it was found that immunity played a key role in determining the ultimate efficacy of Doxorubicin 67. In this and in subsequent reports it was apparent that chemotherapeutic agents are not born equal in terms of interactions with immunity. In response to selected chemotherapeutic agents, Doxorubicin in particular, tumor cells undergo immunogenic cell death, i.e. express alarm signals which trigger effective adaptive immune responses 70, 71. For instance after exposure to Doxorubicin, in a murine model tumor cells released ATP which caused recruitment of mononuclear phagocytes. Under these conditions myeloid cells differentiated into antigen presenting cells which triggered effective adaptive immune responses 72. Moreover, cooperation between chemotherapeutic agents such as Actinomycin D and human and murine monocytes-macrophages had been observed early on in a phenomenon called drug-dependent cellular cytotoxicity 73.

Trabectedin, approved by EMA for soft tissue sarcomas and ovarian cancer, and by FDA for sarcomas, is a DNA binder causing DNA damage and cell cycle arrest in tumor cells, but

this “conventional” effect is only part of its complex mechanism of action; by interacting with DNA binding proteins, Trabectedin affects the transcription of selected genes, which include some inflammatory cytokines/chemokines and angiogenic factors 74. Clinical observations were inconsistent with its antitumor activity being accounted for completely by effects on cancer cells (see below), with delayed, prolonged responses. These clinical observations prompted an analysis of the effect of this drug on immunity 75, 76. This compound triggers activation of caspase 8, the key effector molecule of the extrinsic apoptotic pathway, selectively in monocytes, inducing their apoptotic death. In preclinical models, macrophage depletion was demonstrated to be a key mechanism of its antitumor activity. Reduced TAM infiltration and angiogenesis were observed in biopsies from sarcoma patients treated with Trabectedin 75. Thus, preclinical and clinical evidence suggest that reduction in macrophage numbers is a key component of the antitumor activity of Trabectedin.

The microbial context has emerged as an important determinant of the efficacy of chemotherapy and immunotherapy in murine models 77–79. Priming of myeloid cells by microbial components was essential for the antineoplastic efficacy of platinum with a CpG agonist 77. In the same vein, the antineoplastic activity of anthracyclines was compromised in mice with genetic inactivation of the formyl peptide receptor 1 (FPR1), a sensor of microbial components and tissue damage expressed in myeloid cells 80. A loss-of-function FPR1 allele was associated with lower survival in human mammary carcinoma and colorectal cancer receiving adjuvant chemotherapy. Preclinical models suggest that myeloid cells are prime movers of the contribution of immunity to the antitumor activity of selected chemotherapeutic agents 71 and the latter amplify the efficacy of checkpoint blockade therapy 81. In mouse models repolarization of macrophages in the context of targeted therapies has been reported for imatinib in KIT+ gastrointestinal stromal tumors (GISTs) 82 and sorafenib of hepatocellular carcinoma 83.

The apparent discrepancy in the role of TAM in the response to Doxorubicin (e.g. 67, 84–88) is likely to reflect differences in the murine tumor models utilized (for instance immunogenicity). However, as discussed above, high TAM infiltration is associated with better prognosis in patients with lymphomas treated with Doxorubicin-containing regimens mirroring preclinical data 67.

The positive interaction between chemotherapy and macrophage-mediated host defence is reflected by the prognostic/predictive significance of TAM in pancreatic cancer and colorectal cancer discussed above. Mirroring the in vivo association data, in vitro gemcitabine was found to synergize with macrophages in tumor cell killing 50.

M2 and M2-like polarized macrophages orchestrate tissue repair. Consistently with this general property of cells of the monocyte-macrophage lineage, there is evidence that under selected conditions TAM can limit the effectiveness of cytotoxic agents (Fig. 2). These include platinum compounds, paclitaxel and Doxorubicin itself 32, 84–88. In transplanted mouse models, M2-like macrophages were found to accumulate in perivascular areas after chemotherapy 89. Here they promoted tumor revascularization and relapse and their recruitment was CXCR4/CXCL12 dependent.

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Two general mechanisms emerge as responsible for the antagonistic action of TAM on chemotherapy. In mouse models, chemotherapy-triggered tissue damage has been linked to triggering recruitment of immunosuppressive myeloid cells 32, 84 or to elicit a skewed Th17 protumor response promoted by IL-1 86. An alternative pathway is centered on cancer stem cells (CSC): TAM have been reported to protect murine CSC against cytotoxicity 87, 88. Indeed, macrophages are an essential component of the stem cell niche in a variety of tissues. Thus, the dark side of the interaction of chemotherapy with TAM is a reflection of fundamental properties of macrophages, i.e. being part of tissue stem cell niches, taming adaptive immunity and orchestrating repair responses.

As for chemotherapy, the impact of radiotherapy on myeloid cells can have dual significance. The influx of monocytes which follows radiotherapy in mouse models drives a profibrotic tissue response and may promote tumor recurrence (e.g. 8, 90. Conversely, tumor regressions observed in patients at sites distant from irradiated lesions, called “abscopal” effect 91 call for activation of host immunity as a plausible explanation. In a mouse model, neoadjuvant low-dose γ-irradiation was found to set macrophage functions in an antitumor mode characterized by lack of immunosuppressive and proangiogenic activity and production of T cell attracting chemokines 92. Therefore as for chemotherapy, TAM can reduce or amplify the antitumor effect of radiotherapy depending on context.

5 Hormonal Therapy

There is evidence suggesting that the two classic pathways of tumor promotion, inflammation and sex steroid hormones are linked 1. In carcinoma of the prostate, IL-1β produced by macrophages converts androgen receptor modulators from being inhibitory to stimulatory 93. The occurrence of TAM was increased in prostate cancer patients who had been treated with androgen blockade therapy 94. TAM frequency was correlated with time to tumor progression. In a preclinical model, androgen blocking therapy induced production of macrophage attracting cytokines, CSF-1 in particular 95. Inhibition of the CSF-1R tyrosine kinase had synergistic antitumor activity with androgen inhibition. Thus, targeting TAM is a candidate approach to amplify susceptibility to hormonal intervention.

6 Anti-Angiogenesis

Strategies targeting VEGF are part of the current therapeutic armamentarium. In addition to eliciting angiogenesis, VEGF is a potent attractant for monocytes 96. It acts on monocytes via the VEGFR1/FLT1 receptor 96 97. Interestingly Qian et al, found that FLT1/VEGFR1 is upregulated in metastasis-associated macrophages in a murine mammary carcinoma model 97. Although VEGF has long been known to be chemotactic for monocytes, in this model VEGF did not drive recruitment. VEGF was upstream of autocrine CSF-1-triggered tumor promoting activity of metastasis-associated macrophages.

