Tumor-induced myeloid deviation: when myeloid-derived suppressor cells meet tumor-associated macrophages

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The generation of an inflammatory environment is favorable and often decisive for the growth of both primary tumors and metastases. Tumor cells either express membrane molecules or release tumor-derived soluble factors able to alter myelopoiesis. Tumor-reprogrammed myeloid cells not only create a tolerogenic environment by blocking T cell functions and proliferation, but also directly drive tumor growth by promoting cancer stemness, angiogenesis, stroma deposition, epithelial-to-mesenchymal transition, and metastasis formation. In this Review, we discuss the interplay between immunosuppressive and protumoral myeloid cells and detail their immune-regulatory mechanisms, the molecular pathways involved in their differentiation, as well as their potential role as prognostic and diagnostic biomarkers and prospective targets for innovative approaches to treat tumor-bearing hosts.

Tumor progression depends on the gradual accumulation of genetic and epigenetic aberrations in cancer cells that also modify the cellular composition of the tumor environment, establishing a state of chronic inflammation characterized by the stromal infiltration of immune cells. Myeloid cells play a critical role in sustaining cancer progression (1). Moreover, inflammatory myeloid cells help to create and fuel the mutagenic pressure underlying the genetic instability of neoplastic cells by both direct mechanisms, such as the production of free-radical compounds (2), and indirect processes, such as the disruption of host defense barriers (3).

Tumor growth is assisted by tumor-associated macrophages (TAMs), the major leukocyte population infiltrating cancers (4). Although macrophages have the potential to attack and eliminate tumor cells, TAMs exhibit many protumoral features that are partly shared by macrophages involved in tissue repair, and they interfere with the function and proliferation of immune effectors (5). Thus, a high frequency of TAMs is associated with poor prognosis in many but not all human tumors (6).

Myeloid-derived suppressor cells (MDSCs) have received increased attention, and their presence and frequency in the blood of patients with tumors is emerging as a potential and simple prognostic marker to monitor clinical outcome and response to therapy (7). MDSCs are characterized by their myeloid origin, heterogeneous cell composition, and ability to negatively regulate adaptive and innate immune responses to cancer. Although TAMs and MDSCs are regarded as separate entities (Figure 1), the boundaries between them are not clearly demarcated, and they share many characteristics (8). TAM accumulation in cancerous tissues is sustained by circulating inflammatory monocytes ($CCR2^+Ly6C^+$ cells in mice and $CCR2^+CD14^+CD16^-$ cells in humans; ref. 9), which are distinct from vessel-patrolling monocytes ($Ly6C^{lo}CX3CR1^{hi}$ in mice and $CD14^{dim}CD16^-CX3CR1^{hi}$ in humans). Interestingly, immunosuppressive MDSCs with monocytic features are able to traffic from BM to tumors, mainly through the same chemokine pathway (10). Therefore, the CCR2/CCL2 axis is required for MDSC and TAM accrual and functional specialization. Here, we review the distinctive and common characteristics of TAMs and MDSCs, their role in maintaining cancer growth, and the ongoing development of selective therapeutic approaches.

MDSCs and TAMs result from altered myelopoiesis

The most pervasive and efficient strategy of immune escape likely relies on cancer's ability to create a widespread tolerogenic environment by altering normal hematopoiesis and promoting the expansion of myeloid cells through the constant and progressive release of tumor-derived factors (TDFs), which include metabolites, cytokines, and chemokines (ref. 11 and Figure 2). This "reactive myelopoiesis," leading to MDSC and TAM accumulation, presents marked and distinct molecular features compared with emergency granulopoiesis (12), as emphasized below.

Macrophage composition in different tissues or inflammatory environments depends on a dynamic equilibrium between recruited and tissue-resident macrophages. Tissue-resident macrophages originate at the prenatal stage from the yolk sac and fetal liver (13–15) and acquire selective, tissue-dependent features through the activation of distinctive transcriptional profiles (16–20). During inflammation and under steady-state conditions in some tissues, macrophages are derived from circulating $Ly6C^+CCR2^+$ monocytes, as in the case of colonic mucosal macrophages (21).

In cancer, the evidence to date indicates that TAMs are dynamically replaced by circulating precursors. Both the tissue-

Conflict of interest: The authors have declared the no conflict of interest exists.
Reference information: *J Clin Invest*. 2015;125(9):3365–3376. doi:10.1172/JCI80006.

