

# We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

186,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index  
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?  
Contact [book.department@intechopen.com](mailto:book.department@intechopen.com)

Numbers displayed above are based on latest data collected.  
For more information visit [www.intechopen.com](http://www.intechopen.com)



## Functions of Diverse Myeloid Cells in the Tumor Micro-Environment

A. Sica<sup>1,2\*</sup>, C. Porta<sup>2</sup>, E. Riboldi<sup>2</sup>, M. Erreni<sup>1</sup> and P. Allavena<sup>1\*</sup>

<sup>1</sup>Dpt Immunology and Inflammation,

IRCCS Humanitas Clinical Institute, Rozzano, Milan,

<sup>2</sup>DiSCAFF, University of Piemonte Orientale A. Avogadro, Novara,  
Italy

### 1. Introduction

Myeloid cells are abundant in solid tumors and early infiltrate neoplastic lesions since the first stages of tumourigenesis, usually preceding other leukocytes (e.g. lymphocytes). (Clark et al., 2007) In the last decades there has been growing evidence that infiltrating T lymphocytes (CD3+ CD8+CD45RO+) are associated with favourable prognosis in human colorectal cancer (Laghi et al., 2009; Pages et al., 2005) melanoma, ovarian and breast cancer (Clemente et al., 1996; Mahmoud et al., 2011; Vesely et al., 2011; Zhang et al., 2003) In marked contrast, cells of the innate immunity, like myeloid cells, are most frequently associated with poor clinical outcomes. A number of studies have demonstrated that tumor-associated myeloid cells (TAMCs) have the ability to support tumor cell proliferation and invasion, activate the neo-angiogenic switch, and suppress anti-tumor immune responses. (DeNardo et al., 2009; Mantovani et al., 2004a; Martinez et al., 2009; Pollard, 2004; Qian and Pollard, 2010; Talmadge et al., 2007) Thus, in a simplified scheme, adaptive immunity is usually protective and limit tumour progression, while innate immunity favours disease development. However, research in recent years have added a further level of complexity, as components of the adaptive immunity (e.g. IL-4-producing CD4 T cells and antibody-producing B cells) have been shown to activate innate immune cells in a pro-tumour manner. (DeNardo et al., 2009; Wang and Joyce, 2010) Therefore, the dynamic interplay between tumor-infiltrating cells of the innate and adaptive immunity is of paramount importance for the outcome of tumour progression or regression.

Tumor-associated myeloid cells (TAMCs) include at least four different myeloid populations (**Figure 1**): 1) tumor-associated macrophages (TAMs), considered crucial orchestrators of cancer-related inflammation (Mantovani et al., 2008), promoting angiogenesis, immunosuppression, tissue remodelling and metastasis (Sica, 2010); 2) the angiogenic monocytes expressing the tunica internal endothelial kinase 2 (Tie2), the angiopoietin receptor, playing a key role in tumor angiogenesis (De Palma et al., 2005); 3) the Ly6G and Ly6C subsets of an heterogeneous population of immature myeloid cells, called myeloid-derived suppressor cells (MDSCs) for their ability to suppress T cells

---

\* Corresponding Authors

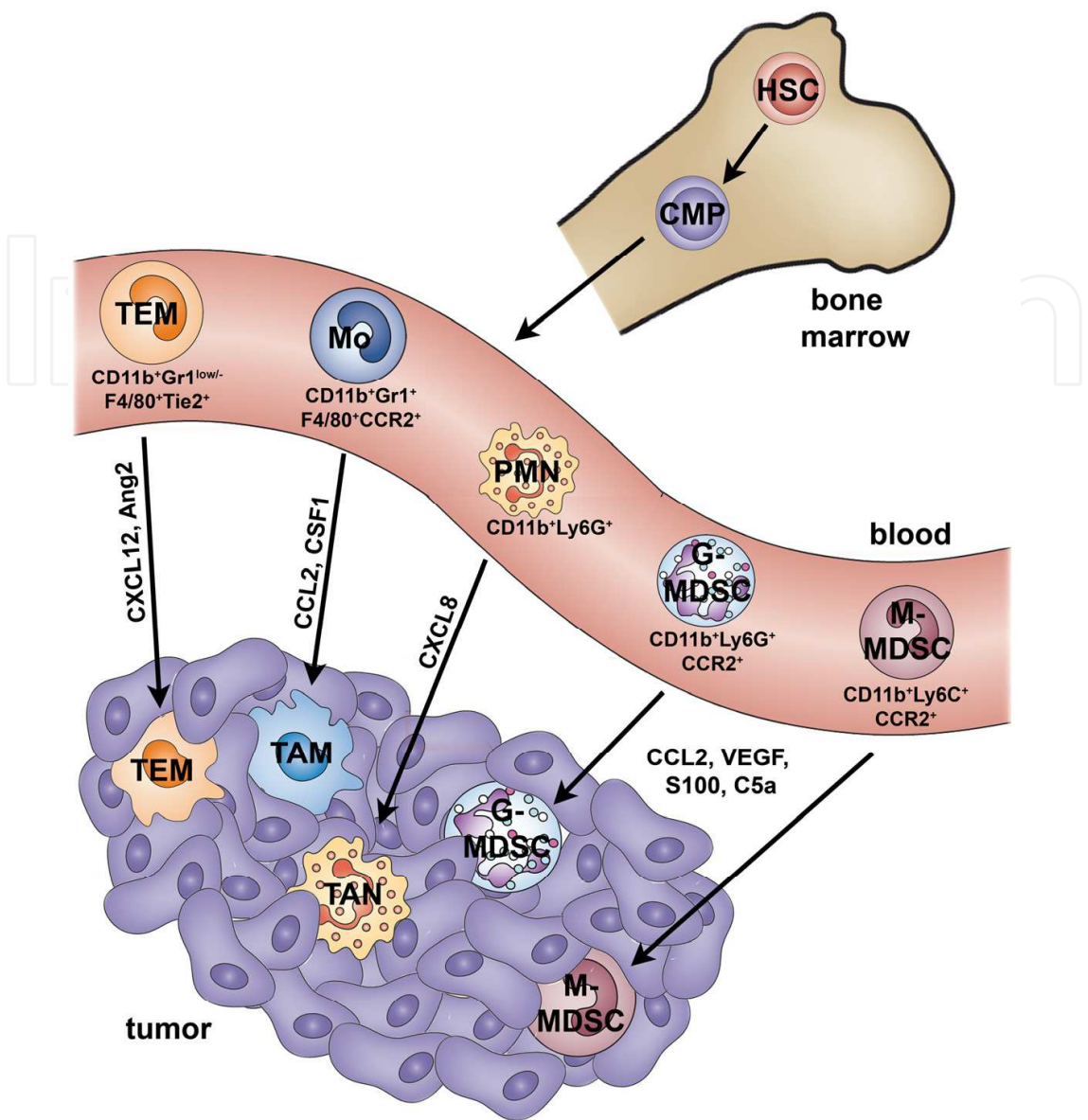


Fig. 1. **Pathways of differentiation and accumulation of TAMCs.** In the bone marrow hematopoietic stem cell (HSC) differentiate into common myeloid progenitors (CMPs), which can subsequently differentiate into different subsets of circulating myeloid cells: monocytes (Mo), Tie2-expressing monocytes (TEM), neutrophils (PMN), and granulocytic and monocytic myeloid-suppressor cells (G-MDSC and M-MDSC). Tumors secrete factors which sustain myelopoiesis, and promote both the recruitment and pro-tumor differentiation of circulating myeloid cells. TAMs are recruited into the tumor site by chemotactic factors (eg. CCL2, CSF-1) and represent the prominent phagocytes population orchestrating cancer-related inflammation. TEMs derive from circulating Tie2<sup>+</sup> monocytes and are recruited in tumors by hypoxia-inducible chemoattractants, such as Ang2 and CXCL12. Tumor-associated neutrophils (TANs) stem from circulating neutrophils and are recruited in tumors by chemokines (e.g. CXCL8). TANs participate in tumor promotion by the expression of crucial pro-angiogenic factors. During tumour progression an heterogeneous population of myeloid cells (G-MDSC and M-MDSC) accumulate in blood and lymphoid organs. MDSCs may be recruited by selected chemoattractants (CCL2, S-100, VEGF, C5a) into the tumor microenvironment, where they contribute to suppression of the adaptive immunity.

functions, which accumulate mainly in blood and lymphoid organs during tumor progression, but may also be recruited to the tumor site (Sica and Bronte, 2007); 4) tumor-associated neutrophils (TANs) that, despite their short half-life, have been recently proven to participate in tumor promotion by the expression of crucial pro-angiogenic factors (Fridlender et al., 2009).