Macrophages, including TAM, are a major source of angiogenic growth factors acting on vascular and lymphatic vessels 13. TAM frequency and vascular density are generally associated in human tumors. Resistance to current anti-VEGF therapies is frequently associated with high levels of myeloid cell infiltration 53. Preclinical evidence suggests that

hypoxia following the destruction of the vascular bed by anti-angiogenic drugs triggers a compensative recruitment of myeloid cells which promote angiogenesis via alternative routes 8, 13, 98.

Angiopoietin-2 is a regulator of vessel wall function which is functionally linked to angiogenesis and TAM function. In addition to providing an escape pathway to VEGF inhibition, Angiopoietin-2 can trigger a proangiogenic phenotype in macrophages 99. In human glioblastoma macrophage infiltration is correlated with poor prognosis and this tumor is resistant to anti-VEGF treatment. In preclinical models, a dual Angiopoietin-2/VEGF bispecific antibody had appreciable antitumor activity and reprogrammed TAM from an M2 protumor to an M1 antitumor phenotype 100, 101. Thus targeting TAM may complement current anti-angiogenic therapies.

7 Immunotherapy with Checkpoint Blockade Inhibitors

Immunotherapy using checkpoint blockade inhibitors is now part of the therapeutic armamentarium in an increasing number of cancers 102. Clinically validated targets include CTLA4 and PD-1/PD-L1, and more will undergo clinical evaluation. Myelomonocytic cells are a key component of the immunosuppressive pathways targeted by checkpoint blockade inhibitors, and may offer tools to predict or increase their activity.

Macrophages express the ligands for checkpoint molecules, including PD-L1, PD-L2, B7H4 and the CTLA4 ligands B7-1 and B7-2. PD-L1 and PD-L2 are upregulated in response to various stimuli including cytokines and hypoxia 103, 104. TAM express high levels of PD-L1 and/or B7H4 in a variety of human tumor types, such as hepatocellular carcinoma 105, 106, glioblastoma 107 and pancreatic cancer 108. It has not been fully elucidated how and to what extent the expression of inhibitory receptors PDL-1, PDL-2 and B7-H4 on macrophages contributes to their immunosuppressive function. The expression of triggers of checkpoint blockade (e.g. PD-L1) by TAM as a predictor of response needs to be carefully assessed 109, 110.

Analysis of the mode of action of CTLA4 blocking mAb revealed an unexpected role of TAM. In preclinical models, FcγR expressing macrophages eliminated CTLA4 expressing, mAb coated Treg cells by ADCC 111, 112. TAM-mediated elimination of anti-CTLA4 sensitized Treg unleashed effective antitumor immunity. The role of macrophage-mediated ADCC in the activity of anti-CTLA4 (Iplimumab) was examined in 15 responding and 14 non-responding matched melanoma patients 113. Responding patients had higher number of $CD16^{+}$ monocytes and at the tumor sites a higher CD68+/CD163+ ratio, used as a correlate of M2 skewing. Moreover, response was associated with a decrease of Treg cells at the tumor site 113. These results are consistent with the preclinical data mentioned above and suggest that macrophages contribute to the action of anti-CTLA4.

Macrophage contribute to immunosuppression observed in the tumor microenvironment. Therefore macrophage targeting may complement the action of checkpoint blockade inhibitors. Indeed in a model of pancreatic cancer, CSF-1 inhibition synergized with

checkpoint immunotherapy 84, 114. Combination based on this principle are undergoing early clinical evaluation (see Table 2).

8 Antibody-Dependent Cellular Phagocytosis (ADCP) and Antibody-Dependent Cellular Cytotoxicity (ADCC)

Phagocytosis is a fundamental mechanism of innate resistance against microbes and of effete cell disposal. It has long been held by students in the field including one of the authors (AM) that phagocytosis does not represent a significant mechanism in the antitumor activity mediated by activated macrophages and that mechanisms of extracellular killing are the ones relevant for macrophage-mediated killing of cancer cells 10. Evidence has now challenged this long held view and has spurred interest into clinical translation.

The CD47-signal regulatory protein α (SIRP-α) pathway plays an important role in homeostasis and in regulating the engulfment of old erythrocytes. Both molecules are members of the Ig superfamily with SIRP-α and CD47 being expressed on macrophages and candidate target cells, respectively 115–117. SIRP-α is a negative regulator which acts as a docking protein for the SHP-1 and SHP-2 phosphatases which dampen intracellular signaling. Therefore CD47 acts physiologically as a “don’t eat me” signal. CD47 is frequently overexpressed in cancer cells 115, 116, 118, 119. Masking CD47 on cancer cells using mAb or engineered SIRP-a triggers ADCP in vitro and results in antitumor activity in diverse mouse tumor models 115, 116, 118–120. Strategies targeting the CD47/SIRP-a pathway using mAb or SIRP-α Fc fusion have proven to act synergistically with diverse anti-tumor mAb including anti-CD20 and anti-Her2. This result is consistent with the ability of SIRP-α to downregulate FcγR signaling 115, 116. In an interesting twist, anti-CD47 mAb treatment was found to activate adaptive immune responses 121–123 by activating accessory cell function. Moreover, CD47 blockade triggered DC-dependent activation of CD8 anti-tumor responses. Interestingly, CD47 blockade targeted pancreatic cancer stem cells and resulted in synergistic antitumor activity with chemotherapy 124. The ultimate efficacy of CD47 blockade requires activation of an adaptive immune response in the B16F10 murine melanoma model 125.

It has long been known that macrophages can kill tumor cells extracellularly via ADCC 126. ADCC, which can be mediated by NK cells and by macrophages is an essential component of the antitumor activity of anti-cancer mAb, including anti-CD20, anti-Her2 and anti-EGFR 127. Polymorphisms in FcγRIIIa and FcγRIIa are correlated with response in lymphoma (rituximab, anti-CD20), colon cancer (cetuximab, anti-EGFR) and breast carcinoma (trastuzumab, anti-HER2) 128–130. Since FcγRII is only expressed in myeloid cells, these data suggest an important role of macrophages in the clinical activity of mAbs. In a preclinical model the antitumor activity of anti-CD20 was dependent on chemokine-mediated macrophage recruitment and on macrophage effector function 131. Interestingly, in a mouse model signals present in the tumor microenvironment which skew TAM function (IL-10 and CSF-1) were found to increase macrophage effector function in the presence of anti-lymphoma mAb 132. Consistently with this observation, TAM infiltration is associated with worse prognosis in lymphoma 63. However, when treatment with anti-CD20 is taken

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into consideration, high TAM infiltration was associated with better outcome 65. Similarly, in a breast cancer model TAM promoted tumor growth but were essential for the therapeutic efficacy of mAb 133. Thus, preclinical models, mechanistic analysis and clinical correlative analysis suggest that the functional polarization of TAM may represent an advantage in terms of mAb triggered effector functions.