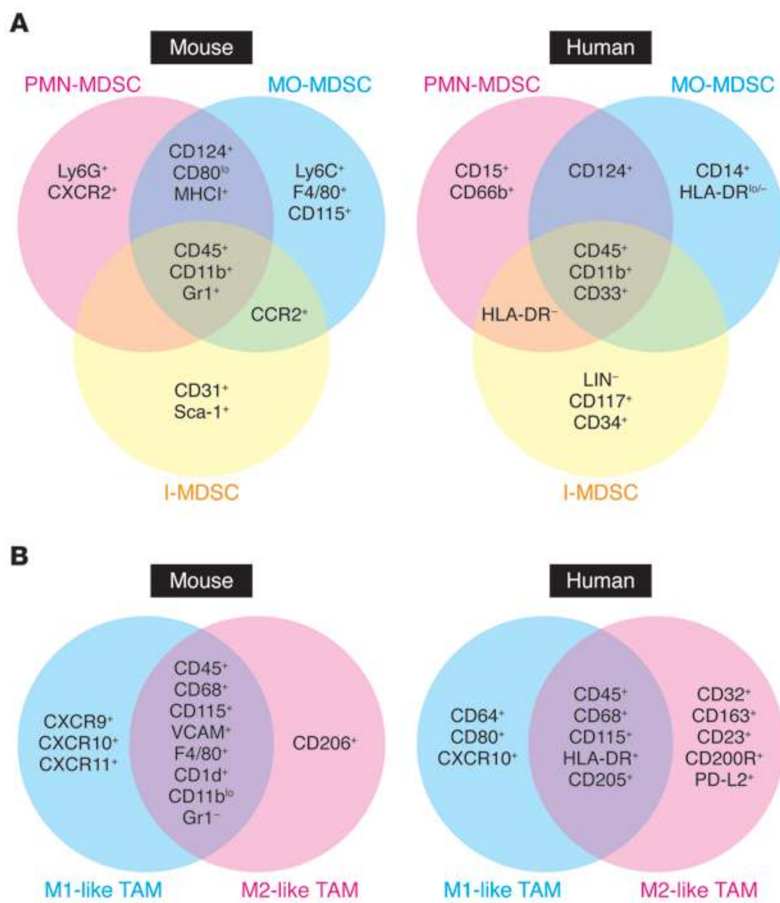

Figure 1. Common phenotypic markers of MDSCs and TAMs. Several phenotypic markers of mouse and human MDSCs (**A**) and TAMs (**B**) have been identified (+ indicates expression, while – indicates lack of expression) and used to define specific cell subgroups, such as PMN-MDSCs, MO-MDSCs, and immature MDSCs (I-MDSCs), as well as M1-like and M2-like TAMs, by both cytofluorimetric and immunohistochemical analyses.

resident macrophages present in normal mammary tissues and TAMs that develop during tumor progression in the MMTV-PyMT breast cancer model are derived from blood-circulating $CCR2^+$ monocytes, but only TAMs display self-renewal capability (22). In fact, TAM differentiation relies on the NOTCH/recombination signal–binding protein for the Ig κ J region (RBPJ) signaling pathway and is cell restricted, as genetic ablation of *RbpJ* caused a reduction in both TAMs and tumor growth (22). In the MMTV-neu mouse model of autochthonous mammary carcinogenesis, in situ cell division of fully differentiated $CD11b^{lo}F4/80^{hi}$ macrophages was the main contributor to the rapid TAM expansion; however, circulating monocyte influx was required in the long term (23). TAM progenitors ($Ly6C^+$ monocytes) can also arise from tumor-induced extramedullary hematopoiesis within the spleen (24), although the relative contribution of BM and spleen to the monocyte reservoir and tumor trafficking is not clear and might be tumor dependent (25).

MDSCs in tumor-bearing hosts: cellular heterogeneity. Normal $CD11b^+Gr1^+$ cells in BM are multipotent cells that can differentiate, depending on the kind and/or extent of cytokine/chemokine stimulation, into cells able to either enhance (e.g., myeloid DCs) or restrain (MDSCs) the immune response (26, 27). However, even in tumor-bearing hosts, BM $CD11b^+Gr1^+$ cells are poorly suppressive, while the same cells isolated from liver, spleen, blood, and tumors are fully competent to inhibit T cell activation (28, 29). These findings suggest that the BM niche is not permissive for a complete, functional maturation of MDSCs.

As further detailed by Marvel and Gabrilovich (30), mouse MDSCs have been divided into two main subgroups with different phenotypic and biological properties: the monocytic (MO-MDSC) and polymorphonuclear/granulocytic (PMN-MDSC) subsets (31). In tumor-bearing mice, MO-MDSCs ($Gr1^{lo/int}CD11b^+Ly6C^{hi}Ly6G^-$) are highly immunosuppressive and exert their effects largely in an antigen-nonspecific manner, whereas PMN-MDSCs ($Gr1^{hi}CD11b^+ Ly6C^{lo}Ly6G^+$) are moderately immunosuppressive and promote T cell tolerance via antigen-specific mechanisms (32, 33). TDFs induce tumor-infiltrating MO-MDSC differentiation into immunosuppressive TAMs. This conversion is primarily mediated by CSF1 (34), but also by molecular pathways controlled by the hypoxia-inducible factor 1α (HIF-1α) (35). HIF-1α may also be stabilized by the lactic acid that is produced by aerobic glycolysis (Warburg effect) in cancer cells (36). Alternatively, lactic acid can be actively produced in immune-regulatory myeloid cells by cytokine-activated, anerobic glycolysis (28, 37).

TAMs in tumor-bearing hosts: cellular plasticity. After arriving at the tumor site, $Ly6C^+CD11c^-MHCII^-CD11b^{hi}VCAM^-$ monocytes undergo sequential phenotypical changes characterized by the downregulation of Ly6C and CD11b and the upregulation of MHC class II (MHCII) molecules, VCAM, and CD11c (22). However, TAM differentiation and distribution is not a defined and preserved track but depends on both anatomical location and the tumor stage: cancers with different histology are infiltrated by TAMs with phenotypic and functionally distinct features (38). It is essential to avoid simplified conclusions regarding TAM onto-