TAMCs originate in the bone marrow where hematopoietic stem cells (HSCs) differentiate into common myeloid precursors (CMPs), which subsequently give rise to different subsets of circulating cells: immature myeloid cells (IMCs) that can be further subdivided in a granulocytic (CD11b<sup>+</sup>/Ly6G<sup>+</sup>) and a monocytic (CD11b<sup>+</sup>/Ly6C<sup>+</sup>) subpopulation, monocytes (CD11b<sup>+</sup>/Gr1<sup>+</sup>/F4/80<sup>+</sup>/CCR2<sup>+</sup>), Tie2-expressing monocytes (CD11b<sup>+</sup>/Gr1<sup>low</sup>/Tie2<sup>+</sup>) and neutrophils (CD11b<sup>+</sup>Ly6G<sup>+</sup>) (Mantovani et al., 2009). Tumors secrete factors which sustain myelopoiesis, promote the recruitment of circulating cells into the tumor mass, and orientate their functional differentiation to their own advantage (Mantovani et al., 2009; Sica and Bronte, 2007). In addition, Dendritic cells (DCs) also belong to the family of myeloid cells stemming from CMPs. Cells with dendritic characteristics are scarcely present in neoplastic tissues (Murdoch et al., 2008). Tumor-associated DCs generally show an immature phenotype and are poor inducers of effective responses to tumor antigens. The properties of these cells have been extensively reviewed elsewhere (Ma et al., 2011; Palucka et al., 2010) and are not discussed here.

## 2. Pro-tumour functions of tumor-associated myeloid cells

### 2.1 Tumor-associated macrophages

TAMs derive from circulating monocytes which are recruited at tumor sites by a number of diverse chemoattractants secreted by tumour and stromal cells. For instance the chemokine CCL2 was discovered as a tumour-derived factor inducing chemotaxis in monocytes (Bottazzi et al., 1983; Zachariae et al., 1990). Other chemokines these include : CCL3, CCL4, CCL5, CXCL12 (Balkwill, 2004; Konishi et al., 1996; Schioppa et al., 2003). Non-chemokine chemotactic factors are also important, for instance: urokinase plasminogen activator (uPa) (Zhang et al., 2011), M-CSF, TGFβ; fibroblast growth factor, FGF; vascular endothelial growth factor, VEGF) (Joyce and Pollard, 2009; Lin et al., 2002; Sica and Bronte, 2007) and antimicrobial peptides (β-defensin-3, BD-3) (Jin et al., 2010). Many of these molecules correlate with TAMs infiltration in different types of tumor, while other (eg. uPa, BD-3) are specifically associated with certain types of cancer, prostate and gastric cancer respectively (Jin et al., 2010; Zhang et al., 2011).

Once in tumours, monocytes differentiate to macrophages, primarily because of the presence of M-CSF produced by tumour cells, and polarize to tumour-educated macrophages by exposure to the local milieu rich in immune-suppressive mediators such as IL-10, TGFβ and VEGF.

Macrophages are versatile cells that are capable of displaying different functional activities, some of which are antagonistic: they can be immuno-stimulatory or immune suppressive, and either promote or restrain inflammation. (Auffray et al., 2009; Gordon and Taylor, 2005; Hamilton, 2008; Mantovani et al., 2004b; Martinez et al., 2009) Macrophage heterogeneity has been simplified in the macrophage polarization concept where the two extreme phenotypes, the M1 and M2 macrophages, have distinct features. (Allavena et al., 2008; Goerdt and

Orfanos, 1999; Gordon and Taylor, 2005; Mantovani et al., 1992; Pollard, 2009; Stein et al., 1992) M1 or classically-activated macrophages are stimulated by bacterial products and Th1 cytokines (e.g. IFN $\gamma$ ); they are potent effectors that produce inflammatory and immunostimulating cytokines to elicit the adaptive immune response, secrete reactive oxygen species (ROS) and nitrogen intermediates and may have cytotoxic activity to transformed cells. M2 or alternatively activated macrophages differentiate in micro-environments rich in Th2 cytokines (e.g. IL-4, IL-13); they have high scavenging activity, produce several growth factors that activate the process of tissue repair and suppress adaptive immune responses. (Gordon and Martinez, 2010; Mantovani et al., 2005; Qian and Pollard, 2010)

While this M1 vs M2 dual subsets simplification offers a mechanistic model of the functional polarization of macrophages, tissue microenvironments are likely to elicit simultaneous activation of different signalling pathways with opposite influence on macrophage functions, contributing to the extensive heterogeneity in patterns of gene expression seen in macrophages (Gratchev et al., 2008; Murray and Wynn, 2011; Ravasi et al., 2002; Riches, 1995; Stout et al., 2005; Tannenbaum et al., 1988). This *in vivo* functional skewing of myeloid populations is an emerging paradigm of tumor-mediated immunosuppression, where myeloid cell plasticity plays as a double-edged sword (Mantovani and Sica, 2010; Sica and Bronte, 2007). In early phases, high production of M1 inflammatory mediators (e.g. tumor necrosis factor, TNF; reactive oxygen species, ROS) appears to support neoplastic transformation (Sica and Bronte, 2007), whereas in established cancers the expression of M2-like phenotypes with immunosuppressive, pro-angiogenic and tissue remodelling activities promotes immune escape, tumor growth and malignancy (Dinapoli et al., 1996; Mantovani and Sica, 2010; Movahedi et al., 2010; Pollard, 2004; Saccani et al., 2006; Sica and Bronte, 2007; Sica et al., 2008; Sica et al., 2000).

In molecular profiling studies, murine TAMs from fibrosarcoma showed several features of M2 macrophages: arginase-I, YM1, FIZZ1, MGL2, VEGF, osteopontin and MMPs, as well as an immunosuppressive phenotype : high IL-10, TGF $\beta$  and low IL-12, RNI and MHC II, which correlate functionally to reduced cytotoxicity and antigen-presenting capacity. (Biswas et al., 2006; Hagemann et al., 2009; Ojalvo et al., 2010) Similar findings were found in human TAMs from ovarian cancer patients. (Allavena et al., 2010) We compared the expression of upregulated genes in human TAMs with the profiling of *in vitro*-polarized M1 and M2 macrophages. Several genes (e.g. osteopontin, fibronectin, scavenger and mannose receptors) were similarly upregulated in TAMs and in M2 macrophages. By the Principal Component Analysis, the global profiling of TAMs fell much closer to that of M2-polarized macrophages. (Solinas et al., 2010)

However, TAMs heterogeneity is starting to emerge, likely depending on the tumour type and micro-environmental cues. (Lewis and Pollard, 2006; Movahedi et al., 2010) Notably, murine TAMs showed also the expression of typical M1 factors such as IFN-inducible chemokines (CCL5, CXCL9, CXCL10, CXCL16). (Biswas et al., 2006; Stout and Suttles, 2005)

TAMs influence fundamental aspects of tumour biology, as shown in **figure 2**. Among the well documented pro-tumour functions of TAMs is the production of trophic and activating factors for tumour and stromal cells (e.g. EGF, FGF, VEGF, PDGF, TGF $\beta$ ). These growth factors directly promote the proliferation of tumour cells and increase the resistance to apoptotic stimuli (Ingman et al., 2006; Kalluri and Zeisberg, 2006; Mantovani et al., 2008; Moussai et al., 2011) The cytokine IL-6, released by TAMs, is important to sustain the



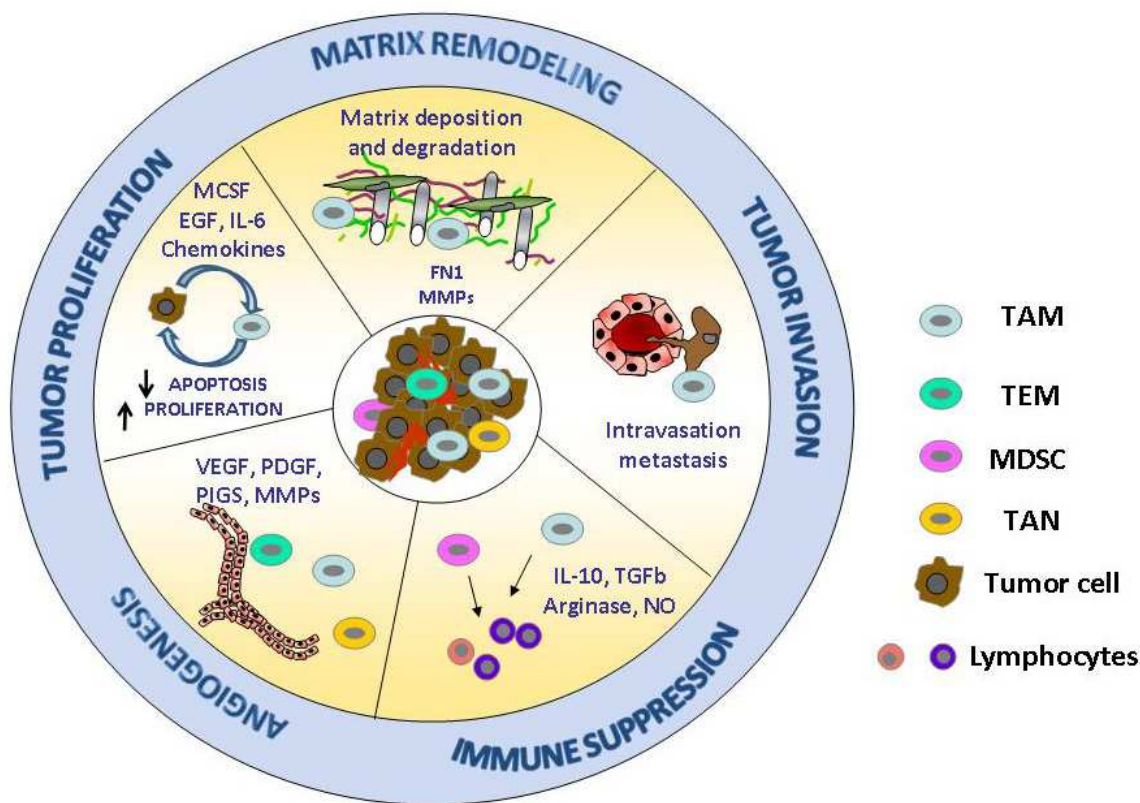


Fig. 2. **Pro-tumour functions of Tumour-Associated Myeloid Cells (TAMCs).** Different types of TAMCs promote the progression of tumors. TAMs rescue neoplastic cells from apoptotic stimuli and stimulate their proliferation, by producing several growth factors and cytokines (e.g. EGF, IL-6). TAMs, TEMs and TAN activate angiogenesis, via VEGF, MMPs and other angiogenic factors. TAMs have an intense proteolytic activity and degrade the extra-cellular matrix, but also produce matrix proteins, such as fibronectin (FN1). TAMs favour tumour cell intravasation and dissemination to distant sites. TAMs and MDSC induce immune suppression by producing suppressive mediators such as IL-10 and TGFb, arginase 1 and nitric oxide (NO).

