**Review**

Inflammatory cells and mediators are an essential constituent of the tumor microenvironment (Mantovani et al., 2008; Hanahan and Weinberg, 2011; Coussens et al., 2013). Cells of the monocyte-macrophage lineage are major components of the host cell infiltrate of tumors, and the analysis of their function has led to the dissection of tumor-promoting inflammatory mechanisms in cancer (Mantovani et al., 1992; Sica and Mantovani, 2012; De Palma and Lewis, 2013; Noy and Pollard, 2014). In primary tumors and in metastatic sites, tumor-associated macrophages (TAMs) engage in complex bidirectional interactions with tumor cells, cancer stem cells (CSCs), fibroblasts, mesenchymal stem cells, endothelial cells, and T, B, and NK cells.

Although macrophages have the potential to kill tumor cells and to elicit tumor-destructive reactions, several lines of evidence indicate that TAMs are drivers of tumor progression in established tumors, promoting cancer cell proliferation and survival, angiogenesis, and lymphangiogenesis and skewing and taming effective T cell responses. There is also evidence that chronic inflammatory circuits may mediate tumor initiation and promote genetic instability (Mantovani et al., 2008; Noy and Pollard, 2014).

Macrophages are essential components of the inflammatory microenvironment of tumors. Conventional treatment modalities (chemotherapy and radiotherapy), targeted drugs, antiangiogenic drugs, and immunotherapy, including checkpoint blockade, all profoundly influence or depend on the function of tumor-associated macrophages (TAMs). Chemotherapy and radiotherapy can have dual influences on TAMs in that a misdirected macrophage-orchestrated tissue repair response can result in chemoresistance, but in other circumstances, TAMs are essential for effective therapy. A better understanding of the interaction of anticancer therapies with innate immunity, and TAMs in particular, may pave the way to better patient selection and innovative combinations of conventional approaches with immunotherapy.

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**The interaction of anticancer therapies with tumor-associated macrophages**

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Macrophages are essential components of the inflammatory microenvironment of tumors. Conventional treatment modalities (chemotherapy and radiotherapy), targeted drugs, antiangiogenic drugs, and immunotherapy, including checkpoint blockade, all profoundly influence or depend on the function of tumor-associated macrophages (TAMs). Chemotherapy and radiotherapy can have dual influences on TAMs in that a misdirected macrophage-orchestrated tissue repair response can result in chemoresistance, but in other circumstances, TAMs are essential for effective therapy. A better understanding of the interaction of anticancer therapies with innate immunity, and TAMs in particular, may pave the way to better patient selection and innovative combinations of conventional approaches with immunotherapy.
Monocytes and macrophages undergo profound functional reprogramming (“activation”) in response to microbial signals, tissue damage, cytokines, and metabolic products, the diverse outcomes of which reflect the extreme plasticity of mononuclear phagocytes. The CCR2–CCL2 pathway is an important determinant of monocyte recruitment and functional orientation of monocytes in tumors. It is not yet clear whether patrolling monocytes, which survey the intravascular space, have a specific function in the development of cancer.

Under homeostatic conditions, macrophages located in different tissues originate from embryonic precursors and acquire distinct morphological and functional features (Fig. 1), with the exception of the adult hematopoietic origin of gut, heart, and dermis macrophages (Bain et al., 2014; McGovern et al., 2014; Molawi et al., 2014). The recent identification of key transcription factors involved in the differentiation of tissue macrophages, such as GATA6 for peritoneal cells (Gautier et al., 2014; Okabe and Medzhitov, 2014; Rosas et al., 2014) and SPI-C for red pulp macrophages (Kohyama et al., 2009), and an increased understanding of the epigenetic landscape of resting and activated macrophages (De Santa et al., 2007; Ostuni et al., 2013; Gosselin et al., 2014; Lavin et al., 2014) will likely pave the way to an increased understanding of macrophage diversity in tissues under resting and inflammatory conditions.
The evidence and consensus about the role of TAMs in tumor-promoting inflammation (Hanahan and Weinberg, 2011) raise the issue of their involvement in current treatment modalities and of their potential as therapeutic targets. Here, we review the impact and significance of the interactions between cells of the monocyte-macrophage lineage and different therapeutic approaches, ranging from conventional chemotherapy to immunotherapy checkpoint blockade, and the ongoing development of macrophage-targeting strategies.