Maximizing TAM-mediated ADCC and ADCP could be part of combination approaches with chemotherapy. In a B cell leukemia model, cyclophosphamide-mediated tissue damage and elicited macrophage recruitment via chemokines and cytokines. In this setting chemotherapy and mAb (alemtuzumab, anti-CD52) synergistically drove cancer cell death and macrophage-mediated disposal 134.

9 Targeting Macrophage Recruitment, Survival and Polarization

Fig. 3 provides a schematic representation of macrophage targeting strategies. In general, macrophage-centered therapeutic approaches are aimed either at inhibiting functions related to tumor promotion or at activating antitumor activity.

Recruitment and localization

As mentioned above, mediators which have been involved in macrophage recruitment in tumors are diverse and include chemokines, complement components, CSF-1, VEGF. Chemokines have long been involved in macrophage accumulation in tumors 11, 15–17, 19, 27, 135. Stumbling blocks in translating anti-chemokine strategies into chemical benefit in inflammatory and neoplastic diseases include the fact that multiple chemokines and chemokine receptors are involved in phagocyte attraction (“robustness” of the system) and the fact that individual chemokines act on multiple cell types. Inhibition of CCL2 with specific antibodies reduced tumor growth and dissemination in different experimental models such as prostate, melanoma, breast, lung and liver cancer; when administered in combination with chemotherapy, anti-CCL2 antibodies improved the therapeutic efficacy 136–140. However, it has been also shown, in a breast cancer mouse model, that withdrawal of anti-CCL2 treatment increased the mobilization of bone marrow monocyte and infiltration in tumors, accelerating lung metastasis 141.

Selective antibodies to CCL2 have entered phase I and II clinical trials. The antibody CNTO 888 (carlumab) showed preliminary antitumor activity in advanced cancer patients and was well tolerated 142, 143. However, no responses were observed in a phase II study in castration resistant prostate cancer 142. Combinations of carlumab with conventional chemotherapy have been studied in a phase Ib clinical trial. The results indicated that carlumab has a good safety profile but no significant tumor responses were observed 144. A recent study demonstrated the feasibility of combining a novel CCR2 antagonist (PF-04136309) with conventional chemotherapy in locally advanced pancreatic cancer patients not eligible for surgery. In a Phase 1b clinical trial, patients with adenocarcinoma of the pancreas received FOLFIRINOX (oxaliplatin and irinotecan plus leucovorin and fluorouracil) alone, or combined with the oral CCR2 antagonist. Patients in the latter group did not experience worse toxicity than chemotherapy alone and had clinical signs of stable disease or partial tumor response in 49% of 33 imaging-evaluated patients 145.

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Analysis of leukocyte migration in CRC metastasis revealed overproduction of CCL5, a monocyte attractant also responsible for functional skewing of TAM 146. Treatment with Maraviroc, an antagonist for the CCR5 cognate receptor of CCR5 approved for clinical use in AIDS, resulted in biological and clinical responses in a small cohort of advanced CRC patients 146. Targeting the CCL5/CCR5 axis deserves further scouting given its long recognized activation for instance in breast cancer 135.

Survival

CSF-1-CSF-1R—The CSF-1 receptor (CSF-1R) is exclusively expressed by the monocytic lineage and represents an obvious target to hit TAM directly or indirectly, acting on precursors. CSF-1 (also known as M-CSF) is the major growth and differentiation factor for cells of the monocyte-macrophage lineages. CSF-1 is abundantly expressed by several tumor types; this ligand-receptor pathways has been extensively investigated in tumor models and constitutes a paradigm of TAM-cancer cell interaction 6, 147. A CSF1 or CSF1R-related signature has been associated with poor survival in different malignancies, such as classical Hodgkin lymphoma, breast cancer and hepatocellular carcinoma 148–150.

CSF-1R is a tyrosine kinase receptor. Antagonists and antibodies to the CSF1R have been developed and tested in preclinical models 84, 151, 152, 32. The humanized monoclonal antibody RG7155 (Emactuzumab) blocks CSF1R activation. In mouse tumor models and in cancer patients, treatment with RG7155 reduced macrophage infiltration in tumors and increased the CD8/CD4 T cell ratio in tumor biopsies of patients 153 (ClinicalTrials.gov, number NCT01494688) (Table 2). In patients with a rare neoplasia (diffuse-type tenosynovial giant cell tumor), characterized by the over-expression of CSF1R, no dose-limiting toxicities were observed. Common adverse events were facial oedema, asthenia and pruritus, 26/28 patients achieved clinically objective responses 154. The small molecule PLX3397 is a CSF-1R inhibitor and can be administered orally. Also this compound was able to induce clinical regression in patients with tenosynovial giant cell tumors (ClinicalTrials.gov number, NCT01004861) 155.

PLX3397 penetrates the blood-brain barrier and was tested in a phase II study in patients with recurrent glioblastoma. The drug was well tolerated and, as a proof-of-principle of its activity, circulating CD14dim/CD16+ monocytes were reduced after treatment. However, the primary endpoint of 6 months progression free survival was met only in 8% of 37 patients. Clearly, the potential of these inhibitors needs to be maximized with combination therapies 156. In preclinical mouse xenograft models with intracranial human glioblastoma, radiotherapy is known to increase CSF-1 ligand expression and tumor infiltration by myeloid cells. Treatment with PLX3397 potentiated the therapeutic effects of radiotherapy, suggesting that radiotherapy of glioblastoma may be improved by combinations with CSF-1R inhibition 157. In a syngeneic mouse model of BRAFV600-driven melanoma, PLX3397 also improved the anti-tumor efficacy of adoptive cell transfer immunotherapy by inhibiting the intratumoral accumulation of immunosuppressive macrophages 158. The CSF-1 inhibitor BZL945 blocked glioma progression and improved survival in preclinical models. Interestingly CSF-1R blockade did not result in TAM depletion but contributed, together with glioma-supplied factors (i.e. GM-CSF and IFNγ), to “re-educate”

macrophages from a pro-tumor phenotype to an anti-tumor effector cell 32. Analysis of glioblastomas recurring after CSF-1R inhibition revealed an interplay between cancer cells and the microenvironment 159. In recurrent tumors, IL-4-driven M2 activation of macrophages drove Stat6 and nuclear factor of activated T cells (NFAT)-mediated induction of insulin-like growth factor 1 (Igf1). Igf1 acted on tumor cells via the PI3K pathway. Blocking Igf1 or PI3K in tumor cells and CSF-1R in macrophages resulted in prolongation of survival in this mouse model.