survival and proliferation of malignant cells in tumours of epithelial and hematopoietic origin. (Bollrath et al., 2009; Fukuda et al., 2011; Grivennikov et al., 2009; Lesina et al., ; Ribatti and Vacca, 2009) TAMs are also a major source of proteolytic enzymes that degrade the ECM, thus favouring the release of matrix-bound growth factors. (Joyce and Pollard, 2009; Mantovani et al., 2008)

TAMs a key effectors of the "angiogenic switch" where the balance between pro- and anti-angiogenic factors, commonly present in tissues, tilts towards a pro-angiogenic outcome. (Baeriswyl and Christofori, 2009; Du et al., 2008; Murdoch et al., 2008; Zumsteg et al., 2009) In hypoxic conditions the transcription factor HIF-1alpha induces in TAMs the production of VEGF and of the angiogenic chemokine CXCL8. (Lewis et al., 2000)

TAMs are probably the most active contributors to the incessant matrix remodelling present within tumours, as they produce several MMPs and other proteolytic enzymes. (Mason and Joyce, 2011) Tumour cells exploit the ECM degradation mediated by TAMs to invade locally, penetrate into vessels and disseminate to give distant metastasis. (Wyckoff et al.,

2007) TAMs aiding cancer cell invasion have been directly visualized in experimental tumours in vivo by multiphoton microscopy: by using fluorescently labelled cells Wyckoff and colleagues showed that tumour cell intravasation occurs next to perivascular macrophages in mammary tumours. (Pollard, 2008; Wyckoff et al., 2007) Further, it has been recently shown that cathepsin protease activity, by IL-4-stimulated TAMs, promotes tumour invasion. (Gocheva et al., 2010) IL-4 is produced by tumour-infiltrating CD4 T cells and there is mounting evidence of its relevance in the polarization of macrophages with pro-tumour functions. (DeNardo et al., 2009; Wang and Joyce, 2010) The chemokine CCL18 produced by TAMs has been recently shown to play a critical role in promoting breast cancer invasiveness by activating tumour cell adherence to ECM. (Chen et al., 2011)

We recently found that human TAMs and in vitro tumour-conditioned macrophages express high levels of the Migration Stimulation Factor (MSF), (Solinas et al., 2010) a truncated isoform of Fibronectin. (Schor et al., 2003) Macrophage-secreted MSF displays potent chemotactic activity to tumour cells in vitro, (Solinas et al., 2010) confirming that the pro-invasive phenotype of cancer cells is modulated by macrophage products released in the tumour-micro-environment.

Further support to the concept of a reciprocal interaction between tumour cells and TAMs was provided by a recent paper where SNAIL-expressing keratinocytes became locally invasive after macrophage recruitment elicited by M-CSF. (Du et al., 2010)

In line with the above experimental evidence, high numbers of infiltrating TAMs have been significantly associated with advanced tumours and poor patient prognosis, in the majority of human tumours. (Bingle et al., 2002; Mantovani et al., 2008; Pollard, 2004; Qian and Pollard, 2010) There are, however, notable exceptions to this pro-tumour phenotype, probably dictated by their functional polarization. One such exception is human colorectal cancer, where some studies reported that TAMs density is associated with better prognosis. (Forssell et al., 2007; Ohno et al., 2003; Sconocchia et al., 2011) The localization of TAMs within colorectal cancers appears of primary importance: the number of peritumoural macrophages with high expression of costimulatory molecules (CD80 and CD86), but not of those within the cancer stroma, was associated with improved disease-free survival. (Ohtani et al., 1997; Sugita et al., 2002)

Specific TAMs subsets identified by surface markers may have predictive values: in lung adenocarcinoma, the number of TAMs CD204+ (scavenger receptor) showed a strong association with poor outcome while the total CD68+ population did not. (Ohtaki et al., 2010)

Macrophage-related gene signatures have been identified in human tumours such as ovarian and breast cancer, soft tissue sarcoma and follicular B lymphoma; (Beck et al., 2009; Finak et al., 2008; Ghassabeh et al., 2006; Lenz et al., 2008) in classic Hodgkin's lymphoma, tumours with increased number of CD68+ TAMs were significantly associated with shortened progression-free survival. (Steidl et al., 2010)

Recent addition to the molecular repertoire of TAMs includes semaphorin 4D (Sema4D) (Sierra et al., 2008) and growth arrest-specific 6 (Gas6) (Loges et al., 2010), which are respectively involved in promoting tumor angiogenesis and cancer cell proliferation.

## 2.2 Tie2-expressing monocytes/macrophages (TEMs)

Tie2-expressing monocytes/macrophages (TEMs) are a small subset of myeloid cells characterized by the expression of the angiopoietin receptor Tie2 and powerful pro-angiogenic activity (De Palma and Naldini, 2009; De Palma et al., 2005; Murdoch et al., 2007; Venneri et al., 2007). They derive from circulating Tie2-expressing monocytes which are recruited in tumors by hypoxia-induced endothelial-derived chemotactic factors, such as Ang-2 and CXCL12 (Coffelt et al., 2011; Murdoch et al., 2007; Venneri et al., 2007; Welford et al., 2011b). The CXCL12-CXCR4 axis is a well known circuit driving accumulation of TAMs in hypoxic areas of solid tumors (Schioppa et al., 2003). In addition, it has been demonstrated that pharmacological inhibition of CXCR4 is associated with a significant reduction of TEM recruitment into mammary tumors (Welford et al., 2011b). Both ablation and adoptive transfer studies have demonstrated that TEMs are crucial promoters of tumor angiogenesis (De Palma et al., 2005; De Palma et al., 2003; Venneri et al., 2007). In two models of mammary tumours and orthotopic human gliomas, Ganciclovir-driven ablation of Tie2<sup>+</sup> monocytes induced a significant reduction of both tumour mass and vasculature, demonstrating their importance in tumour angiogenesis and growth (De Palma et al., 2005; De Palma et al., 2003; Venneri et al., 2007). In line, adoptive transfer studies demonstrated that subcutaneous co-injection of tumor cells with TEMs increases tumor vascularization (De Palma et al., 2005).

Strikingly, gene expression analysis highlighted that TEMs are highly related to TAMs, but express a more pronounced M2-skewed gene signature, with higher expression of M2 genes, including arginase 1 (Arg1), scavenger receptors (CD163; Mannose receptor 1, Mrc1; Macrophage scavenger receptor 2, Msr2; stabilin-1) and lower levels of pro-inflammatory molecules (IL-1 $\beta$ ; prostaglandin endoperoxide synthase 2/cyclooxygenase 2, PTGS2/COX2; IL-12; TNF; inducible nitric oxide synthase, iNOS; CCL5; CXCL10; CXCL11) (Pucci et al., 2009). These results suggested that Tie2<sup>+</sup> monocytes could be a distinct lineage of myeloid cells, committed to execute physiologic pro-angiogenic and tissue-remodeling programs, which can be co-opted by tumors (Andreu et al., 2010). Noteworthy, human Tie2<sup>+</sup> circulating monocytes express high levels of pro-angiogenic genes (e.g. VEGF-A; Matrix metalloproteinase 9, MMP9; COX2; wingless-related MMTV integration site 5A, WNT5A) and are powerful inducers of endothelial cells activation (Coffelt et al., 2010). In agreement, sub-cutaneous tumors growing in Ang-2-overexpressing mice showed increased number of TEMs associated with enhanced microvessels density (Coffelt et al., 2010). Tie2 engagement by Ang-2 in both mouse and human TEMs not only elicits a chemotactic response but also enhances their pro-tumoral activities (Coffelt et al., 2010). It was also recently demonstrated that Ang-2 levels in 4T1 mammary tumors correlates with both TEM-derived IL-10 and Treg infiltration, resulting in suppression of T cells proliferation (Coffelt et al., 2011). In contrast, Ang-2 inhibited the expression of M1 cytokines (IL-12 and TNF $\alpha$ ) in TEMs exposed to hypoxia (Murdoch et al., 2007).