The yin-yang of cancer therapies

Cancer cell–centered therapeutic strategies and immunotherapy profoundly influence the function of TAMs by directly modulating their activity or by affecting components of the tumor microenvironment (e.g., effective adaptive immune responses). During cancer therapy, TAMs can have a yin-yang function in that they either contribute to the ultimate efficacy of anticancer strategies or have a tumor-promoting function by orchestrating a misdirected tissue repair response, as we discuss below for different therapeutic strategies.

Chemotherapy. Conventional chemotherapeutic agents can inhibit or activate effective antitumor responses, including those mediated by cells of the monocyte-macrophage lineage (Fig. 3). Immunity can contribute to the antineoplastic efficacy of selected chemotherapeutic agents (e.g., cyclophosphamide and doxorubicin; Mantovani et al., 1979), but the underlying mechanisms have remained elusive. It is conceivable that chemotherapeutic agents elicit a misdirected macrophage–orchestrated tissue repair response by causing tissue damage in the tumor (Mantovani et al., 2013), which may
result in promotion of tumor growth and limitation of antineoplastic efficacy. In vitro and/or in vivo evidence for a tumor-protective function of macrophages is available for some antitumor agents and tumor types (Table 1), including doxorubicin (earlier called Adriamycin), platinum compounds, 5-fluorouracil (5-FU), gemcitabine, paclitaxel (PTX), and combinations of cyclosphamide, methotrexate, and 5-FU (e.g., Paulus et al., 2006; DeNardo et al., 2011; Shree et al., 2011; Dijkstra et al., 2013; Affara et al., 2014). The pathways responsible for the tumor-promoting function of TAMs after chemotherapy are diverse. In different settings, these include increased recruitment of immunosuppressive TAMs by CSF-1 (DeNardo et al., 2011), protumor polarization (Dijkstra et al., 2013; Pyontek et al., 2013), activation via IL-1 of a tumor-promoting Th17 response (Bruchard et al., 2013), and protection against chemotherapy toxicity of CSCs (Jinushi et al., 2011; Mitchem et al., 2013).

These results suggest that in many instances chemotherapy is self-defeating by eliciting a misdirected tissue repair response orchestrated by TAMs. In apparent contrast with these results, immune responses are also essential for the optimal antitumor activity of some drugs (Fig. 3). Selected chemotherapeutic agents, doxorubicin in particular, cause immunogenic cell death of tumor cells, which leads to activation of effective adaptive responses (Kroemer et al., 2013). Myeloid-derived suppressor cells (MDSCs) are operationally defined as an immature heterogeneous population including cells belonging to the monocytic and neutrophil lineage (Gabrilovich et al., 2012). In a model of mammary carcinoma, doxorubicin was found to reduce the number of MDSCs and to pave the way to effective adoptive T cell transfer (Alizadeh et al., 2014). Doxorubicin-damaged cancer cells released ATP, which caused myeloid cell recruitment and differentiation into antigen-presenting cells, ultimately resulting in effective antitumor adaptive immunity (Ma et al., 2013). Cyclophosphamide-treated leukemic cells released activating cytokines (CCL4, CXCL8, vascular endothelial growth factor [VEGF], and TNF), which recruited monocytes/macrophages and enhanced their phagocytic activity. Co-administration of cyclophosphamide and a therapeutic antibody against B cell leukemia synergistically collaborated to induce tumor cell death and disposal by activated macrophages (Pallasch et al., 2014).

In the mouse, functional conditioning by the microbiome has emerged as a key component shaping the function of myeloid cells in tumors and their role in response to chemotherapy (platinum and cyclophosphamide; Iida et al., 2013; Viaud et al., 2013). In particular, microbial education of myelomonocytic cells is essential to prime for the antitumor activity of platinum combined with CpG (Fig. 3; Iida et al., 2013).