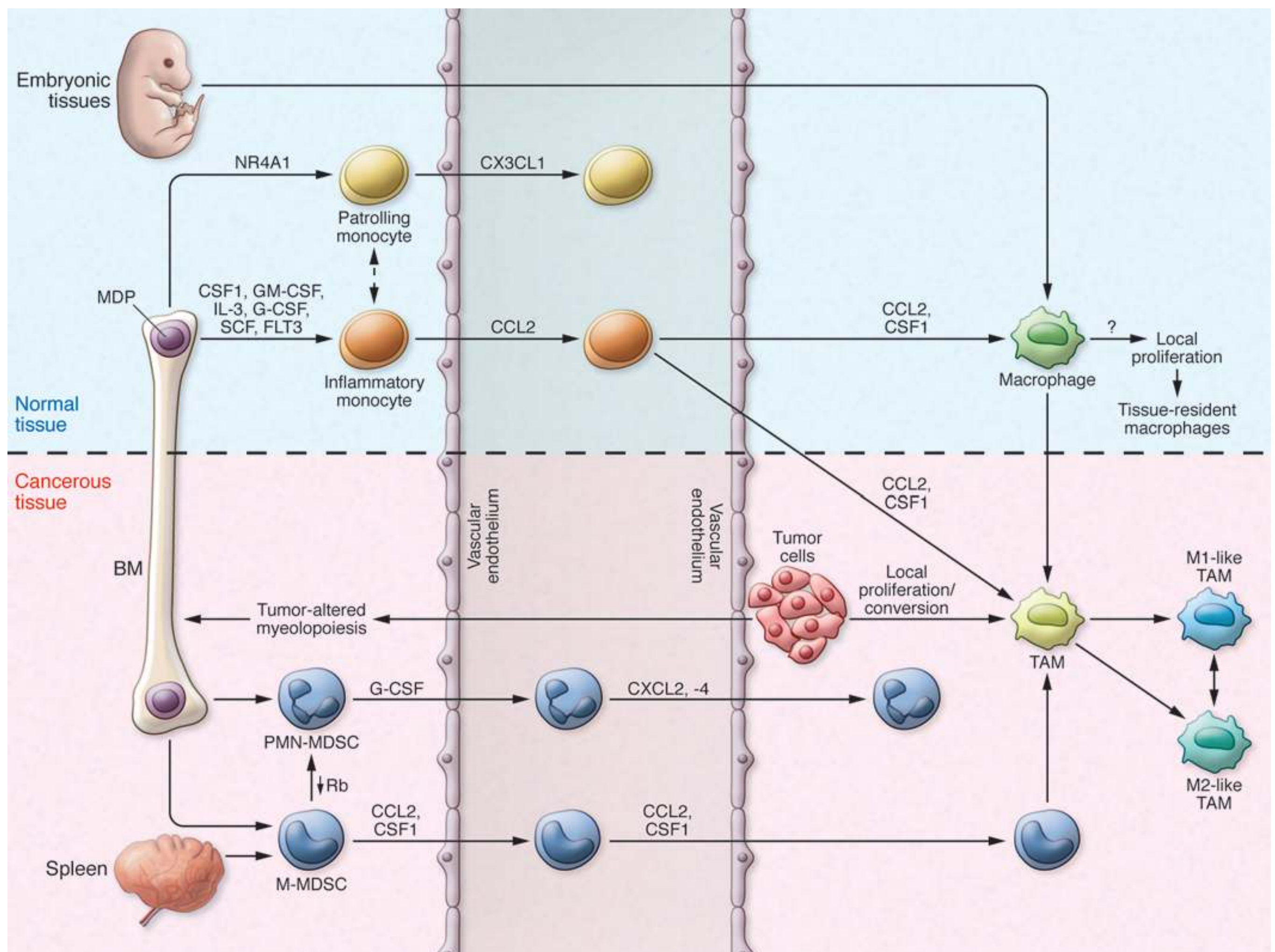

Figure 2. MDSC and TAM development in tumor-bearing mice. Under steady-state conditions, resident macrophages may originate from either embryonic tissues or inflammatory monocytes. Resident macrophages are programmed by local factors, and molecular switches support their differentiation. Circulating monocytes can be divided into two subsets: patrolling monocytes (Ly6C^{lo}CX3CR1^{hi}) and inflammatory monocytes (Ly6C^{hi}CD11b^{+}CD11c^{-}MHCII^{-}VCAM1^{-}CCR2^{+}), originating from macrophage and DC precursors (MDPs) in BM. Inflammatory monocytes migrate from blood to tissue under the guidance of CCL2/CCR2 chemokine signaling. Tumor cells secrete several factors that modify physiological myelopoiesis, promoting MDP differentiation into PMN-MDSCs (CD11b^{+}Ly6G^{+}) and MO-MDSCs (CD11b^{+}Ly6C^{hi}CCR2^{+}CD115^{+}F4/80^{lo}). MO-MDSCs also originate from the spleen under conditions of emergency and reactive myelopoiesis. MO-MDSCs and inflammatory monocytes migrate to tumor tissues via CCL2/CCR2 and CSF1 signaling and differentiate into TAMs (Ly6C^{-}CD11b$^{+/lo}$CD68^{+}CD1d^{+}MHCII$^{hi/lo}$F4/80^{+}VCAM1^{+}) in the presence of specific signals released by tumor cells within the local environment. However, the TAM phenotypic profile depends on cancer histology and stage, which might influence marker distribution. TAMs also proliferate locally, with different rates in various tumors. Furthermore, TAMs are inherently plastic, with an activation state falling along a continuum between the two extremes of M1- and M2-like phenotypes. Rb, retinoblastoma.

genetic analysis; for instance, the adoptive transfer of fully differentiated macrophages to alternate tissues demonstrated that the local environment is sufficient to reprogram both the macrophage chromatin landscape and gene expression, similar to what happens to less mature, BM-derived myeloid precursors (20).

The definition of TAM function that is based on a rigid dichotomy in which inducible NOS–positive (iNOS, also known as NOS2) macrophages (M1) are antitumoral and ARG1-positive macrophages (M2) are protumoral is no longer satisfactory and was recently revised (39). The M1 and M2 designations should only describe macrophages activated by either IFN-γ and LPS or IL-4 and IL-13, respectively, and M1 and M2 should be viewed as the extremes of a continuum that emphasize the extremes of macrophage plasticity. M1 and M2 extremes exhibit specific, characteristic expression of metabolic enzymes (iNOS vs. ARG1), cytokines (IL-12^{hi}IL-10^{lo} vs. IL-12^{lo}IL-10^{hi}), chemokines (CXCL9 and CXCL10 vs. CCL17 and CCL22), and marker genes (*Nos2*, *IL12b*, and *Ciita* vs. *Arg1*, *Retnla*, and *Chi3l3*), as well as transcription factors (NF-κB, STAT1, and IRF5 vs. STAT6, MYC, IRF4, KLF4, and PPARγ) (39). M1 macrophages are functionally proinflammatory and cytotoxic, whereas M2 macrophages act preferentially in antiinflammatory responses and tissue repair; however, when applied to TAMs, this classification is excessively simplistic and can generate misunderstandings and serious errors in data interpretation. For instance, mammary carcinoma–derived TAMs exhibit M2-related gene expression that is IL-4 independent and primarily orchestrated by

NOTCH signaling (22) or lactic acid–stabilized HIF-1α (36). M1-like TAMs are detectable in early-stage cancers as well as in regressing cancers and necrotic areas of growing tumors (40). Furthermore, monocytes isolated from the blood of patients with renal cell carcinoma (RCC) simultaneously express both tumor-suppressing genes, such as *TNF* and *IL1A*, and tumor-promoting genes, such as *VEGFA*, *MMP9*, and *HIF1A*, a mixed profile that was confirmed in macrophages of RCC specimens (41). Thus, TAM classification will require the integration of a multiparameter analysis of cell surface markers, exclusion of ambiguous identifications, and comparison of the TAM transcriptome with the gene profile of resident macrophages isolated from the same tissues (39).