The CSF-1R inhibitor GW2580 enhanced the activity of gemcitabine in a transgenic pancreatic ductal adenocarcinoma model. Mechanistically, the study demonstrated that macrophages induced the up-regulation of cytidine deaminase causing resistance to gemcitabine 160. This and other studies suggest that macrophage targeting could be a complementary strategy to enhance the efficacy of conventional chemotherapy. Along the same line in a transgenic mouse model of mammary adenocarcinoma treatment with Paclitaxel up-regulated the production of CSF-1, IL-34 (another growth factor using the CSF-1R), and the chemokine CCL8. Blockade of the CSF-1–CSF-1R loop, either by anti-CSF-1 antibodies or a CSF-1R inhibitor, enhanced the therapeutic efficacy of chemotherapy, inhibited metastasis, and increased CD8 T cell infiltration in tumors 84. In a mouse model of ovarian cancer, the administration of the CSF-1R kinase inhibitor GW2580 in the late stages of disease (peritoneal dissemination) resulted in dramatically reduced ascites volume and decreased number of infiltrating macrophages 161. In prostate cancer, androgen blockade therapy induced cancer cells to express CSF-1 and other cytokines that caused increased infiltration of TAM. Combination treatment of androgen blockade with inhibitors of CSF-1R achieved more durable therapeutic responses compared with hormonal therapy alone 95. Collectively, preclinical results strongly suggest that targeting the CSF1-CSF-1R axis has the potential to complement conventional therapeutic strategies.

Recent evidence has been provided of specific factors important for the survival and expansion of myeloid cells in cancer. The retinoic-acid-related orphan receptor (RORC1) expressed in myeloid cells drives cancer-related myelopoiesis in response to colony-stimulating factors. Its ablation in the myeloid compartment impairs tumor development and the generation of suppressive MDSCs, while promoting M1-polarized TAMs with antitumor activity. Thus, RORC1 is a potential molecule to target myeloid cells in cancer 162.

Biphosphonates—Bisphosphonates have cytotoxic potential on myeloid cells and are used for the treatment of osteoporosis and prevention of complications associated with bone metastases. They are inhibitors of the farnesyldiphosphonate synthase, a key enzyme responsible for cholesterol synthesis and protein prenylation, and have high affinity for bone hydroxyapatite where they are internalized by bone macrophages (osteoclasts), causing their apoptosis 163, 164. Tissue macrophages other than the ones in bone, including TAM, have been reported to be affected by bisphosphonates 165, in particular by clodronate in a liposomal formulation 166, 167. The current clinical usage of bisphosphonates in the treatment of solid malignancies is in combination with chemotherapy or hormonal therapy. In postmenopausal women with breast cancer, recurrence and overall mortality were significantly reduced. Moreover, clodronate was reported to reduce the incidence of bone and visceral metastases in human mammary carcinomas, an observation which points to

actions unrelated to the bone metastatic niche 168. In patients with bone metastatic hormone-naïve prostate cancer, Zoledronate reduced skeletal–related events and improved progression-free survival time (see for review 169). The relative importance of targeting macrophages in particular in the bone metastatic niche versus modifying bone resistance to osteolysis in the clinical activity of bisphosphonates remains to be assessed.

Trabectedin—As discussed above, Trabectedin, originally developed as an antiproliferative agent, was found to cause a partial depletion of circulating monocytes and TAM 75, 76. These studies stemmed from the clinical observation of delayed, persistent responses in cancer patients (see above). Monocyte depletion includes the monocytic component of MDSCs 75. Trabectedin activates a TRAIL dependent pathway of apoptosis75. Monocytes are exquisitely sensitive to TRAIL triggering of apoptosis because, unlike other leukocytes like neutrophils, they express very low levels of TRAIL decoy receptors 170 . In murine tumors and in human sarcomas Trabectedin-induced TAM reduction was associated with decreased angiogenesis. In murine tumors increase T cell infiltration was also observed. These observations raise the issue of combinations of Trabectedin with inhibitors of angiogenesis and of checkpoint blockade inhibitors.

Functional activation

Microbial preparations and microbe-derived molecules (e.g. MDP) activate macrophages for tumor cytotoxicity and have undergone clinical testing in the ‘70s. The only remainder of the bacterial era of immunotherapy is intravescical BCG in recurrent bladder carcinoma. In addition to or in concert with microbial moieties such as LPS, IFNγ is a classic inducer of macrophage killing of tumor cells and M1 activation 10. With the aim to avoid unwarranted systemic macrophage activation, IFNγ was administered ip first in advanced ovarian cancer patients and then in patients with minimal residual disease 171, 172. I.p. IFNγ resulted in activation of tumor cytotoxicity and clinical responses. It remains unclear whether the potential of IFNγ immunotherapy under these conditions has been fully exploited.

More specific, although unexpected, macrophage targeting came from the administration of an anti-CD40 antibody in a preclinical model of pancreatic cancer 173. Alternatively activated, M2-like macrophages were re-educated in the tumor microenvironment, and acquired antigen-presenting capabilities, leading to re-establishment of tumor immune surveillance and short-term reduction in tumor volume 173. This preclinical evidence spurred a clinical trial with a fully human CD40 agonist antibody in combination with gemcitabine in advanced pancreatic cancer patients, with partial responses 174. Repolarization of proangiogenic macrophages was achieved in mice by expressing the host-produced antiangiogenic and immunomodulatory protein histidine-rich glycoprotein (HRG), which induced downregulation of placental growth factor (PlGF) in macrophages, supporting the evaluation of PlGF-blockade strategies 175. A modified Vitamin D binding protein (EF-022) is undergoing early clinical evaluation based on effects on macrophage activation (Table 2).

ADCC and ADCP are strategies capitalizing on the effector function of TAM (see above). Macrophage ADCP activated by interfering with the SIRPa-CD47 pathway may be more

than a mechanism of effector function. In a recent study focused on glioblastoma, where the M1/M2 ratio had prognostic significance, anti-CD47-elicited ADCP resulted in functional skewing of murine macrophages in an M1 direction 125.

In a pancreatic cancer preclinical model 114, administration of the Bruton's tyrosine kinase (BTK) inhibitor Ibrutinib reset macrophages toward a phenotype that promoted CD8+ T M1-like cell cytotoxicity and curbed PDAC growth 176, a strategy that is currently under evaluation in combination with checkpoint inhibitors clinical trials 176.