Thus, chemotherapeutic agents engage in a complex interaction with cells of the monocyte-macrophage lineage. Different contexts, including microbial education of innate immunity, the presence of potentially effective adaptive responses, and
inherent characteristics of different drugs, dictate the outcome of this yin-yang interaction (Fig. 3). The identification of the pathways responsible for the protumor function of TAMs in well-controlled tumor models (e.g., DeNardo et al., 2011; Germano et al., 2013; Pyonteck et al., 2013) has paved the way to clinical evaluation of therapeutic approaches that combine chemotherapy with macrophage-blocking strategies.

**Targeted therapies.** In spite of their specificity for cancer-associated molecular targets, targeted therapies can influence immune responses, and this interplay can in turn influence their antineoplastic effectiveness. Imatinib is a prototype for immune responses, and this interplay can in turn influence their associated molecular targets, targeted therapies can influence targeted therapies.

**Antiangiogenesis.** The capacity to elicit new vessel formation is a fundamental property of cancer cells, resulting in an abnormal vascular bed with hypoxic and normoxic microdomains (Hanahan and Weinberg, 2011). TAMs have proangiogenic activity, and macrophage infiltration in tumors is generally associated with high vascular density (Coffelt et al., 2010).

Antiangiogenic therapies based on inhibitors of the VEGF pathway frequently induce transitory responses in patients. In mouse tumor models and in cancer patients, refractoriness to angiogenic treatments is associated with higher numbers of CD11b+ cells or TEMs infiltrating tumor tissues (Mazzieri et al., 2011; Lu-Emerson et al., 2013; Gabrusiewicz et al., 2014). Destruction of the vessel network caused by antiangiogenic treatments creates a strongly hypoxic microenvironment, up-regulation of HIF1/2 signaling, and, as a compensatory mechanism, an increase in myeloid cell recruitment. Alternative vascular growth factors, other than VEGF, are essential for tumor recurrence, and CD11b+‘Gr1’+ myeloid cells produce the proangiogenic factor Bv8 (prokineticin-1; Chung et al., 2010). In preclinical models, depletion of TAMs, either by clodronate-loaded liposomes or CSF-1R inhibition, increased the antitumor effects of VEGF-targeted therapies (Zeisberger et al., 2006; Priceman et al., 2010). These data provide a rationale for combining antiangiogenic drugs with macrophage-targeting strategies. Furthermore, disruption of the angiopoietin-2–TIE2 axis (Mazzieri et al., 2011) is a promising approach to complement chemotherapy. Combining antiangiopoietin-2 with low-dose metronomic chemotherapy effectively inhibited the repopulation of myeloid cell and blocked metastatic growth in mice (Srivastava et al., 2014).

**Radiotherapy.** After irradiation, an influx of myeloid cells occurs with release of inflammatory cytokines (e.g., IL–1) and proinflammatory immunosuppressive mediators (TGFβ). Recruitment of macrophages ultimately leads to tumor recurrence (Moeller et al., 2004; Shiao and Coussens, 2010; Moding et al., 2013; Russell and Brown, 2013; Xu et al., 2013). Thus, as for chemotherapy, a misdirected tissue repair response can promote tumor recurrence and progression. However, recent studies have shown that the efficacy of fractionated radiotherapy may involve the activation of the immune system. When tumor cells undergo immunogenic cell death (Kroemer et al., 2013), the innate immune system is activated to present tumor-released antigens to the adaptive immune system. The pro-immunogenic effects of fractionated irradiation induced objective responses, even in lesions

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**Table 1.** Pathways responsible for the tumor-protective function of TAMs against chemotherapy