Factors driving TAM and MDSC recruitment, expansion, and activation during tumor growth

In tumor-bearing hosts, MDSC and TAM generation requires the integration of at least two types of signals: factors that expand myeloid precursors, followed by factors that activate immune-regulatory programs. Myeloid cells are activated and localize to specific tumor areas with different kinetics during primary tumor formation. CSF1, granulocyte-CSF (G-CSF), and granulocyte-macrophage CSF (GM-CSF) are the three chief regulators of myeloid lineage proliferation and differentiation. G-CSF promotes the differentiation of myeloid precursors into PMN-MDSCs. Expansion of $Ly6G^+$ PMN-MDSCs occurs very early during tumorigenesis in the MMTV-PyMT mouse model, and these cells are detectable in the blood, spleen, and lungs of mice at the onset of oncogene-driven malignant conversion (42). In this model, tumor-released G-CSF stimulated reactive granulopoiesis at the expense of erythropoiesis by expanding hematopoietic stem cells and granulocyte/macrophage progenitors, but not common myeloid progenitors. This peculiar precursor signature in the BM is reproduced by either G-CSF or GM-CSF inoculation (31, 42) as well as by transplantable, GM-CSF–secreting tumors (31), suggesting a shared action of both cytokines on myeloid progenitors. G-CSF also mediates the lung infiltration of PMN-MDSCs, a step required for the formation of the premetastatic niche (43).

GM-CSF and IL-6 activate the immune-suppressive program in BM-derived progenitors by regulating the C/EBPβ transcription factor (28) and affect myeloid function during very early stages of pancreatic ductal adenocarcinoma (PDAC) progression. After initiation of the transforming program controlled by the active KRAS oncogene in mouse PDAC models, there are progressive waves of myelomonocytic cell recruitment, with $CD11b^+Gr1^+$ cells and TAMs being among the first to be accrued (44). Along with transformed epithelial cells, $CD11b^+Gr1^+$ cells contribute to the local release of IL-6 and IL-11, which activate protumoral STAT3 in cancer cells (45, 46). Moreover, KRAS-dependent release of GM-CSF primed $CD11b^+Gr1^+$ cells to suppress tumor-specific $CD8^+$ T cells and promoted progression to invasive PDAC; only the blockade of either GM-CSF production or $CD11b^+Gr1^+$ cell activity restored antitumor immunity (47). Other unknown factors might promote systemic $CD11b^+Gr1^+$ cell expansion in tumors driven by the viral SV40 oncogene, but GM-CSF was nonetheless required for the full in vivo maturation of $CD11b^+Gr1^+$ cell–suppressive activity (48). Further highlighting the role of GM-CSF, mesenchymal breast cancer cells activate TAMs by the combined activity of GM-CSF and lactate; in turn, TAMs release CCL18, which supports epithelial-to-mesenchymal transition (EMT) and metastasis formation (49).

The master factor for TAM recruitment and programming in the tumor microenvironment is CSF1. Genetic deletion of CSF1 either slowed tumor initiation or decreased disease progression and distal metastatic spread, both of which were associated with TAM loss or reduction (50, 51). Indeed, elevated CSF1 levels correlated with marked macrophage infiltration in human metastatic breast cancer (52). In addition to CSF1 and CCL2, several other TDFs attract circulating monocytes to the tumor site. For instance, chemokines, such as CCL5, CXCL12, and CX3CL1 (53) as well as growth factors and noncanonical chemotactic peptides, such as VEGF, TGF-β, bFGF, and the antimicrobial peptide β-defensin 3, are involved in monocyte recruitment and macrophage differentiation (54).

IL-4 and IL-13 participate in both TAM and MDSC survival and the acquisition of an immune-suppressive phenotype. They bind different receptors sharing the IL-4Rα chain that is responsible for recruiting and phosphorylating STAT6, which induces the transcription of genes involved in the immune-suppressive program, including *Arg1* (55). GM-CSF released by mouse and human gliomas upregulate IL-4Rα in MDSCs (56), which further fuel a positive loop for MDSC-mediated immune-suppressive activity by releasing IL-13 and IFN-γ, with the last cytokine maintaining IL-4Rα surface expression (57). Accordingly, IL-4R genetic depletion impaired MDSC-dependent immune suppression in vivo (57), and administration of aptamers targeting IL-4Rα triggered MDSC and TAM apoptosis and delayed tumor progression (58). Additionally, IL-4 in the tumor microenvironment (secreted by tumor cells or Th2-polarized infiltration T cells) (59, 60) induces local macrophages to produce WNT7β, thereby promoting tumor invasion (61).

Metabolic environmental signals can also modulate the intratumoral distribution of myeloid cells. Macrophages can survive in a hypoxic environment, but the high lactate levels produced via the Warburg effect can influence their spatial dissemination within specific areas of tumors as well as their dismissal (62). Hypoxia induces semaphorin 3A (SEMA3A), which interacts with a holoreceptor composed of neuropilin 1 (NRP1) and plexin A1/A4 to trigger VEGFR1 phosphorylation and macrophage recruitment (63). A TAM retention signal within hypoxic areas is delivered by SEMA3A through plexin A1/A4; conversely, NRP1 is downregulated in cancer, and its genetic inactivation in macrophages enhances TAM trapping within normoxic areas, resulting in the ablation of their immunosuppressive and proangiogenic activity (63). Partial correction of tumor hypoxia did not affect the relative distribution of TAM subsets or overall M2 marker expression, but rather downregulated the hypoxia-sensitive genes and proangiogenic activity of TAMs residing in the hypoxic areas (64).