Usage of nonsteroidal anti-inflammatory drugs (NSAIDs), aspirin in particular, is associated with protection against occurrence of many tumors and metastasis 177–179. PGE2 is well-known to have immune-suppressive effects, for instance on dendritic cells, and to favour the development of myeloid suppressor cells (MDSC) 180. Intriguingly, in a recent report PGE2 transactivated the CSF-1 receptor 181. Prostaglandins (e.g. PGE2) have also been reported to be involved in the M2-like polarization of macrophages in part through activation of the cAMP pathway 182, 183. Thus, it is tempting to speculate that targeting the tumor-associated myeloid cells plays a major role in the protective function of NSAIDs against primary cancer and metastasis.

10 Concluding Remarks and Perspectives

Cells of the monocyte-macrophage lineage are an essential element of the inflammatory component of the ecological niche of cancer and play a key role in progression. Progress has been made in defining the molecular landscapes and mechanisms of macrophage differentiation and diversity in tissues including cancer (e.g. 21, 184 24). Macrophage diversity includes the presence within tumors of TAM with different functional profiles, dictated by hypoxia. Current general paradigms on TAM reflect assessment at a population level. Deconvoluting TAM diversity at a single cell level and integrating information represent a challenge and may provide new vistas on cancer-related inflammation.

Macrophage can exert dual influences on the effect of conventional cytoreductive therapies, radiotherapy and chemotherapy. Moreover, TAM contribute to creating an immunosuppressive environment in tumors through multiple routes including triggers of checkpoint blockade in T cells. It will be important to assess whether TAM can provide predictive biomarkers for cytoreductive therapies and immunotherapy and contribute to personalized patient care.

Macrophage-centered therapeutic approaches are entering the clinical arena. These include blocking TAM-sustained tumor promotion and taking advantage of macrophage antitumor effector potential (ADCC, ADCP, M1-like polarization). While macrophage targeting strategies may per se result in therapeutic benefits, it is out tenet that macrophage therapeutics are borne to complement conventional citoreductive therapies, angiogenesis inhibitors and immunotherapy.

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Box 1

Diversity and Nomenclature of myelomonocytic cells

Diversity and plasticity are hallmarks of cells of the monocyte-macrophage lineage 20,21,185–187. Here we outline key aspects of monocyte-macrophage diversity and nomenclatures. In general there are considerable differences between mouse and man in terms of markers defining monocyte-macrophage diversity. Circulating monocytes originate from bone marrow precursors. In man, two main monocyte subsets have been identified based on expression of CD14 and CD16 ($CD14^+CD16^-$ and $CD14^+Cd16^+$). In the mouse, two monocyte subsets, which have been referred to as “inflammatory” or “patrolling”, based on the expression of Ly6C, CD11b, CCR2 and CX3CR1 20.

During cancer progression, immature bone marrow-derived elements of the myeloid lineage appear in the circulation. These cells are potent suppressors of adaptive immune responses. These immature myeloid cells with immunosuppressive activity have been operationally defined myeloid derived suppressor cells188,52. MDSCs are heterogeneous, being related to monocytes (MoMDSC) or neutrophils (Neu MDSC); they both express the immature myeloid cell marker CD33, but only MoMDSC at high levels. MoMDSCs can differentiate into TAM52.

Tissue macrophages originate either from embryonic precursors which seed peripheral locations and self-sustain or from circulating monocytes 189,21. In tissues, in response to diverse signals, cells of the monocyte-macrophage lineage undergo diverse forms of functional reprogramming. In particular, Interferonγ (IFNγ), produced in type 1 immune responses driven by TH1 cells and type 1 innate lymphoid cells (ILC1), and bacterial products activate the tumor cell killing activity and tissue damaging properties of these cells. Cytokines (IL-4 and IL-13), produced during type 2 immune responses driven by TH2 cells and ILC2 cells, activate an alternative form of macrophage activation oriented to resistance against parasites and to tissue repair and remodeling. Mirroring nomenclatures in current usage (e.g. TH1 and TH2), these two alternative forms of macrophage activation have been frequently referred to as M1 (or classic) and M2 (or alternative). The extremes and continuum between M1 and M2 do not recapitulate the whole spectrum of macrophage plasticity and indeed plasticity and flexibility of phenotypes is now recognized also for T cells and ILC cells. For a discussion of nomenclature issues the reader is referred to ref 34,190. As discussed here and represented in Fig. 1, in neoplastic tissues the signals orchestrating macrophage function are diverse and differ considerably in different tumors or different parts of the same tumor, with different phenotypes which in many cases do not fit the M1/M2 scheme. We and others use M2 to concisely refer to TAM phenotypes driven by IL-4 or IL-13 34 and M2-like to refer to a universe of diverse phenotypes which share, as functional output, tumor promotion and suppression of effective adaptive immunity. The inherent imperfection and utilization value of these oversimplified nomenclatures are discussed elsewhere 190.

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Bullet Points

- Tumor-associated macrophages (TAM) are a key component of the cancer microenvironment.
- TAM contribute to tumor growth and progression.
- Macrophages can have a dual influence on cancer, depending on stage in progression, tissue and microbiome.
- TAM can limit the antitumor activity of conventional chemotherapy and radiotherapy by orchestrating a tumor promoting, tissue repair response to damage and providing a protective niche for cancer stem cells
- On the other hand, macrophages contribute to the antitumor activity of selected chemotherapeutic agents such as Doxorubicin, under selected conditions
- Macrophage depletion plays a key role in the antitumor activity of a clinically approved agent Trabectedin.
- Macrophages mediate antibody-dependent cellular cytotoxicity (ADCC) and phagocytosis (ADCP) and play a key role in the antitumor activity of anticancer monoclonal antibodies.
- Therapeutic strategies targeting macrophages as tumor promoters and/or aiming at their activation and reeducation are undergoing clinical assessment.
- Macrophage-centered strategies have the potential to synergize with, and complement cytoreductive therapies, anti-angiogenic agents and checkpoint blockade inhibitors.