| Tumor                  | Drug          | Mechanism                              | Reference               |
|------------------------|---------------|----------------------------------------|-------------------------|
| Mammary, carcinoma     | PTX           | CSF1-dependent increased recruitment of TAMs | DeNardo et al., 2011    |
| Mammary, carcinoma     | PTX, doxorubicin, etoposide | Increased protease activity           | Shree et al., 2011      |
| Cervical, carcinoma    | Platinum      | Promote polarization of TAMs           | Dijkstra et al., 2013   |
| EL-4 lymphoma and other transplanted solid tumors | Gemicitabine + 5-FU | Skewed Th17 response                  | Bruchard et al., 2013   |
| Pancreatic, carcinoma  | Gemcitabine   | Induction of drug-metabolizing enzyme   | Weizman et al., 2014    |
| Lung, colon, pancreas  | Various       | Protection of CSCs against toxicity    | Jinushi et al., 2011; Mitchem et al., 2013 |
that were distant from the treated site (abscopal effect; Durante et al., 2013; Golden et al., 2013, 2014). It is notable that local radiation therapy also proved efficacious in patients who previously progressed after anti–cytotoxic T-lymphocyte antigen 4 (CTLA-4) treatment (Postow et al., 2012; Grimaldi et al., 2014).

In an interesting twist, neoadjuvant low-dose γ irradiation was found to normalize the tumor vasculature and to enhance recruitment of tumor-specific T cells in various cancer models (Klug et al., 2013). Interestingly, under these conditions, low-dose irradiation skewed macrophage function to an antitumor mode, with production of T cell–attracting chemokines and down-regulation of immunosuppressive and angiogenic mediators. Thus, as discussed below for chemotherapy and antimacrophage strategies (Affara et al., 2014), the interplay between TAMs and adaptive CD8+ T cell antitumor responses during low-dose irradiation is a key determinant of therapeutic outcome.

### Monoclonal antibodies and immune checkpoint blockade.

B cells and antibodies can trigger protumor functions of cells of the monocyte-macrophage lineage (Coussens et al., 2013; Affara et al., 2014). However, monoclonal antibodies directed against tumor antigens (e.g., CD20 and HER-2) represent invaluable targeted therapies in the clinic. TAMs are potent effectors of antibody–dependent cellular cytotoxicity (ADCC) and contribute to the antitumor activity of antitumor monoclonal antibodies such as anti-CD20 and anti–HER-2 (Sliwkowski and Mellman, 2013; Furness et al., 2014).

The intensity and duration of T cell responses are tightly regulated by immune checkpoints, which are essential to prevent autoimmune reactions (Pardoll, 2012). Molecules involved in checkpoint regulation include CTLA-4, programed death 1 (PD1), T cell immunoglobulin and mucin domain–containing protein-3 (TIM-3), and lymphocyte activation gene (LAG–3), whose expression is high in intratumor lymphocytes. Monoclonal antibodies that interfere with checkpoint blockade can activate effective immune response against selected tumors, and in some patients, these antibodies have shown clinical efficacy (Pardoll, 2012; Makkouk and Weiner, 2015).

The role of myelomonocytic cells in the action of checkpoint blockade monoclonal antibodies may well have been underestimated. Cells of the monocyte–macrophage lineage or lineages, including TAMs, express the ligands for the inhibitory receptor programmed cell death protein-1 (PD-L1 and PD-L2) and CTLA-4. Moreover, TAMs from HCC express the B7 family member B7H4 (Kryczek et al., 2006). It remains unclear whether, and to what extent, these inhibitory molecules contribute to the immunosuppressive activity of TAMs.

The mode of action of immune checkpoint blockade is not completely understood. Recent evidence suggests that anti–CTLA-4 antibodies act via Fcγ receptor–expressing macrophages (Selby et al., 2013; Simpson et al., 2013). Evidence in mouse models suggests that elimination of T reg cells by macrophage-mediated ADCC is an essential component of the therapeutic activity of anti–CTLA-4 (Selby et al., 2013; Simpson et al., 2013). It will be important to assess the clinical significance of these observations from the perspective of identifying patients responsive to immune checkpoint blockade.

### Macrophage targeting

Two general strategies have been used to target myelomonocytic cells in tumors: inhibition of recruitment and/or elimination (the latter achieved by direct killing) and reeducation (Fig. 3). The plasticity and flexibility of myelomonocytic cells (Hagemann et al., 2008; Mosser and Edwards, 2008) provides a basis for strategies aimed at “resetting” TAMs in an antitumor mode. Agents in this broad category include the classic Th1 cytokine IFNγ, which early on showed objective responses in minimal residual ovarian cancer (Colombo et al., 1992; Pujade-Lauraine et al., 1996); bacterial products, with intravesical BCG being part of the armamentarium in bladder cancer; TLR agonists (e.g., CpG oligonucleotides, which are undergoing preclinical and clinical evaluation [e.g., Iida et al., 2013]); and antibodies that activate via the CD40 molecule.