## 2.3 Myeloid-Derived Suppressor Cells (MDSCs)

MDSCs represent an heterogeneous population of cells whose common characteristics are an immature state and the ability to suppress T-cell responses both *in vitro* and *in vivo* (Gabrilovich and Nagaraj, 2009; Ostrand-Rosenberg and Sinha, 2009).



MDSC recruitment and expansion are regulated by several cytokines, chemokines and transcription factors (Sica and Bronte, 2007). It has been demonstrated that among chemokine receptors, CCR2 plays a pivotal role in the recruitment and turnover of MDSC to the tumour site (Sawanobori et al., 2008). Furthermore, the C5a complement component, which interacts with a G protein-coupled receptor, has been shown to play a role in MDSC recruitment and activation in a cervix cancer model (Markiewski et al., 2008). Some factors which are found in the tumour microenvironment, such as pro-inflammatory S-100 proteins, are also crucial for MDSC recruitment. Sinha and co-workers demonstrated that MDSCs can produce S-100 proteins by themselves, providing evidence for an autocrine loop that promotes MDSC recruitment (Cheng et al., 2008; Sinha et al., 2008).

MDSCs possess several mechanisms for immune suppression: 1) depletion of arginine, mediated by Arg1 and iNOS; 2) production of ROS; 3) post-translational modifications of T cell receptor (TCR) mediated by peroxynitrite generation; 4) depletion of cysteine; 5) production of TGF $\beta$ ; 6) induction of Tregs (Bronte et al., 2005; Huang et al., 2006; Movahedi et al., 2008; Nagaraj et al., 2007; Srivastava et al., 2010; Terabe et al., 2003; Yang et al., 2006; Youn et al., 2008). In healthy individuals, IMCs differentiate in mature granulocytes, macrophages or dendritic cells, whereas in pathological conditions they expand into MDSCs. MDSCs have been observed in cancer, chronic infectious diseases, and autoimmunity. In tumor-bearing mice, MDSCs accumulate within primary and metastatic tumors, in the bone marrow, spleen and peripheral blood. In cancer patients, MDSCs have been identified in the blood.

Recent studies have contributed to partially clarify the biology of MDSCs. In mice, two major subsets were identified on the basis of their morphology and the expression of Ly6 family glycoproteins: monocytic MDSCs (M-MDSCs) and granulocytic MDSCs (G-MDSCs). M-MDSCs are CD11b<sup>+</sup> Ly6G<sup>-</sup> Ly6C<sup>high</sup> cells with monocyte-like morphology, while G-MDSCs are CD11b<sup>+</sup> Ly6G<sup>+</sup> Ly6C<sup>low</sup> with granulocyte-like morphology (Ostrand-Rosenberg and Sinha, 2009). Cells with similar phenotype, precursors of myeloid cells, are present in physiological conditions, but they are devoid of immunosuppressive activity. These cells, therefore, should not be named MDSCs (Youn and Gabrilovich, 2010). Other markers of MDSC subsets are: IL-4R $\alpha$  (CD124), F4/80, CD80, and CSF-1R (CD115) (Sica and Bronte, 2007). The characterization of MDSCs deeply suffers from the lack of specific markers. However, recent characterizations have identified human MDSCs as CD34<sup>+</sup> CD33<sup>+</sup> CD11b<sup>+</sup> HLA-DR<sup>-</sup> cells (Ostrand-Rosenberg and Sinha, 2009). The ability to differentiate into mature DCs and macrophages *in vitro* has been shown to be restricted to M-MDSCs (Youn et al., 2008). M-MDSC-mediated immune suppression does not require cell-cell contact, but utilizes up-regulation of iNOS and Arg1, as well as production of immunosuppressive cytokines (Gabrilovich and Nagaraj, 2009). On the contrary, G-MDSCs suppress antigen-specific responses using mechanisms, including the release of ROS, that require prolonged cell-cell contact between MDSC and T cell (Gabrilovich and Nagaraj, 2009). The C5a subunit of the complement system appears a key regulator of MDSC functions, by modulating their migration and ROS production (Markiewski et al., 2008).

Several factors produced by tumors have been implicated in the differentiation of MDSCs, including granulocyte monocytes-colony stimulating factor (GM-CSF), macrophage-monocytes-colony stimulating factor (M-CSF), IL-6, IL-1 $\beta$ , VEGF and PGE2 (Gabrilovich and Nagaraj, 2009; Marigo et al., 2010). The transcription factor CCAT/enhancer binding protein

$\beta$  (C/EBP $\beta$ ) proved to be the key player in the process of MDSC development (Marigo et al., 2010). It has been proposed that two signals are needed for the expansion and function of MDSCs: one factor (e.g. GM-CSF) prevents the differentiation in mature myeloid cells, and a second signal, provided by pro-inflammatory molecules such as IFN $\gamma$ , activate MDSCs (Condamine and Gabrilovich, 2011).

A remarkable relation exists between MDSCs and TAMs. MDSCs are able to skew TAMs differentiation toward a tumor-promoting type-2 phenotype (Sinha et al., 2007). The cross-talk between MDSCs and macrophages requires cell-cell contact, then MDSCs release IL-10 to reduce IL-12 production by macrophages. MDSCs from an IL-1 $\beta$  enriched tumor microenvironment produce more IL-10 and are more potent down-regulators of macrophage-released IL-12 (Bunt et al., 2009). Circulating MDSCs can differentiate into Gr1<sup>+</sup>F4/80<sup>+</sup> TAMs in the tumor site (Kusmartsev and Gabrilovich, 2005) and this conversion is driven by tumor hypoxia (Corzo et al., 2010).

Because of their tumor-promoting activities, MDSCs are associated with type-2 immune responses, however accumulating evidence shows that MDSCs have characteristics of both M1 and M2 macrophages (Sica and Bronte, 2007). As an example, MDSCs express both Arg1 and iNOS, where these enzyme are differentially expressed by M1 (iNOS) and M2 (Arg1) macrophages. A recent study, investigating the molecular mechanisms behind MDSC differentiation, demonstrated an essential role of paired-immunoglobulin receptors (PIRs) in the differentiation of M1 or M2 MDSCs (Ma et al., 2011). The balance between PIR-A and PIR-B modulates MDSC polarization. In support of this, growth of Lewis lung carcinoma was significantly retarded in PIR-B-deficient mice (*Lilrb3*<sup>-/-</sup>) and PIR-B-deficient M-MDSCs expressed high levels of the M1 molecules iNOS.

MDSCs contribute to tumor growth also by non-immune mechanisms, including the promotion of angiogenesis. MDSCs isolated from murine tumors express high levels of metalloproteases, including MMP9 (Murdoch et al., 2008). MMP9 increases the bioavailability of VEGF sequestered in the extracellular matrix. Further in the tumor microenvironment and in proangiogenic culture conditions, MDSCs acquire endothelial markers such as CD31 and VEGF receptor 2 (VEGFR2) and the ability to directly incorporate into tumor endothelium (Yang et al., 2004). In agreement, tumor refractoriness to anti-VEGF therapy was shown to be mediated by CD11b<sup>+</sup>Gr1<sup>+</sup> myeloid cells (Shojaei et al., 2007a; Shojaei et al., 2007b).

## 2.4 Tumor-Associated Neutrophils (TANs)

Tumor-associated neutrophils (TANs) have received little interest by immunologists, also based on their short life span. However, new evidence contradicts this view, in that cytokines like IL-1 or microenvironment conditions such as hypoxia can prolong PMN survival (Sica et al., 2011). TANs are present in various tumors, including kidney, breast, colon, and lung (Houghton, 2010), and are recruited by locally secreted chemotactic factors. As an example, several carcinoma cells produce CXCL8, a prototypic chemoattractant for neutrophils (Bellocq et al., 1998). Furthermore, tumor-derived TGF $\beta$  promotes neutrophils migration both directly and indirectly, by regulating the expression of adhesion molecules in the endothelium (Flavell et al., 2010).

Neutrophils are able to produce various cytokines and chemokines that can influence not only immune and antimicrobial responses, but other processes such as hematopoiesis, wound healing, and angiogenesis (Cassatella et al., 2009; Mantovani, 2009; Piccard et al., 2011; Zhang et al., 2009). Despite little attention has been paid to TANs, clinical evidence indicates that their presence is a negative prognostic indicator. A correlation between TANs infiltrate and poor outcome has been described in renal cell carcinoma, bronchoalveolar cell carcinoma, and breast cancer (Jensen et al., 2009; Yang et al., 2005). In agreement, preclinical studies experimenting PMN depletion confirmed the detrimental nature of TANs (Pekarek et al., 1995; Tazawa et al., 2003).

Neutrophils contribute to tumor growth by promoting angiogenesis, cell proliferation, and metastasis (Houghton, 2010). Similarly to macrophages, a recent report described the functional plasticity of neutrophils (Fridlender et al., 2009). The authors investigated the effects of SM16, a TGF $\beta$  receptor kinase antagonist in murine lung cancer and mesothelioma models using syngeneic tumor xenografts and the orthotopic LSL-K-ras tumor model. Depletion of neutrophils by a specific anti-Ly6G antibody resulted in a significantly reduced effect of SM16, suggesting that neutrophils participate to the antitumor activity of TGF $\beta$  blockade, most likely by the production of oxygen radicals. Also, depletion of neutrophils affected the activation of CD8<sup>+</sup> CTLs. Fridlender and colleagues propose a new paradigm in which resident TANs acquire a protumor phenotype, largely driven by TGF $\beta$ , to become “N2 neutrophils”. If TGF $\beta$  is blocked, neutrophils acquire an antitumor phenotype to become “N1 neutrophils” (Fridlender et al., 2009).