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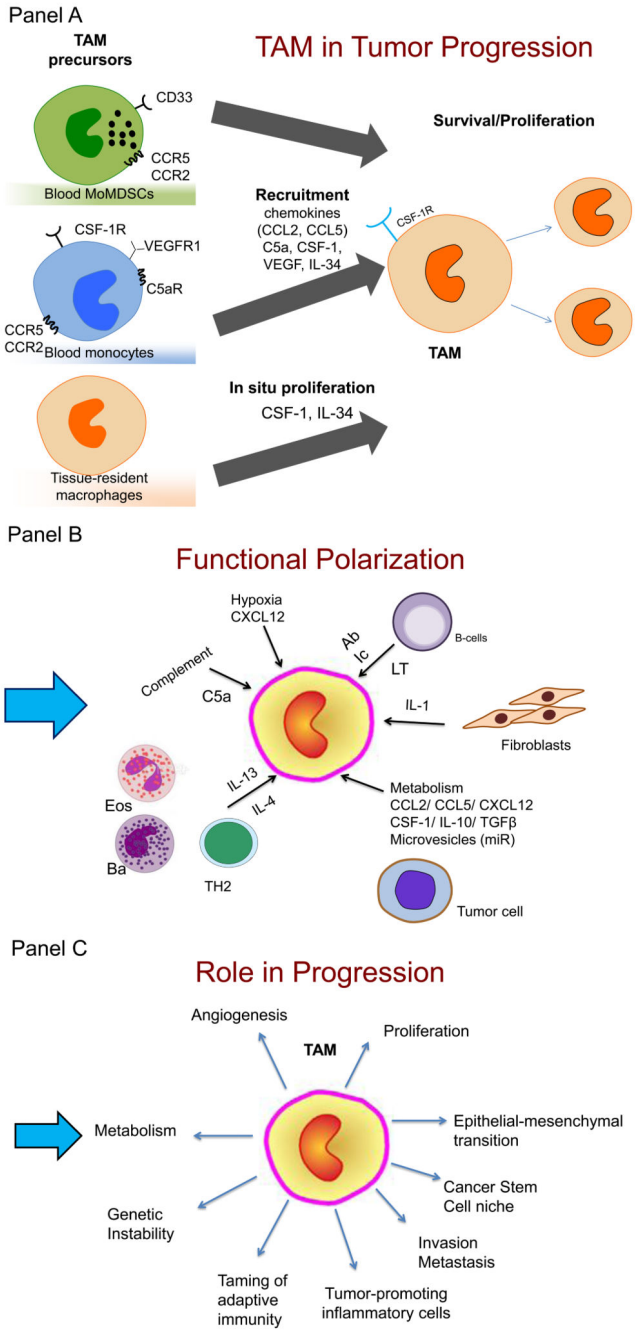

Figure 1. A schematic representation of the role of tumor-associated macrophages in tumor progression.

Panel A. Monocytes and MoMDSC (see Box 1) are recruited in tumors in response to diverse chemoattractants including CSF1, chemokines and complement components. In tumors, monocytes differentiate into macrophages (Tumor-associated macrophages, TAM). In some tumors, in situ proliferation may occur and local tissue resident macrophages of embryonic origin may contribute to TAM. Signals in the tumor microenvironment skew the function of TAM.

Panel B. Pathways and molecules polarizing TAM differ in different tumors. These include: IL-4 and IL-13 derived from TH2 cells, eosinophils (Eos) and basophils (Bas); cytokines and metabolites from tumor cells; antibodies (Ab) from B cells and immune complexes (Ic); stromal cell-derived factors (IL-1, LT).

Panel C. TAM affect virtually all aspects of tumor cell biology, including provision of a niche for cancer stem cells (CSC); angiogenesis; epithelial to mesenchymal transition (EMT); invasion and metastasis; proliferation; genetic instability.

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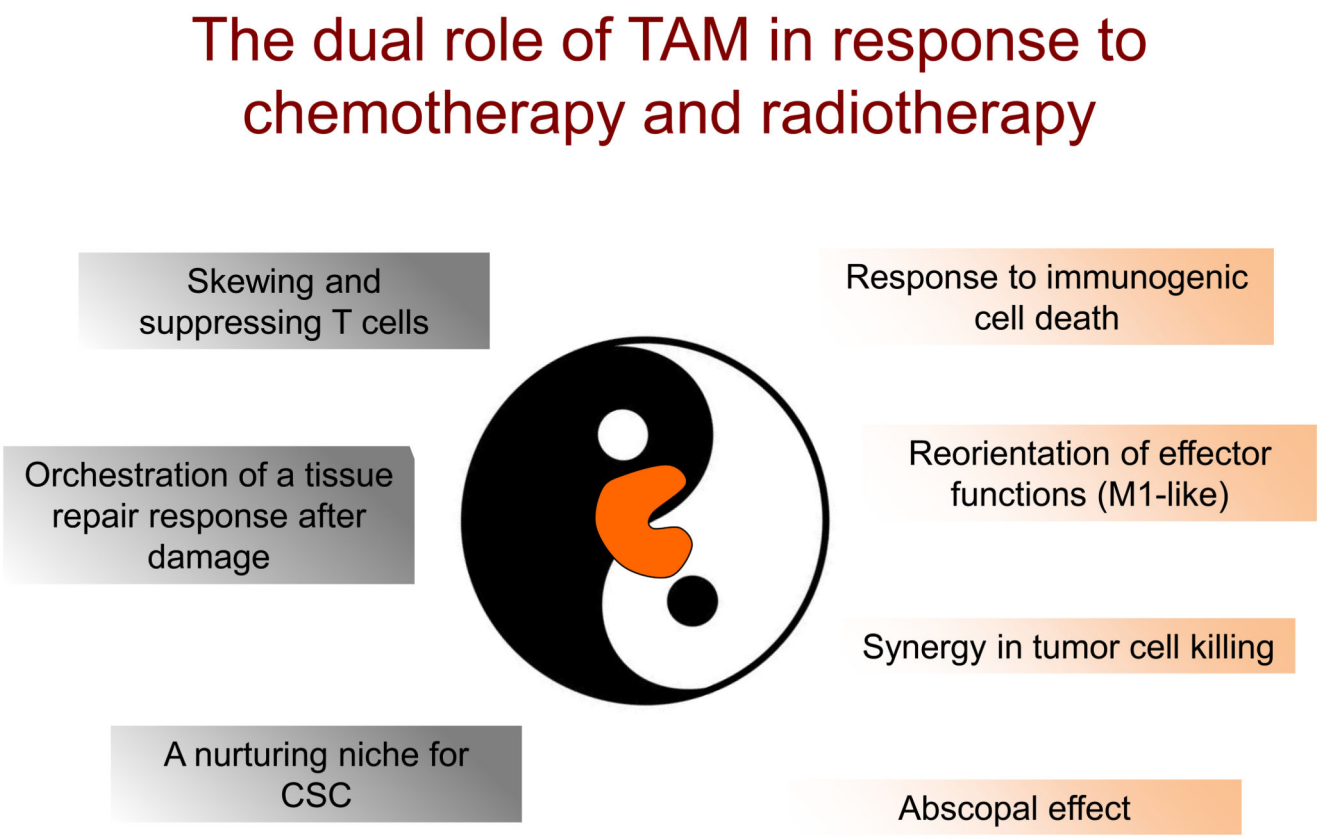

Figure 2. Bimodal function of TAM in response to chemotherapy and radiotherapy.