Myeloid cells and cancer promotion

MDSC and TAM activity is not simply a buildup of an immune-suppressive environment that keeps T cells at bay and protects tumors from the effector arm of the immune system, but includes mechanisms that sustain and promote tumor growth and metastasis (Figure 3), as detailed below.

MDSC- and TAM-induced immune dysfunction. TAMs and MDSCs exert their immunosuppressive effects in an antigen-

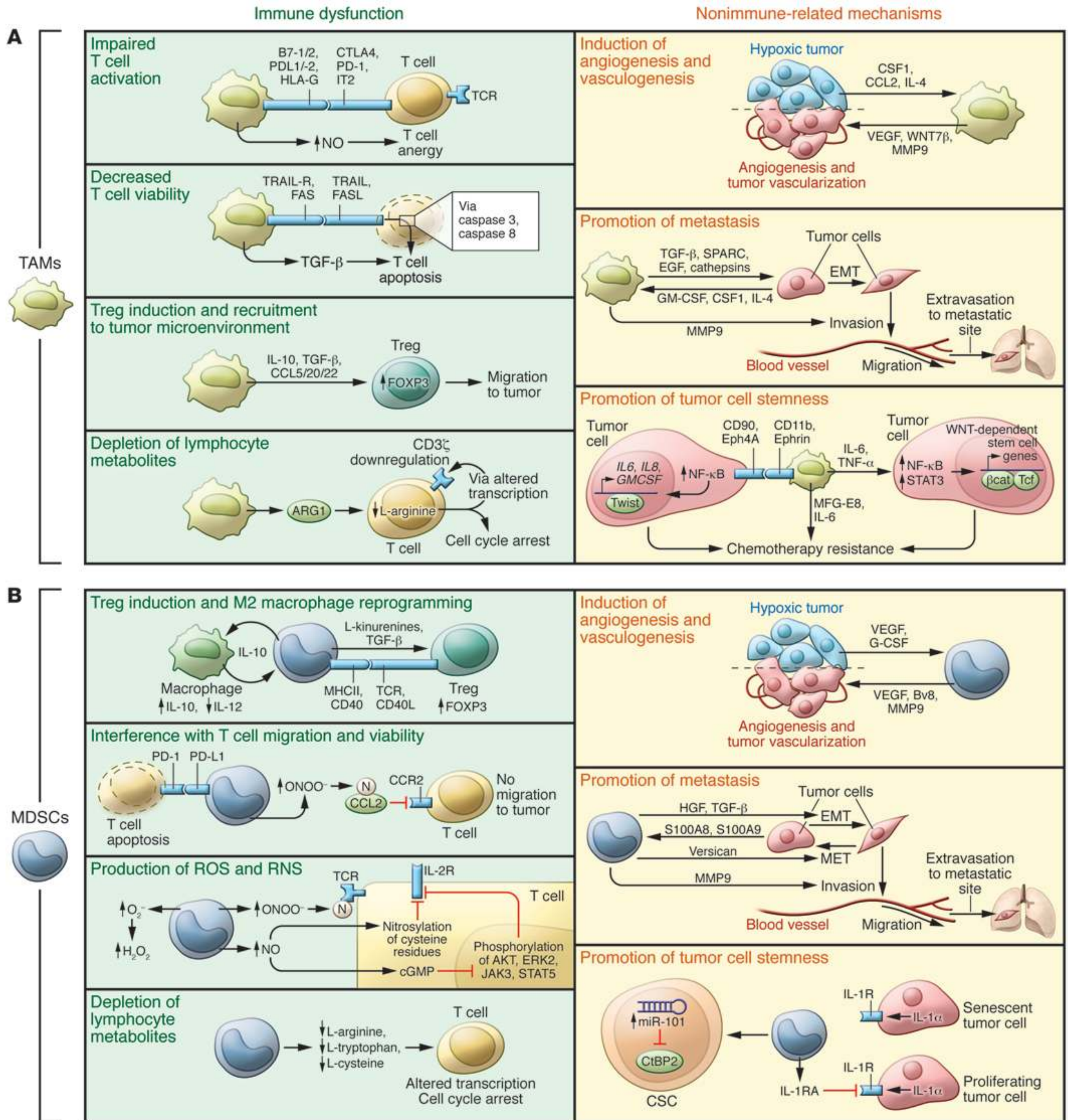

Figure 3. TAM- and MDSC-dependent mechanisms driving tumor progression. TAMs and MDSCs sustain tumor growth, progression, and dissemination by promoting immune dysfunction (green slices) but also by nonimmune-related mechanisms (yellow slices). (**A**) TAMs alter immune responses in tumor-bearing hosts by four main mechanisms: 1) inhibition of T cell activation; 2) inhibition of T cell viability; 3) promotion of Treg induction and recruitment; and 4) consumption of metabolites essential for T cell fitness. TAMs promote tumor angiogenesis and vasculogenesis by the release of VEGF and WNT7β, which favor the generation of new blood vessels and sustain metastasis. Finally, TAMs maintain the cancer cell reservoir by secreting IL-6 and TNF-α and produce MFG-E8 to protect CSCs from chemotherapy. (**B**) MDSCs inhibit the immune response in tumor-bearing mice by four processes: 1) MDSCs drive the differentiation of immune cells toward regulatory cells; 2) MDSCs interfere with T cell migration and viability; 3) MDSCs alter T cell fitness by turning on intracellular ARG1, NOS2, and NOX2 expression to produce NO, ROS, and RNS ($ONOO^-$, O_2^-, H_2O_2); and 4) MDSCs deplete essential metabolites for T lymphocyte fitness. MDSCs can also promote tumor angiogenesis and vasculogenesis via VEGF and MMP9 secretion. MDSCs produce elevated levels of TGF-β and HGF in primary tumors, inducing EMT, and secrete versican in the metastatic niche, promoting MET. Finally, MDSCs maintain tumor cell stemness by both IL-1RA production and by inducing the upregulation of miR-101 in cancer stem cells. cGMP, cyclic GMP; βcat, β-catenin; N, nitrosylated/nitrated; Tcf, HNF1 homeobox A.

specific and -nonspecific manner, deploying strategies that can be either direct or indirect, with the latter involving the generation or expansion of other regulatory cell populations, such as $CD4^+CD25^+$ Tregs (65).