It was suggested that N1- and N2-type neutrophils are cells with a different degree of activation (i.e. fully activated or weakly activated neutrophils, respectively) rather than two alternatively activated cell subtypes (Gregory and Houghton, 2011). It is also object of debate the existence of two distinct populations, namely N2-polarized TANs and granulocytic MDSCs, that seem to overlap for many characteristics. In the absence of specific markers, it cannot be determined if N2 neutrophils within the tumors are granulocytic MDSCs recruited from the spleen or whether they are blood-derived neutrophils converted to an N2 phenotype by the tumor microenvironment. In support to the existence of N2-polarized TANs, Fridlender et al. emphasize that TGF $\beta$ -blockade does not alter blood neutrophils, splenic myeloid cells (CD11b<sup>+</sup>), or splenic MDSCs, selectively acting on the intratumor activation of neutrophils. Also, TANs characterized in Fridlender’s study have clear features of mature neutrophils, while MDSCs mostly exhibit an immature morphology (Mantovani, 2009).

### 3. Therapeutic approaches targeting TAMCs

The frequent association of TAMCs with poor prognosis makes these cells reasonable targets of biological anti-cancer therapies. Further, in the last few years there has been increasing evidence that TAMCs are strongly implicated in the failure of conventional chemotherapy and anti-angiogenic therapy. (Ferrara, 2010; Welford et al., 2011a) Accumulation of myeloid CD11b<sup>+</sup>Gr1<sup>+</sup> cells (including TAMs, MDSC and immature cells) in tumours renders them refractory to angiogenic blockade by VEGF antibodies. (Shojaei and Ferrara, 2008) This effect was traced to a VEGF-independent pathway driven by the G-CSF-induced protein Bv8. (Shojaei et al., 2007b) Further, pharmacological inhibition of TEMs in tumour-bearing mice markedly increased the efficacy of therapeutic treatment with a vascular-disrupting agent.

### 3.1 TAMs and TEMs

Elimination of TAMs at tumor sites, or inhibition of their survival could result in improved prognosis. Earlier and more recent studies of macrophage depletion in experimental settings have been successful to limit tumour growth and metastatic spread (Aharinejad et al., 2009; Lin et al., 2001; Mantovani et al., 1992), and to achieve better therapeutic responses (De Palma et al., 2007; Ferrara, 2010; Gabrilovich and Nagaraj, 2009; Marigo et al., 2008; Welford et al., 2011a)

A number of studies have shown that the bisphosphonate clodronate encapsulated in liposomes is an efficient reagent for the depletion of macrophages in vivo. Clodronate-depletion of TAMs in tumour-bearing mice resulted in reduced angiogenesis and decreased tumour growth and metastatization.(Brown and Holen, 2009; Zeisberger et al., 2006) Moreover, the combination of clodronate with sorafenib, an available inhibitor of tyrosine protein kinases (e.g,VEGFR and PDGFR), significantly increased the efficacy of sorafenib alone in a xenograft model of hepatocellular carcinoma. In clinical practice, bisphosphonates are employed to treat osteoporosis; current applications in cancer therapy include their use to treat skeletal metastases in Multiple Myeloma, prostate and breast cancer. Treatment with zoledronic acid was associated with a significant reduction of skeletal-related events and, possibly, direct apoptotic effects in tumour cells. (Martin et al., 2010; Morgan et al., ; Zhang et al., 2010)

Our group reported that the anti-tumour agent of marine origin, Trabectedin (Yondelis), was unexpectedly found to be highly cytotoxic to mononuclear phagocytes, including TAMs. This cytotoxic effect is remarkably selective, as neutrophils and lymphocytes were not affected. (Allavena et al., 2005; D'Incalci and Galmarini, 2010)

A second approach is to inhibit the recruitment of circulating monocytes in tumour tissues.

The M-CSF receptor (M-CSFR) is exclusively expressed by monocytes-macrophages. In patients with advanced tumours, clinical studies are under way to check the feasibility and possibly clinical efficacy of inhibitors to the CSF-1R. Among the many chemokines expressed in the tumour micro-environment, CCL2 (or Monocyte Chemotactic Protein-1) occupies a prominent role and has been selected for therapeutic purposes. Pre-clinical studies have shown that anti-CCL2 antibodies or antagonists to its receptor CCR2, given in combination with chemotherapy, were able to induce tumour regression and yielded to improved survival in prostate mouse cancer models (Li et al., 2009; Loberg et al., 2007; Popivanova et al., 2009)

In the opposite direction, another approach is to exploit the tumor-homing ability of TAMCs: after all, they are at the right place at the right time. Indeed, delivery of cytokines and cytotoxic proteins to tumors by means of gene modified cells represents a promising strategy to treat cancer. It was recently shown that TEMs could be used to deliver interferon-alpha (IFN $\alpha$ ), a potent cytokine with angiostatic and antiproliferative activity (De Palma et al., 2008), thanks to the preferential homing of TEMs to the tumors (De Palma and Naldini, 2009).

A fourth and more recent approach is to 're-educate' TAMs to exert anti-tumour responses protective for the host, ideally by using factors able to revert TAMs into M1-macrophages, with potential anti-tumour activity. It is becoming accepted that macrophages are flexible and able to switch from one polarization state to the other. (Pelegrin and Surprenant, 2009)



This was achieved in experimental mouse tumours, by injecting the TLR9 agonist CpG-oligodeoxynucleotide (CpG-ODN), coupled with anti-IL-10 receptor.(Guiducci et al., 2005) or the chemokine CCL16 (Cappello et al., 2004). CpG-ODN synergized also with an agonist anti-CD40 mAb to revert TAMs displaying anti-tumour activity. (Buhtoiarov et al., 2011) A remarkable anti-tumour effect of re-directed macrophages has been recently reported in human pancreatic cancer with the use of agonist anti-CD40 mAb. (Beatty et al., 2011) Still in the same direction, a recent report showed that the plasma protein histidine-rich glycoprotein (HRG) known for its inhibitory effects on angiogenesis (Juarez et al., 2002; Olsson et al., 2004) is able to skew TAMs polarization into M1-like phenotype by down-regulation of the placental growth factor (PlGF), a member of the VEGF family. In mice, HRG promoted anti-tumour immune responses and normalization of the vessel network. (Rolny et al., 2011)

Direct activation with IFN $\gamma$ , a prototypical M1-polarizing cytokine, has been shown to re-educate TAMs (Duluc et al., 2009) and there is evidence for antitumor activity of this molecule in minimal residual disease (Mantovani and Sica, 2010). Inhibition of STAT3 activity, required for IL-10 biological functions and gene transcription, restored production of pro-inflammatory mediators (IL-12 and TNF- $\alpha$ ) by infiltrating leukocytes and promoted tumour inhibition (Kortylewski et al., 2005). Recent results suggest that SHIP1 functions in vivo to repress M2 macrophage skewing. Consistent with this, Ship1-/- mice display enhanced tumor implant growth (Rauh et al., 2005). In agreement, inhibition of the M2 polarizing p50 NF- $\kappa$ B activity resulted in restoration of M1 inflammation and tumor inhibition in different cancer mouse models (fibrosarcoma, melanoma)(Saccani A. et al Cancer Res 2006) (Porta et al., 2009)

### 3.2 MDSC

The translational potential of MDSC research is dual. The immunosuppressive activity of MDSCs could be exploited to inhibit immune responses in autoimmune diseases and organ transplantation. Conversely, elimination of MDSCs could be essential in cancer patients undergoing active (vaccination) or passive (adoptive transfer of *ex-vivo* expanded anti-tumor T cells) immunotherapy. A possible approach to contrast MDSC pro-tumoral activities consists in the promotion of MDSC differentiation into mature cells devoid of suppressive activity. Vitamin A represents an interesting candidate to restore immunosurveillance. In fact, Vitamin A metabolites stimulate the differentiation of myeloid progenitor cells into DCs and macrophages and reduce MDSC accumulation (Gabrilovich et al., 2001; Kusmartsev et al., 2003). A clinical trial testing the effects of all-*trans*-retinoic acid (ATRA) in patients with metastatic renal cell carcinoma showed the efficacy of this compound in reducing MDSCs in peripheral blood. The decrease in MDSC number correlated with improved-antigen-specific T cell responses (Mirza et al., 2006). It has been reported that some chemotherapeutic drugs, such as gemcitabine, are able to eliminate MDSCs, without affecting T cells, B cells, NK cells, and macrophages (Ko et al., 2007; Suzuki et al., 2005). Another strategy is aimed to inhibit MDSC suppressive function. Compounds under investigation for this ability belong to COX2 inhibitors, phosphodiesterase 5 (PDE5) inhibitors, and NO-releasing non-steroidal anti-inflammatory drugs (NSAIDs) (Gabrilovich and Nagaraj, 2009). Preclinical evidence supports the use of IL-1 antagonists in treating

human metastatic disease. Blocking IL-1 activity, mainly IL-1 $\beta$ , reduces both metastasis and tumor growth (Dinarello, 2010). Recently, it was shown that the effect is also mediated by the decrease of MDSC accumulation and suppressive activity (Ostrand-Rosenberg and Sinha, 2009). It has also been reported that CD11b<sup>+</sup> Gr1<sup>+</sup> cells enhance tumor refractoriness to anti-VEGF antibody (bevacizumab) treatment (Shojaei et al., 2007a). In this situation, MDSCs release the pro-angiogenic protein Bv8 that surrogates VEGF in the stimulation of tumor angiogenesis (Shojaei et al., 2007b). Because Bv8 is also important in MDSC mobilization and homing to the tumor site, this is an interesting candidate for cancer therapy.