A fully human CD40 agonist antibody CP-870,893 was administered in combination with gemcitabine chemotherapy to 21 patients with advanced pancreatic cancer, with partial clinical effects (Beatty et al., 2013). In a mouse model of pancreatic cancer, anti–CD40 was found to modify macrophage phenotype with up-regulation of MHC class II and CD86 (Beatty et al., 2011). Similarly, the plasma protein histidine-rich glycoprotein (HRG) was reported to skew TAM polarization into a phenotype with antitumor activity by down-regulation of the placental growth factor (PIGF), a member of the VEGF family. In mice, HRG promoted antitumor immune responses and normalization of the vessel network (Rolny et al., 2011).

Blocking macrophage recruitment and survival has been extensively investigated in preclinical models and is undergoing clinical evaluation. TAMs typically originate from blood monocytes that are continuously recruited from the circulation (Mantovani et al., 1992; Franklin et al., 2014), although a certain degree of self-renewal in some tumors has been reported (Bottazzi et al., 1990; Tymozuk et al., 2014). Among chemoattractants that regulate the influx of circulating monocytes in tumor tissue, chemokines have been extensively studied, in particular CCL2 (Weitzenfeld and Ben-Baruch, 2014). Antibodies to CCL2 are now being tested in clinical trials. CNTO 888 (carlumab) showed preliminary antitumor activity in advanced cancer patients and was well tolerated (Pienta et al., 2013; Sandhu et al., 2013). Combinations of carlumab with conventional chemotherapy regimens are being studied in clinical trials (Brana et al., 2014). Recent results caution against the possibility that interruption of anti–CCL2 therapy may lead to enhanced metastasis (Bonapace et al., 2014). In a breast cancer model, cessation of anti–CCL2 therapy was associated with monocyte release from the bone marrow, increased mobilization and infiltration of cancer...
cells, and angiogenesis driven by IL-6 and VEGF. In addition, recent results suggest that complement components are key players in cancer-related inflammation and orchestrate macrophage recruitment in part via CCL2 (Bonavita et al., 2015). Thus, targeting complement with available tools (e.g., anti-C5a) should be taken into consideration.

TAMs localized in different compartments of the same tumor lesion can have considerably different functional properties (Movahedi et al., 2010), with a protumor phenotype prevailing in avascular areas. Semaphorin 3A (Sema3A), which is induced by hypoxia, interacts with the holoreceptor, including neuropilin1 (Nrp1) and plexin A1/plexin A4, triggering VEGFR1 phosphorylation and macrophage attraction (Casazza et al., 2013; Laoui et al., 2014). Interestingly, at hypoxic sites, Nrp1 was down-regulated and Sema3A delivered a stop and retention signaling via plexin A1/plexin A4, thus sequestering TAMs in hypoxic niches. Genetic inactivation of Nrp1 resulted in enhanced trapping of TAMs in the normoxic part of the tumor, with inhibition of their immunosuppressive and angiogenic activity (Casazza et al., 2013; Laoui et al., 2014). These results suggest that localization of TAMs in normoxic versus hypoxic regions of tumors may be a strategy to inhibit the protumor phenotype.

CSF-1 is abundantly produced by several tumor types and represents a prime target for antimacrophage strategies using antisense oligonucleotides (Aharinejad et al., 2004; Paulus et al., 2006), monoclonal antibodies, or kinase inhibitors. Antagonists of the CSF-1R tyrosine kinase have been developed and tested in preclinical models, including acute myeloid leukemia, melanoma mammary carcinoma, and glioblastoma, with promising results (e.g., Goswami et al., 2005; Manthey et al., 2009; DeNardo et al., 2011; Pyonteck et al., 2013). Interestingly, CSF-1R inhibition in glioblastoma did not reduce TAM numbers but blocked their tumor-promoting functions (Pyonteck et al., 2013). In an important proof-of-principle study, an anti-CSF-1R antibody (RG7155) was recently demonstrated to reduce macrophage infiltration in mouse tumor models and in patients. In patients with a rare sarcoma with high production of CSF1 (diffuse-type giant cell tumor), treatment with this antibody resulted in objective clinical responses (Ries et al., 2014).