Indirect strategies of immune suppression. The mechanisms for Treg expansion and conversion are not completely understood but involve cell-to-cell contact (including CD40 and CD40L interactions) and the production of soluble factors such as TGF-β, IFN-γ, and IL-10 (66–68). To sustain the immune-suppressive environment, TAMs and MDSCs secrete an array of chemokines acting on CCR5 and CCR6, which are involved in Treg recruitment (67–69). MDSCs also skew macrophages toward an M2 phenotype, characterized by impaired production of functional IL-12, through a cell contact–dependent mechanism (70). The downregulation of IL-12 is further exacerbated by the macrophages themselves, because TAMs stimulate an additional IL-10 release by MDSCs, thereby creating a self-perpetuating negative loop. Therefore, both MDSCs and TAMs can regulate the intratumoral IL-10/IL-12 balance, which is critical for priming T lymphocyte responses, as reviewed elsewhere (54, 71–73). Interestingly, IL-10 receptor blockade enhanced tumor responses to paclitaxel and carboplatin, enabling $CD103^+$ DCs to produce IL-12 and support antitumor $CD8^+$ T cells (74).

Direct immune suppression strategies. Direct immune-suppressive mechanisms rely on the activity of enzymes, chemokines, and receptors in myeloid cells. L-arginine and L-tryptophan consumption — which is dependent on the activity of ARG1 (73) and iNOS (75) or indoleamine 2,3-dioxygenase 1 (IDO1) and IDO2 (76), respectively — or L-cysteine deprivation (77) promotes T cell proliferation arrest and functional inhibition by downregulation of the CD3ζ chain in the T cell receptor (TCR) complex. The production of NO can inhibit T cell signaling downstream of IL-2R and induce T cell apoptosis by different mechanisms in an antigen-independent manner (78, 79). Another TAM/MDSC-related immune-suppressive mechanism is based on the production of ROS and reactive nitrogen species (RNS). ROS comprise superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) and are generated in high amounts by the activity of NADPH oxidase (NOX) family members, in which NOX2 is the key player (80). ROS affect T cell fitness by downregulating CD3ζ chain expression and reducing cytokine secretion, as observed in pancreatic cancer (81). RNS, such as peroxynitrite ($ONOO^-$), are byproducts of the combined activity of iNOS, ARG1, and NOX2 and can alter the formation of a correct peptide-MHC complex in MHCI molecules or induce modification of the immunodominant tumor-antigen peptides, thereby affecting TCR recognition and T cell activation (82). RNS can act on α and β chains of the TCR, promoting dissociation of the CD3ζ chain from the TCR complex and preventing TCR signaling (83). Last, RNS also modify trafficking of leukocytes that promote homing of immune-suppressive subsets (but not T cells) through aromatic amino acid nitration and nitrosylation of chemokines (CCL2, CCL5, CCL21, CXCL12) or chemokine receptors (CXCR4) (84, 85). Myeloid cells also promote immune dysfunction by expressing membrane surface ligands of T cell–inhibitory receptors, such as programmed death ligand 1/2 (PD-L1/2), which bind programmed death 1 (PD-1) (86–88) and B7-1/2, which bind to cytotoxic T lymphocyte antigen 4 (CTLA4) (89) and CD28 as well as FASL (90). Moreover, TAMs express nonclassical HLA-G and HLA-E molecules that can inhibit T cell activation upon their ligation to the inhibitory leukocyte Ig–like receptor LIT-2 (91).

MDSC- and TAM-dependent protumoral aid

Cancer stemness. MDSCs finely tune tumor senescence by promoting cellular stemness. At tumor onset in different autochthonous tumor models, neoplastic cells showed a senescent phenotype, a condition limiting tumor progression that was reversed by MDSCs (92). MDSC-secreted IL-1RA was the main molecular mediator of this reprogramming activity, and interference with MDSC trafficking to the tumor (i.e., by CXCL1/2 and CXCR2 targeting) enhanced chemotherapy-induced cellular senescence (92). In human ovarian carcinoma, MDSCs regulated senescence by inducing tumor cell expression of miR-101, which downregulated the stemness repressor C-terminal–binding protein 2 (CTBP2), ultimately triggering cancer stem cell (CSC) sphere formation and enhancing metastatic potential (93). Finally, in a mouse model of pancreatic cancer, MO-MDSCs directly induced expansion of aldehyde dehydrogenase 1^+ ($ALDH1A1^+$) pancreatic CSCs; a similar effect was observed with human $CD14^+HLA\text{-}DR^-$ MDSCs from patients with PDAC (94).

In pancreatic tumors, TAM depletion arrests the proliferation of tumor-initiating cells (95). Indeed, TAMs can sustain CSC proliferation by releasing proinflammatory cytokines such as TNF-α and IL-6, which reinforce tumor cell proliferation through NF-κB and STAT3 signaling pathways (96, 97). These same molecular pathways may be activated through a direct TAM-to-CSC contact via CD90 and ephrin A4 receptors (98). Finally, the crosstalk between CSCs and TAMs induced TAM secretion of milk fat globule EGF factor 8 (MFGE8) and IL-6, which favored CSC reservoir survival during chemotherapeutic treatment (99).