### 3.3 TANs

TAN depletion represents a potential therapeutic approach for cancer cure (Tazzyman et al., 2009). However, since oncologic patients are already immunocompromized individuals, a complete ablation of neutrophils is not desirable. Alternatively, given that activated neutrophils can kill tumor cells through the release of toxic substances, it would be of interest to modulate TAN phenotype, with a switch from N2- towards N1-polarization. Nevertheless, this plan would lead to the generation of highly cytotoxic cells and could result in excessive tissue damage, potentially lethal. A more manageable therapeutic strategy can target neutrophils recruitment to tumors. Inhibition of CXCR2-mediated PMN chemotaxis with a specific antibody or a CXCR2 antagonist has been successfully tested in pre-clinical experimentation (Gregory and Houghton, 2011). The description of the pivotal role of TGF $\beta$  in the promotion of a protumor phenotype of TAN suggests that therapies contrasting this cytokine could contribute to re-educate neutrophils in the tumor microenvironment (Flavell et al., 2010). Interestingly, a recent study showed that the CCL2-driven accumulation of TAMs limits the influx of neutrophils in solid tumors by a yet unidentified mechanism. If TAMs accumulation is suppressed, neutrophils are recruited to the tumor providing a secondary source of MMP-9. Therefore, in the absence of TAMs, TANs provide alternative paracrine support for tumor angiogenesis and progression (Pahler et al., 2008). Hence, the elimination of TAMs alone may be insufficient to eradicate myeloid cell support to tumor growth.

## 4. Conclusions

Recent results indicate that tumour development promotes expansion and functional skewing of different myeloid cell populations, leading to accumulation of protumoral TAMC populations, which include TAMs, TEMs, MDSCs and TANs. These myeloid cell populations display distinct specialized functions, as well as overlapping activities (eg. angiogenesis). It is becoming evident that TAMCs appear to constitute a robust pro-tumour system and the functional elimination of a single myeloid population may be insufficient to eradicate their support to tumor growth. New strategies able to target different myeloid cell populations, simultaneously, are therefore desirable.

New evidence indicates that pathways promoting polarized functions of either macrophages (eg. M1 vs M2) or neutrophils (N1 vs N2) may share common constituents. (Mantovani, 2009) Understanding of this convergent pathways may offer common target/s and strategies to therapeutically affect the pro-tumoral networks established by TAMCs.

## 5. References

- Aharinejad, S., Sioud, M., Lucas, T. and Abraham, D. (2009) Targeting stromal-cancer cell interactions with siRNAs. *Methods Mol Biol*, 487, 243-266.
- Allavena, P., Chieppa, M., Bianchi, G., Solinas, G., Fabbri, M., Laskarin, G. and Mantovani, A. (2010) Engagement of the mannose receptor by tumoral mucins activates an immune suppressive phenotype in human tumor-associated macrophages. *Clin Dev Immunol*, 2010, 547179.
- Allavena, P., Sica, A., Garlanda, C. and Mantovani, A. (2008) The Yin-Yang of tumor-associated macrophages in neoplastic progression and immune surveillance. *Immunol Rev*, 222, 155-161.
- Allavena, P., Signorelli, M., Chieppa, M., Erba, E., Bianchi, G., Marchesi, F., Olimpio, C.O., Bonardi, C., Garbi, A., Lissoni, A., de Braud, F., Jimeno, J. and D'Incalci, M. (2005) Anti-inflammatory properties of the novel antitumor agent yondelis (trabectedin): inhibition of macrophage differentiation and cytokine production. *Cancer Res*, 65, 2964-2971.
- Andreu, P., Johansson, M., Affara, N.I., Pucci, F., Tan, T., Junankar, S., Korets, L., Lam, J., Tawfik, D., DeNardo, D.G., Naldini, L., de Visser, K.E., De Palma, M. and Coussens, L.M. (2010) FcRgamma activation regulates inflammation-associated squamous carcinogenesis. *Cancer Cell*, 17, 121-134.
- Auffray, C., Sieweke, M.H. and Geissmann, F. (2009) Blood monocytes: development, heterogeneity, and relationship with dendritic cells. *Annu Rev Immunol*, 27, 669-692.
- Baeriswyl, V. and Christofori, G. (2009) The angiogenic switch in carcinogenesis. *Semin Cancer Biol*, 19, 329-337.
- Balkwill, F. (2004) Cancer and the chemokine network. *Nat Rev Cancer*, 4, 540-550.
- Beatty, G.L., Chiorean, E.G., Fishman, M.P., Saboury, B., Teitelbaum, U.R., Sun, W., Huhn, R.D., Song, W., Li, D., Sharp, L.L., Torigian, D.A., O'Dwyer, P.J. and Vonderheide, R.H. (2011) CD40 agonists alter tumor stroma and show efficacy against pancreatic carcinoma in mice and humans. *Science*, 331, 1612-1616.
- Beck, A.H., Espinosa, I., Edris, B., Li, R., Montgomery, K., Zhu, S., Varma, S., Marinelli, R.J., van de Rijn, M. and West, R.B. (2009) The macrophage colony-stimulating factor 1 response signature in breast carcinoma. *Clin Cancer Res*, 15, 778-787.
- Bellocq, A., Antoine, M., Flahault, A., Philippe, C., Crestani, B., Bernaudin, J.F., Mayaud, C., Milleron, B., Baud, L. and Cadranel, J. (1998) Neutrophil alveolitis in bronchioloalveolar carcinoma: induction by tumor-derived interleukin-8 and relation to clinical outcome. *Am J Pathol*, 152, 83-92.
- Bingle, L., Brown, N.J. and Lewis, C.E. (2002) The role of tumour-associated macrophages in tumour progression: implications for new anticancer therapies. *J Pathol*, 196, 254-265.
- Biswas, S.K., Gangi, L., Paul, S., Schioppa, T., Saccani, A., Sironi, M., Bottazzi, B., Doni, A., Vincenzo, B., Pasqualini, F., Vago, L., Nebuloni, M., Mantovani, A. and Sica, A. (2006) A distinct and unique transcriptional program expressed by tumor-associated macrophages (defective NF-kappaB and enhanced IRF-3/STAT1 activation). *Blood*, 107, 2112-2122.
- Bollrath, J., Phesse, T.J., von Burstin, V.A., Putoczki, T., Bennecke, M., Bateman, T., Nebelsiek, T., Lundgren-May, T., Canli, O., Schwitalla, S., Matthews, V., Schmid, R.M., Kirchner, T., Arkan, M.C., Ernst, M. and Greten, F.R. (2009) gp130-mediated