As mentioned above, TAMs influence the tumor response to chemotherapy. In a transgenic mouse model of mammary adenocarcinoma, PTX up-regulated CSF-1, IL-34 (a growth factor using the CSF-1 receptor), and CCL8 in tumor cells. Blockade of the CSF1–CSFIR loop, either with anti-CSF1 antibodies or a CSF1-R inhibitor, in combination with chemotherapy, enhanced the therapeutic efficacy, inhibited metastases, and increased the recruitment of CD8 T cells in tumors (DeNardo et al., 2011). A more in-depth, mechanistic analysis of the interplay between TAMs and CD8+ T cells in the context of combined therapies revealed a more complex circuit (Ruffell et al., 2014). TAMs were the main source of IL-10, and anti–IL-10R was as effective as anti-CSF1 when combined with PTX and carboplatin. However, perhaps unexpectedly, macrophage-derived IL-10 did not directly affect CD8+ T cell function; rather, it inhibited IL-12 expression in intratumor DCs, thus blocking activation of an effective adaptive response (Ruffell et al., 2014). Similar to the approach of targeting macrophages to re-activate T cell effector function, selective expression of an IFNα transgene in monocytes inhibited tumor progression in mammary carcinoma and enhanced cytotoxic T cells (Escobar et al., 2014). It is possible that intratumor DCs also play a role as an intermediary component between TAMs and adaptive immunity. The enhancement of chemotherapeutic responses by macrophage depletion in different settings therefore provides a rationale for clinical testing of combined therapeutic approaches.

It has recently been demonstrated that targeting of TAMs plays a key role in the antitumor activity of a clinically approved drug (Germano et al., 2013). Trabectedin was originally derived from the marine organism Ecteinascidia turbinata and is approved in Europe (by the EMEA) for the treatment of sarcomas and ovarian carcinoma. It is selectively cytotoxic for human and mouse monocytes, including TAMs, and induces caspase-dependent apoptosis (Fig. 3; Germano et al., 2013). Evidence in the mouse and in sarcoma patients suggests that macrophage depletion is a key mechanism of action of the antitumor activity of this agent. In biopsies from sarcoma patients treated with trabectedin, a significant decrease of TAMs and vessel networks was noted (Germano et al., 2013). These results provide proof-of-principle for macrophage targeting in human cancer and may have implications for the design of combination therapies.

Concluding remarks
Myelomonocytic cells have emerged as an essential component of tumor-promoting inflammation (Mantovani et al., 1992, 2008; Hanahan and Weinberg, 2011; Coussens et al., 2008; Hanahan and Weinberg, 2011; Coussens et al., 2013; Noy and Pollard, 2014). Evidence suggests that cells of the monocyte-macrophage lineage can have a dramatic impact on the outcome of current treatment modalities. It will be important to definitively assess whether TAMs or TAM-related biomarkers can serve to guide diverse therapeutic approaches, including checkpoint blockade strategies. Development of effective strategies targeting myelomonocytic cells in tumor tissues or reeducating or relocating them will require a better understanding of their molecular pathways and diversity. The identification of genetic and epigenetic mechanisms (e.g., Ostuni et al., 2013; Okabe and Medzhitov, 2014; Rosas et al., 2014) underlying macrophage diversity in tissues and their different forms of activation is likely to pave the way to reeducation strategies. Although these strategies are in their infancy, early clinical trials are ongoing, and there is proof-of-principle that targeting TAMs can be clinically beneficial (Germano et al., 2013; Ries et al., 2014). However, macrophage-targeting strategies are unlikely to be effective per se and will need to be combined with conventional therapeutic approaches, capitalizing on an improved understanding of their interaction with mononuclear phagocytes.
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