Angiogenesis. MDSCs and TAMs play a crucial role in promoting the angiogenic switch. During hypoxia adaptation, tumor cells, which sense O_2 levels through HIF prolyl hydroxylase 1–3 (PHD1–3) to control HIF-1α stability, release VEGF and thereby stimulate angiogenesis (100). Similarly, TAMs, in response to hypoxia, release mediators such as VEGF, bFGF, CXCL8/IL-8, and glycolytic enzymes (101, 102). Secreted VEGF also orchestrates peripheral expansion, trafficking, and functional commitment of MDSCs (103). In the tumor microenvironment, TAMs and MDSCs release proteases (cathepsin and MMP9), which support angiogenesis by freeing heparin-bound growth factors, such as VEGF-A, and by inducing extracellular matrix remodeling, which promotes invasion (51). Recruitment of MDSCs mediates resistance to anti-VEGF Ab–mediated therapy, as MDSCs can support new vessel growth, even in the presence of anti-VEGF Ab (104), by releasing the proangiogenic bombina variegata peptide 8 (105).

EMT–mesenchymal-to-epithelial transition and metastatic spreading. Myeloid cells play an active role in promoting the spread of distal tumor cells. In mammary tumors, TAMs promote metastatic diffusion via a paracrine loop involving CSF1 and EGF, which induces macrophages and tumor cells to cluster around blood vessels, where macrophages create a gate for tumor cell intravasation into the circulation, thus producing a tumor microenvironment for metastasis (TMEM) (106–108). The proinflammatory proteins S100A8 and S100A9, potent MDSC

Table 1. Synopsis of therapeutic interventions to limit monocyte and macrophage protumoral activity

Drug	Type of cancer	Effects on myeloid cells	References
5-Fluorouracyl	Thymoma (mouse)	MO-MDSC apoptosis	29, 141
Gemcitabine	Lung, breast, and sarcoma cancers (mouse)	MO-MDSC apoptosis	29, 142
Aptamers targeting CD124 (IL-4Rα)	Mammary cancer (mouse)	MO-MDSC and TAM depletion	58
Anti-CCL2 mAb	Mammary carcinoma, prostate cancer, other solid tumors (mouse and human)	MO-MDSC recruitment and angiogenesis alteration	111, 153, 154
CSF1R antagonist	Prostate tumor lung carcinoma, diffuse-type giant cell tumor, and tenosynovial giant cell tumors, glioma (mouse and human)	MO-MDSC expansion and TAM recruitment	140, 152, 156
Anti-CSF1R mAb (RG7155)	Mouse colon carcinoma, human diffuse-type giant cell tumor	Circulating monocyte subsets, tissue macrophage and TAM depletion	149
Lipid nanoparticles delivering CCR2-targeting siRNA	Thymoma and CRC (mouse)	Ly6C^{hi} inflammatory monocytes and TAM depletion	159
Bisphosphonates	Mammary tumor (mouse)	TAM depletion, inhibition of MDSC expansion	160, 161
Combined therapy with IL-12, IL-16, CpG DNA, and anti–IL-10R mAb	Lung and breast cancer (mouse)	TAM reprogramming[A]	150
CD40 agonist and gemcitabine	PDAC (mouse and human)	TAM reprogramming[A]	157
Anti-CD40 mAb with IL-2	RCC (mouse)	TAM reprogramming[A] in lung metastasis but not in primary tumor	151
Histidine-rich glycoprotein	Pancreatic and breast cancer, fibrosarcoma (mouse)	TAM reprogramming[A]	158
Trabectedin	Lung and ovarian carcinomas, soft tissue sarcoma (mouse and human)	MO-MDSCs and macrophage depletion	90

[A]In these studies, the indicated treatments did not affect TAM numbers; rather, TAMs were reprogrammed toward an antitumor, M1-like phenotype and function.

chemoattractants, have been implicated in tumor progression (109); S100A8/A9-induced serum amyloid A3 directly recruited MDSCs to premetastatic lungs, stimulated NF-κB signaling in a TLR4-dependent manner, and facilitated metastatic spreading (110). Moreover, MO-MDSCs and inflammatory monocytes are recruited through the CCL2/CCR2 axis to a metastatic environment in which they can differentiate into metastasis-associated macrophages (MAMs) (52, 111). Hypoxia in primary tumors can trigger MDSC-induced dysfunction in NK cells within the lung premetastatic niche, a defined site to which hematopoietic cells migrate before the tumor cells can seed the niche (112). PMN-MDSCs can also be armed by IL-17 released from γδ T cells infiltrating the primary breast cancers and assist lymph node and lung metastasis, in part through the inhibition of CD8$^+$ T cell function (113). MDSCs and TAMs also assist the metastatic process by inducing tumor cell EMT. MDSCs attracted by CXCL5 induced EMT in melanoma cells by releasing HGF and TGF-β at the primary tumor site; targeting of PMN-MDSCs in this model resulted in marked impairment of primary tumor growth (114). TAM recruitment induces EMT by both TGF-β release in a variety of solid tumors (115) and IL-8 in hepatocellular carcinoma (116). Additionally, a positive correlation was found between intratumoral macrophage densities, EMT markers, intraepithelial TGF-β levels, and tumor grade of non–small-cell lung cancer (NSCLC) patient samples (115). Because metastatic cells reacquire morphological and phenotypic traits of epithelial cells at the metastatic site, it is conceivable that premetastatic myeloid cells also control a mesenchymal-to-epithelial transition (MET) that promotes cancer cell colonization of and survival in the new organ, likely by releasing the proteoglycan versican (117).

Prognostic significance of myeloid cells in cancer patients

Three main myeloid classes with distinct lineage commitments have been identified in the blood of cancer patients: monocytic, granulocytic, and immature MDSCs. Each class contains more than one subset (118). Although the role of MDSCs has been acknowledged in primary tumor formation (119), extensive data connect MDSC expansion to more advanced cancer stages (120). MDSC numbers are associated with clinical stage in bladder carcinoma (121), pancreatic adenocarcinoma (122), hepatocellular carcinoma (123, 124), gastric cancer (125), NSCLC (126), and head and neck squamous cell carcinoma (127), as well as in hematological malignancies such as non-Hodgkin lymphoma (128). Collectively, these results indicate that expansion of MDSCs in cancer patients is a general phenomenon accompanying tumor progression. MDSC levels also correlated with response to therapy (126, 129, 130) or surgery (121); however, a deep analysis of clinical outcome in patients showed that MDSC frequency in blood is associated with prognosis, independent of tumor burden (131, 132). In patients with either stage IV breast cancer or stage IV colorectal cancer (CRC), a significant correlation was observed between high numbers of circulating MDSCs and poor prognosis. In fact, survival estimates for patients with high numbers of immature MDSCs (lineage$^-$HLA-DR$^{lo/-}$CD11b$^+$CD33$^+$) in the blood prior to starting standard chemotherapy were associated with shorter overall survival (OS) (133). Finally, high levels of MDSCs, cytokines, and chemokines (PDGF, IL-4, IL-8, IL-17, FGF-2, CCL5, and VEGF) in patients with PDAC are associated with progressive disease (134).