Macrophages orchestrate immune responses that can either hamper (left) or foster (right) the effectiveness of conventional anticancer strategies.

On the left: cytotoxic agents enhance tumor infiltration by immunosuppressive macrophages, which activate chemoprotective T cells and tame adaptive immune responses; chemotherapy or radiotherapy-induced tissue damage triggers the recruitment of immunosuppressive myeloid cells, which orchestrate a misdirected tissue-repair response, promoting tumor growth and revascularization; macrophages, an essential component of tissue stem cell niches, can protect CSC against cytotoxicity.

On the right: selected chemotherapeutic agents (e.g Doxorubicin) increase the immunogenicity of malignant cells (immunogenic cell death), which stimulate myeloid cells to differentiate into antigen presenting cells and trigger effective adaptive immune responses; anticancer agents like Gemcitabine can directly skew macrophage effector functions towards an antitumor mode and increase their cytotoxicity, resulting into a favorable synergism; neoadjuvants low-dose γ-irradiation set macrophage functions in an antitumor mode, promoting regression at sites distant from irradiated lesions (abscopal effect). CSC: cancer stem cells.

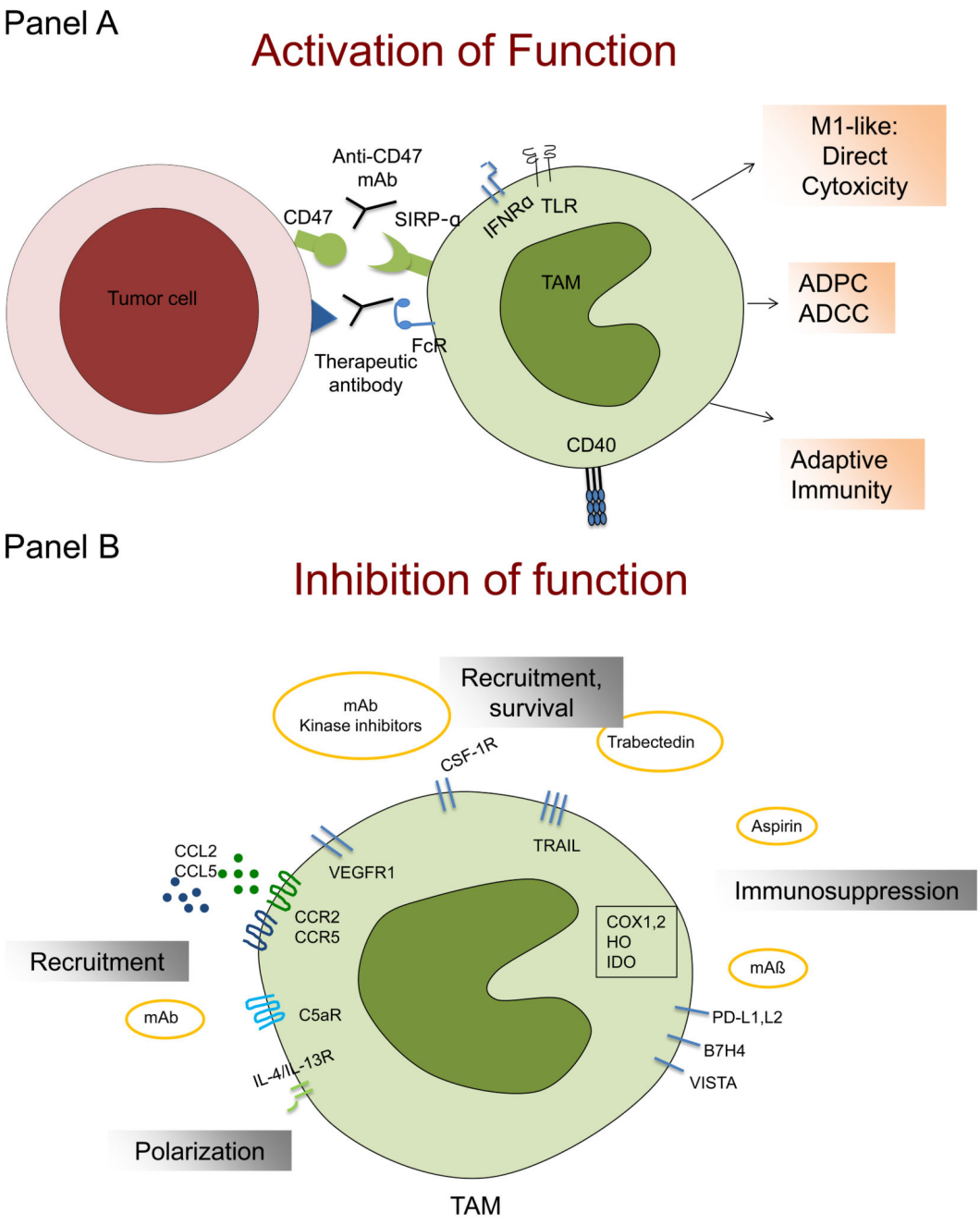

Figure 3. Schematic representation of strategies targeting macrophages in tumor settings.

Macrophage-centered therapeutic approaches are aimed either at activating their antitumor activity (Panel A) or inhibiting their recruitment and functions related to tumor promotion (panel B).

Panel A: the concerted action of microbial moieties (acting via TLRs) and IFNγ induces M1-like functional polarization and can activate macrophage killing of tumor cells; macrophage-mediated antibody-dependent cytotoxicity (ADCC) can mediate the therapeutic effect of therapeutic antibodies; interference with the SIRPα-CD47 pathway activates

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macrophage-mediated antibody-dependent phagocytosis (ADCP) and results in functional skewing of macrophages in an M1 direction and antitumor activity; an anti-CD40 antibody re-educates M2-like macrophages in the tumor microenvironment, leading to re-establishment of tumor immune surveillance.

Panel B: Inhibition of monocyte-attracting molecules, including chemokines (e.g CCL2, CCL5), VEGF, CSF-1 and complement mediators (C5a) with specific monoclonal antibodies (e.g. carlumab, emactuzumab) or antagonists (e.g. maraviroc) prevent macrophage recruitment to the tumor microenvironment, reducing tumor growth and dissemination; inhibitors of CSF-1 have also the potential to inhibit macrophage survival; Trabectedin activates a caspase-dependent pathway of apoptosis, selectively in cells of the monocyte lineage, causing a partial depletion of circulating monocytes and TAM; the protective function of NAIDS, aspirin in particular, against primary cancer and metastasis relies on the inhibition of prostaglandin production, which have immunosuppressive properties; TAM contribute to suppression of adaptive immunity by expression of immunosuppressive molecules, such as IDO, cyclooxygenases (COX1,2), TGFβ and IL-10. Moreover, TAM express triggers of checkpoint blockade, such PD-L1, PD-L2, B7H4 and VISTA.