- Stat3 activation in enterocytes regulates cell survival and cell-cycle progression during colitis-associated tumorigenesis. *Cancer Cell*, 15, 91-102.
- Bottazzi, B., Polentarutti, N., Acero, R., Balsari, A., Boraschi, D., Ghezzi, P., Salmona, M. and Mantovani, A. (1983) Regulation of the macrophage content of neoplasms by chemoattractants. *Science*, 220, 210-212.
- Bronte, V., Kasic, T., Gri, G., Gallana, K., Borsellino, G., Marigo, I., Battistini, L., Iafrate, M., Prayer-Galetti, T., Pagano, F. and Viola, A. (2005) Boosting antitumor responses of T lymphocytes infiltrating human prostate cancers. *J Exp Med*, 201, 1257-1268.
- Brown, H.K. and Holen, I. (2009) Anti-tumour effects of bisphosphonates--what have we learned from in vivo models? *Curr Cancer Drug Targets*, 9, 807-823.
- Buhtoiarov, I.N., Sondel, P.M., Wigginton, J.M., Buhtoiarova, T.N., Yanke, E.M., Mahvi, D.A. and Rakhmilevich, A.L. (2011) Anti-tumour synergy of cytotoxic chemotherapy and anti-CD40 plus CpG-ODN immunotherapy through repolarization of tumour-associated macrophages. *Immunology*, 132, 226-239.
- Bunt, S.K., Clements, V.K., Hanson, E.M., Sinha, P. and Ostrand-Rosenberg, S. (2009) Inflammation enhances myeloid-derived suppressor cell cross-talk by signaling through Toll-like receptor 4. *J Leukoc Biol*, 85, 996-1004.
- Cappello, P., Caorsi, C., Bosticardo, M., De Angelis, S., Novelli, F., Forni, G. and Giovarelli, M. (2004) CCL16/LEC powerfully triggers effector and antigen-presenting functions of macrophages and enhances T cell cytotoxicity. *J Leukoc Biol*, 75, 135-142.
- Cassatella, M.A., Locati, M. and Mantovani, A. (2009) Never underestimate the power of a neutrophil. *Immunity*, 31, 698-700.
- Chen, J., Yao, Y., Gong, C., Yu, F., Su, S., Chen, J., Liu, B., Deng, H., Wang, F., Lin, L., Yao, H., Su, F., Anderson, K.S., Liu, Q., Ewen, M.E., Yao, X. and Song, E. (2011) CCL18 from tumor-associated macrophages promotes breast cancer metastasis via PITPNM3. *Cancer Cell*, 19, 541-555.
- Cheng, P., Corzo, C.A., Luetkeke, N., Yu, B., Nagaraj, S., Bui, M.M., Ortiz, M., Nacken, W., Sorg, C., Vogl, T., Roth, J. and Gabrilovich, D.I. (2008) Inhibition of dendritic cell differentiation and accumulation of myeloid-derived suppressor cells in cancer is regulated by S100A9 protein. *J Exp Med*, 205, 2235-2249.
- Clark, C.E., Hingorani, S.R., Mick, R., Combs, C., Tuveson, D.A. and Vonderheide, R.H. (2007) Dynamics of the immune reaction to pancreatic cancer from inception to invasion. *Cancer Res*, 67, 9518-9527.
- Clemente, C.G., Mihm, M.C., Jr., Bufalino, R., Zurrida, S., Collini, P. and Cascinelli, N. (1996) Prognostic value of tumor infiltrating lymphocytes in the vertical growth phase of primary cutaneous melanoma. *Cancer*, 77, 1303-1310.
- Coffelt, S.B., Chen, Y.Y., Muthana, M., Welford, A.F., Tal, A.O., Scholz, A., Plate, K.H., Reiss, Y., Murdoch, C., De Palma, M. and Lewis, C.E. (2011) Angiopoietin 2 Stimulates TIE2-Expressing Monocytes To Suppress T Cell Activation and To Promote Regulatory T Cell Expansion. *J Immunol*, 186, 4183-4190.
- Coffelt, S.B., Tal, A.O., Scholz, A., De Palma, M., Patel, S., Urbich, C., Biswas, S.K., Murdoch, C., Plate, K.H., Reiss, Y. and Lewis, C.E. (2010) Angiopoietin-2 regulates gene expression in TIE2-expressing monocytes and augments their inherent proangiogenic functions. *Cancer Res*, 70, 5270-5280.



- Condamine, T. and Gabrilovich, D.I. (2011) Molecular mechanisms regulating myeloid-derived suppressor cell differentiation and function. *Trends Immunol*, 32, 19-25.
- Corzo, C.A., Condamine, T., Lu, L., Cotter, M.J., Youn, J.I., Cheng, P., Cho, H.I., Celis, E., Quiceno, D.G., Padhya, T., McCaffrey, T.V., McCaffrey, J.C. and Gabrilovich, D.I. (2010) HIF-1 $\alpha$  regulates function and differentiation of myeloid-derived suppressor cells in the tumor microenvironment. *J Exp Med*, 207, 2439-2453.
- D'Incalci, M. and Galmarini, C.M. (2010) A review of trabectedin (ET-743): a unique mechanism of action. *Mol Cancer Ther*, 9, 2157-2163.
- De Palma, M., Mazziere, R., Politi, L.S., Pucci, F., Zonari, E., Sitia, G., Mazzoleni, S., Moi, D., Venneri, M.A., Indraccolo, S., Falini, A., Guidotti, L.G., Galli, R. and Naldini, L. (2008) Tumor-targeted interferon- $\alpha$  delivery by Tie2-expressing monocytes inhibits tumor growth and metastasis. *Cancer Cell*, 14, 299-311.
- De Palma, M., Murdoch, C., Venneri, M.A., Naldini, L. and Lewis, C.E. (2007) Tie2-expressing monocytes: regulation of tumor angiogenesis and therapeutic implications. *Trends Immunol*, 28, 519-524.
- De Palma, M. and Naldini, L. (2009) Tie2-expressing monocytes (TEMs): novel targets and vehicles of anticancer therapy? *Biochim Biophys Acta*, 1796, 5-10.
- De Palma, M., Venneri, M.A., Galli, R., Sergi, L., Politi, L.S., Sampaolesi, M. and Naldini, L. (2005) Tie2 identifies a hematopoietic lineage of proangiogenic monocytes required for tumor vessel formation and a mesenchymal population of pericyte progenitors. *Cancer Cell*, 8, 211-226.
- De Palma, M., Venneri, M.A., Roca, C. and Naldini, L. (2003) Targeting exogenous genes to tumor angiogenesis by transplantation of genetically modified hematopoietic stem cells. *Nat Med*, 9, 789-795.
- DeNardo, D.G., Barreto, J.B., Andreu, P., Vasquez, L., Tawfik, D., Kolhatkar, N. and Coussens, L.M. (2009) CD4(+) T cells regulate pulmonary metastasis of mammary carcinomas by enhancing protumor properties of macrophages. *Cancer Cell*, 16, 91-102.
- Dinapoli, M.R., Calderon, C.L. and Lopez, D.M. (1996) The altered tumoricidal capacity of macrophages isolated from tumor-bearing mice is related to reduce expression of the inducible nitric oxide synthase gene. *J Exp Med*, 183, 1323-1329.
- Dinarello, C.A. (2010) Why not treat human cancer with interleukin-1 blockade? *Cancer Metastasis Rev*, 29, 317-329.
- Du, F., Nakamura, Y., Tan, T.L., Lee, P., Lee, R., Yu, B. and Jamora, C. (2010) Expression of snail in epidermal keratinocytes promotes cutaneous inflammation and hyperplasia conducive to tumor formation. *Cancer Res*, 70, 10080-10089.
- Du, R., Lu, K.V., Petritsch, C., Liu, P., Ganss, R., Passegue, E., Song, H., Vandenberg, S., Johnson, R.S., Werb, Z. and Bergers, G. (2008) HIF1 $\alpha$  induces the recruitment of bone marrow-derived vascular modulatory cells to regulate tumor angiogenesis and invasion. *Cancer Cell*, 13, 206-220.
- Duluc, D., Corvaisier, M., Blanchard, S., Catala, L., Descamps, P., Gamelin, E., Ponsoda, S., Delneste, Y., Hebbard, M. and Jeannin, P. (2009) Interferon- $\gamma$  reverses the immunosuppressive and protumoral properties and prevents the generation of human tumor-associated macrophages. *Int J Cancer*, 125, 367-373.
- Ferrara, N. (2010) Role of myeloid cells in vascular endothelial growth factor-independent tumor angiogenesis. *Curr Opin Hematol*, 17, 219-224.

- Finak, G., Bertos, N., Pepin, F., Sadekova, S., Souleimanova, M., Zhao, H., Chen, H., Omeroglu, G., Meterissian, S., Omeroglu, A., Hallett, M. and Park, M. (2008) Stromal gene expression predicts clinical outcome in breast cancer. *Nat Med*, 14, 518-527.
- Flavell, R.A., Sanjabi, S., Wrzesinski, S.H. and Licona-Limon, P. (2010) The polarization of immune cells in the tumour environment by TGFbeta. *Nat Rev Immunol*, 10, 554-567.
- Forssell, J., Oberg, A., Henriksson, M.L., Stenling, R., Jung, A. and Palmqvist, R. (2007) High macrophage infiltration along the tumor front correlates with improved survival in colon cancer. *Clin Cancer Res*, 13, 1472-1479.
- Fridlender, Z.G., Sun, J., Kim, S., Kapoor, V., Cheng, G., Ling, L., Worthen, G.S. and Albelda, S.M. (2009) Polarization of tumor-associated neutrophil phenotype by TGF-beta: "N1" versus "N2" TAN. *Cancer Cell*, 16, 183-194.
- Fukuda, A., Wang, S.C., Morris, J.P.t., Folias, A.E., Liou, A., Kim, G.E., Akira, S., Boucher, K.M., Firpo, M.A., Mulvihill, S.J. and Hebrok, M. (2011) Stat3 and MMP7 contribute to pancreatic ductal adenocarcinoma initiation and progression. *Cancer Cell*, 19, 441-455.
- Gabrilovich, D.I. and Nagaraj, S. (2009) Myeloid-derived suppressor cells as regulators of the immune system. *Nat Rev Immunol*, 9, 162-174.
- Gabrilovich, D.I., Velders, M.P., Sotomayor, E.M. and Kast, W.M. (2001) Mechanism of immune dysfunction in cancer mediated by immature Gr-1+ myeloid cells. *J Immunol*, 166, 5398-5406.
- Ghassabeh, G.H., De Baetselier, P., Brys, L., Noel, W., Van Ginderachter, J.A., Meerschaut, S., Beschin, A., Brombacher, F. and Raes, G. (2006) Identification of a common gene signature for type II cytokine-associated myeloid cells elicited in vivo in different pathologic conditions. *Blood*, 108, 575-583.
- Gocheva, V., Wang, H.W., Gadea, B.B., Shree, T., Hunter, K.E., Garfall, A.L., Berman, T. and Joyce, J.A. (2010) IL-4 induces cathepsin protease activity in tumor-associated macrophages to promote cancer growth and invasion. *Genes Dev*, 24, 241-255.
- Goerdts, S. and Orfanos, C.E. (1999) Other functions, other genes: alternative activation of antigen-presenting cells. *Immunity*, 10, 137-142.
- Gordon, S. and Martinez, F.O. (2010) Alternative activation of macrophages: mechanism and functions. *Immunity*, 32, 593-604.
- Gordon, S. and Taylor, P.R. (2005) Monocyte and macrophage heterogeneity. *Nat Rev Immunol*, 5, 953-964.
- Gratchev, A., Kzhyshkowska, J., Kannookadan, S., Ochsenreiter, M., Popova, A., Yu, X., Mamidi, S., Stonehouse-Usselman, E., Muller-Molinet, I., Gooi, L. and Goerdts, S. (2008) Activation of a TGF-beta-specific multistep gene expression program in mature macrophages requires glucocorticoid-mediated surface expression of TGF-beta receptor II. *J Immunol*, 180, 6553-6565.
- Gregory, A.D. and Houghton, A.M. (2011) Tumor-Associated Neutrophils: New Targets for Cancer Therapy. *Cancer Res*.
- Grivennikov, S., Karin, E., Terzic, J., Mucida, D., Yu, G.Y., Vallabhapurapu, S., Scheller, J., Rose-John, S., Cheroutre, H., Eckmann, L. and Karin, M. (2009) IL-6 and Stat3 are required for survival of intestinal epithelial cells and development of colitis-associated cancer. *Cancer Cell*, 15, 103-113.