In recent years, immunotherapy has emerged as a therapeutic option for the treatment of cancer. IMA901 is a therapeutic vac-

cine for RCC that consists of HLA-A*02–restricted, tumor-derived peptides. In patients with advanced RCC, the levels of five of six MDSC subsets were expanded at baseline, and two of these subsets were prognostic for OS following IMA901 administration. These results indicate that MDSCs are potential biomarkers of response to the vaccine (135).

Immune checkpoint inhibitors represent a new drug category that is dramatically changing the treatment options for cancer (136). Lower MDSC frequencies correlated with prolonged OS in ipilimumab-treated patients (132, 137), whereas a decrease in MDSCs after treatment correlated with improved progression-free survival (PFS) in advanced melanoma patients receiving neoadjuvant ipilimumab (138). To date, it is not clear whether ipilimumab targets MDSCs or, conversely, whether the lower MDSC levels observed following ipilimumab treatment simply reflect tumor shrinkage in response to immune-mediated rejection.

While some studies demonstrated a correlation of extensive TAM infiltration with poor prognosis in breast, cervix, and bladder carcinomas, conflicting results were obtained in other solid tumors like prostate, NSCLC, and brain cancers (139). Along the same line, a recent meta-analysis of the literature showed inconsistent results (6), since elevated TAM numbers were associated with worse OS in patients with gastric, urogenital, or head and neck cancers, but with better prognosis in patients with CRC.

It appears that, while the expansion of MDSCs is often associated with poor prognosis, expansion of TAMs is not always a negative prognostic factor. When TAM evaluation is carried out at the molecular level, another layer of complexity appears. As discussed above, monocytes from patients with RCC have a distinct transcriptional profile, with upregulation of protumor and antitumor genes. The tumor-promoting function of RCC monocytes and TAMs required IL-1/IL-1R signaling, which also supported progression of RCC xenografts (41). These results are the first indication in human cancers that TAM induction is not mediated by the tumor microenvironment and suggest that patients' monocytes are already primed in the blood. Finally, CSF1R inhibition in a mouse model of proneural glioblastoma (GBM) increased survival by inducing regression of established tumors. Interestingly, a gene signature induced by CSF1R inhibition in murine TAMs was associated with increased survival in patients with proneural GBM (140).

Conclusions and future perspective

Targeting MO-MDSCs and TAMs can open new therapeutic opportunities to control tumor progression and block metastatic diffusion. The main strategies used thus far involve the inhibition of recruitment, depletion, or reprogramming of target cell populations. Some first-generation chemotherapeutic agents, such as 5-fluorouracil (141) and gemcitabine (29, 142), are able to control MO-MDSC accumulation, probably because these cells are more sensitive than tumor cells to low-dose chemotherapy (29). Low-dose irradiation also increases $CD8^+$ T cell trafficking and normalizes tumor vasculature in many cancer models by reprogramming TAMs toward a more inflammatory M1 type that releases NO (143). However, TAMs can either positively or negatively influence the antitumor activity of cytotoxic chemotherapy and radiotherapy (144), and targeting of immunosuppressive myeloid cells can have different effects on cancer progression (145, 146). Additionally, the microbiome can condition different myeloid cells, including TAMs, within murine tumors to contribute to the antitumor efficacy of both chemotherapy and immunotherapy (147, 148). Novel biologic drugs recognizing MDSC and TAM antigens or disrupting their function have been developed for selective targeting of these cell populations. As shown in Table 1, these compounds include Abs and/or aptamers (58, 111, 149–151) as well as molecular antagonists of essential receptors and/or molecular pathways (152). Among chemokines, targeting of CCL2 with a mAb (carlumab, CNTO 888) has proven to be beneficial in patients (153, 154); however, abrupt discontinuation of the therapy may result in a rebound effect causing increased metastatic disease (155). The inhibition of the CSF1/CSF1R axis with Abs (RG7155) or RTK inhibitors (imatinib mesylate) affects macrophage recruitment and differentiation and has shown encouraging results in clinical trials (149, 156). Considering the role of macrophages in regulating the tissue architecture and in mediating innate immune defense, there are concerns about side effects from the extended depletion of these cells. In this context, Abs activating immune stimulators (CD40), combinations of cytokines and Abs, or administration of histidine-rich glycoprotein appeared to modify macrophage polarization toward an antitumor phenotype, without affecting overall macrophage levels (150, 151, 157, 158).

Future investigations will need to focus on the mechanisms driving macrophage polarization toward either proimmune or protumoral phenotypes. Gene expression, proteomic, and metabolomic profiles are increasing our understanding of TAM and MDSC biology and offer potential therapeutic strategies for impeding tumor-induced immune dysfunctions. The identification of functional markers could guide the development of a new class of drugs targeting specific subsets of macrophages and MDSCs, thereby reducing the side effects of ablative therapy. In conclusion, while MDSC/TAM targeting will likely be insufficient to eradicate tumors, interference with patients' immune dysfunctions is a prerequisite and fundamental step for improving the efficacy of passive and active immunotherapeutic protocols.

Acknowledgments

This work was supported by grants from the Italian Ministry of Health; the Italian Ministry of Education, Universities and Research (FIRB cup: B31J11000420001); the Italian Association for Cancer Research (AIRC) (6599, 12182, 14103, and 12886); and the Fondazione Cassa di Risparmio di Verona, Vicenza, Belluno e Ancona.

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