TLR: Toll-like receptors; IFNRα: interferon receptor alpha; FcR: Fc receptor, mAb: monoclonal antibody; VEGF: vascular endothelial growth factor; CSF-1: colony-stimulating factor; NAIDS: nonsteroidal anti-inflammatory drugs; IDO: indoleamine 2,3 dioxygenase; TGFβ: transforming growth factor β; IL-10: interleukin 10.

Table 1

High density of TAMs as an outcome predictor in patients with neoplastic disease receiving chemotherapy.

Author	Year	Tumor Type	Therapy	Marker	Outcome Prediction	Ref.
Farinha P	2005	Follicular lymphoma	BP-VACOP[#]	CD68	Positive	63
Taskinen M	2007	Follicular lymphoma	AVP AVP + rituximab	CD68 CD68	Negative Positive	65
Steidl C	2010	Classic Hodgkin Lymphoma	ABVD[*]	CD68	Negative	61
Tan KL	2012	Classic Hodgkin Lymphoma[§]	ABVD *vs* Stanford V	CD68 CD163	Negative	62
Kridel R	2015	Follicular lymphoma	CHOP + rituximab CVP + rituximab	CD163 CD163	Negative Positive	66
Algars A	2012	Colorectal cancer –stage III	Unspecified[°]	Clev/Stab	Positive	49
Di Caro G	2015	Pancreatic cancer	No adjuvant Post-surgical adjuvant[^]	CD68	Negative Positive	50
Malesci A	submitted	Colorectal cancer –stage III	No adjuvant Post-surgical adjuvant[@]	CD68	None Positive	-

[#]Farinha, multiagent chemotherapy followed by involved region radiation

[*]Steidl, ABVD / ABVD-like / radiation therapy, second line therapy (autologous stem cell transplantation; CVPP; GDP; field radiation)

[§]Tan, locally advanced and advanced stage CHL; E2496 Intergroup trial, a multicenter phase 3 randomized controlled trial

[°]Algars, fluorurail-based adjuvant therapy is the standard regimen for stage III colorectal cancer. CD68, p=0.09 for better survival in this cohort

[^]Di Caro; gemcitabine-based adjuvant treatment

[@]Malesci, 5-flurouracil based adjuvant therapy

Table 2

Clinical trials targeting macrophages in tumors (from http://clinicaltrials.gov)

COMPOUND	CLINICAL PHASE	SPONSOR	TUMOR TYPE	COMBINATION
PLX3397 (CSF-1R inhibitor)	Phase 1/2 (O)	Plexxikon	Sarcoma, Nerve Sheath tumors	Sirolimus
	Phase 2 (O)		Melanoma	
	Phase 1 (O)		Prostate Cancer	Radiation therapy, Anti-androgen Therapy
	Phase 1/2 (O)		Solid tumors PVNS * and GCT-TS *	Pembrolizumab
	Phase 3 (O)		Breast cancer	Eribulin
	Phase 1B/2 (O)		Leukemia, Sarcoma, Neurofibroma	
	Phase 1/2 (O)		Acute Myeloid Leukemia	
	Phase 1/2 (O)		Glioblastoma	Radiation therapy; Drug: Temozoloide
	Phase 1/2 (O)		Solid tumors	Paclitaxel
	Phase 1/2 (O)		Breast cancer	Standard therapy **
PLX7486 (CSF-1R inhibitor)	Phase 1 (O)		Pancreatic adenocarcinoma	Gemcitabine; nab-Paclitaxel
LY3022855 (CSF-1R inhibitor)	Phase 1 (not yet open)	Eli Lilly	Solid tumors	Durvalumab; Tremelimumab
	Phase 1 (O)		Breast and Prostate cancer	
IMC-CS4 Anti-CSF-1R Ab	Phase 1 (O)	Eli Lilly	Solid tumors	
RO5509554 (RG7155) Anti-CSF-1R Ab	Phase 1 (O)	Hoffmann-La Roche	Solid tumors	MPDL3280A (anti-PD-L1 Ab)
	Phase 1 (O)		Solid tumors	Paclitaxel
AMG820 Anti-CSF-1R Ab	Phase 1/2 (not yet open)	Amgen	Pancreatic cancer; Colorectal cancer; Non-small cell lung cancer	Pembrolizumab
	Phase 1 (C)		Solid tumors	
Hu5F9-G4 Anti-CD47 Ab	Phase 1 (O)	Stanford University	Myeloid leukemia	
CC-90002 Anti-CD47 Ab	Phase 1 (O)	Celgene	Myeloid leukemia Myelodysplastic syndromes	
	Phase 1 (O)		Hematologic malignancies	
TTI-621	Phase 1 (O)	Trillium Ther.	Hematologic malignancies	

COMPOUND	CLINICAL PHASE	SPONSOR	TUMOR TYPE	COMBINATION
CD47 Fc fusion Protein				
CP-870,893 Agonist CD40 Ab	Phase 1 (C)	Pfizer (UPenn)	Melanoma	
	Phase 1 (C)		Solid neoplasms	Paclitaxel + Carboplatin
	Phase 1 (C)		Adenocarcinoma Pancreas	Gemcitabine
RO7009789 Agonist CD40 Ab	Phase 1 (O)	Hoffmann-La Roche	Solid neoplasms	Anti-PD-L1
	Phase 1 (O)		Solid neoplasms	Vanucizumab
	Phase 1 (O)		Adenocarcinoma Pancreas	Nab-Paclitaxel and Gemcitabine
Xilonix Anti-IL-1a Ab	Phase III (O)	XBiotech	Colorectal cancer	
Carlumab Anti-CCL2 Ab	Phase 2 (C)	Centocor	Prostate cancer	
CNTO888 Anti-CCL2 Ab	Phase 1 (C)	Centocor	Solid tumors	Gemcitabine + Paclitaxel + Carboplatin
NCT02052492 **EF-022** Vit D Binding Protein Macrophage Activator	Phase 1	Efranat	Solid tumors	

(O) Ongoing; (C) Completed;

* Pigmented Villonodular Synovitis (PVNS) or Giant Cell Tumor of the Tendon Sheath (GCT-TS);

** Standard therapy

Drug: AMG 386
Drug: AMG 479 (Ganitumab) plus Metformin
Drug: MK-2206 with or without Trastuzumab
Drug: AMG 386 and Trastuzumab
Drug: T-DM1 and Pertuzumab
Drug: Pertuzumab and Trastuzumab
Drug: Ganetespib
Drug: ABT-888
Drug: Neratinib
Drug: Pembrolizumab

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