- Guiducci, C., Vicari, A.P., Sangaletti, S., Trinchieri, G. and Colombo, M.P. (2005) Redirecting in vivo elicited tumor infiltrating macrophages and dendritic cells towards tumor rejection. *Cancer Res*, 65, 3437-3446.
- Hagemann, T., Biswas, S.K., Lawrence, T., Sica, A. and Lewis, C.E. (2009) Regulation of macrophage function in tumors: the multifaceted role of NF-kappaB. *Blood*, 113, 3139-3146.
- Hamilton, J.A. (2008) Colony-stimulating factors in inflammation and autoimmunity. *Nat Rev Immunol*, 8, 533-544.
- Houghton, A.M. (2010) The paradox of tumor-associated neutrophils: fueling tumor growth with cytotoxic substances. *Cell Cycle*, 9, 1732-1737.
- Huang, B., Pan, P.Y., Li, Q., Sato, A.I., Levy, D.E., Bromberg, J., Divino, C.M. and Chen, S.H. (2006) Gr-1+CD115+ immature myeloid suppressor cells mediate the development of tumor-induced T regulatory cells and T-cell anergy in tumor-bearing host. *Cancer Res*, 66, 1123-1131.
- Ingman, W.V., Wyckoff, J., Gouon-Evans, V., Condeelis, J. and Pollard, J.W. (2006) Macrophages promote collagen fibrillogenesis around terminal end buds of the developing mammary gland. *Dev Dyn*, 235, 3222-3229.
- Jensen, H.K., Donskov, F., Marcussen, N., Nordsmark, M., Lundbeck, F. and von der Maase, H. (2009) Presence of intratumoral neutrophils is an independent prognostic factor in localized renal cell carcinoma. *J Clin Oncol*, 27, 4709-4717.
- Jin, G., Kawsar, H.I., Hirsch, S.A., Zeng, C., Jia, X., Feng, Z., Ghosh, S.K., Zheng, Q.Y., Zhou, A., McIntyre, T.M. and Weinberg, A. (2010) An Antimicrobial Peptide Regulates Tumor-Associated Macrophage Trafficking via the Chemokine Receptor CCR2, a Model for Tumorigenesis. *PLoS ONE*, 5, e10993.
- Joyce, J.A. and Pollard, J.W. (2009) Microenvironmental regulation of metastasis. *Nat Rev Cancer*, 9, 239-252.
- Juarez, J.C., Guan, X., Shipulina, N.V., Plunkett, M.L., Parry, G.C., Shaw, D.E., Zhang, J.C., Rabbani, S.A., McCrae, K.R., Mazar, A.P., Morgan, W.T. and Donate, F. (2002) Histidine-proline-rich glycoprotein has potent antiangiogenic activity mediated through the histidine-proline-rich domain. *Cancer Res*, 62, 5344-5350.
- Kalluri, R. and Zeisberg, M. (2006) Fibroblasts in cancer. *Nat Rev Cancer*, 6, 392-401.
- Ko, H.J., Kim, Y.J., Kim, Y.S., Chang, W.S., Ko, S.Y., Chang, S.Y., Sakaguchi, S. and Kang, C.Y. (2007) A combination of chemoimmunotherapies can efficiently break self-tolerance and induce antitumor immunity in a tolerogenic murine tumor model. *Cancer Res*, 67, 7477-7486.
- Konishi, T., Okabe, H., Katoh, H., Fujiyama, Y. and Mori, A. (1996) Macrophage inflammatory protein-1 alpha expression in non-neoplastic and neoplastic lung tissue. *Virchows Arch*, 428, 107-111.
- Kortylewski, M., Kujawski, M., Wang, T., Wei, S., Zhang, S., Pilon-Thomas, S., Niu, G., Kay, H., Mule, J., Kerr, W.G., Jove, R., Pardoll, D. and Yu, H. (2005) Inhibiting Stat3 signaling in the hematopoietic system elicits multicomponent antitumor immunity. *Nat Med*, 11, 1314-1321.
- Kusmartsev, S., Cheng, F., Yu, B., Nefedova, Y., Sotomayor, E., Lush, R. and Gabrilovich, D. (2003) All-trans-retinoic acid eliminates immature myeloid cells from tumor-bearing mice and improves the effect of vaccination. *Cancer Res*, 63, 4441-4449.

- Kusmartsev, S. and Gabrilovich, D.I. (2005) STAT1 signaling regulates tumor-associated macrophage-mediated T cell deletion. *J Immunol*, 174, 4880-4891.
- Laghi, L., Bianchi, P., Miranda, E., Balladore, E., Pacetti, V., Grizzi, F., Allavena, P., Torri, V., Repici, A., Santoro, A., Mantovani, A., Roncalli, M. and Malesci, A. (2009) CD3+ cells at the invasive margin of deeply invading (pT3-T4) colorectal cancer and risk of post-surgical metastasis: a longitudinal study. *Lancet Oncol*, 10, 877-884.
- Lenz, G., Wright, G., Dave, S.S., Xiao, W., Powell, J., Zhao, H., Xu, W., Tan, B., Goldschmidt, N., Iqbal, J., Vose, J., Bast, M., Fu, K., Weisenburger, D.D., Greiner, T.C., Armitage, J.O., Kyle, A., May, L., Gascoyne, R.D., Connors, J.M., Troen, G., Holte, H., Kvaloy, S., Dierickx, D., Verhoef, G., Delabie, J., Smeland, E.B., Jares, P., Martinez, A., Lopez-Guillermo, A., Montserrat, E., Campo, E., Braziel, R.M., Miller, T.P., Rimsza, L.M., Cook, J.R., Pohlman, B., Sweetenham, J., Tubbs, R.R., Fisher, R.I., Hartmann, E., Rosenwald, A., Ott, G., Muller-Hermelink, H.K., Wrench, D., Lister, T.A., Jaffe, E.S., Wilson, W.H., Chan, W.C. and Staudt, L.M. (2008) Stromal gene signatures in large-B-cell lymphomas. *N Engl J Med*, 359, 2313-2323.
- Lesina, M., Kurkowski, M.U., Ludes, K., Rose-John, S., Treiber, M., Kloppel, G., Yoshimura, A., Reindl, W., Sipos, B., Akira, S., Schmid, R.M. and Algul, H. Stat3/Socs3 activation by IL-6 transsignaling promotes progression of pancreatic intraepithelial neoplasia and development of pancreatic cancer. *Cancer Cell*, 19, 456-469.
- Lewis, C.E. and Pollard, J.W. (2006) Distinct role of macrophages in different tumor microenvironments. *Cancer Res*, 66, 605-612.
- Lewis, J.S., Landers, R.J., Underwood, J.C., Harris, A.L. and Lewis, C.E. (2000) Expression of vascular endothelial growth factor by macrophages is up-regulated in poorly vascularized areas of breast carcinomas. *J Pathol*, 192, 150-158.
- Li, X., Loberg, R., Liao, J., Ying, C., Snyder, L.A., Pienta, K.J. and McCauley, L.K. (2009) A destructive cascade mediated by CCL2 facilitates prostate cancer growth in bone. *Cancer Res*, 69, 1685-1692.
- Lin, E.Y., Gouon-Evans, V., Nguyen, A.V. and Pollard, J.W. (2002) The macrophage growth factor CSF-1 in mammary gland development and tumor progression. *J Mammary Gland Biol Neoplasia*, 7, 147-162.
- Lin, E.Y., Nguyen, A.V., Russell, R.G. and Pollard, J.W. (2001) Colony-stimulating factor 1 promotes progression of mammary tumors to malignancy. *J Exp Med*, 193, 727-740.
- Loberg, R.D., Ying, C., Craig, M., Day, L.L., Sargent, E., Neeley, C., Wojno, K., Snyder, L.A., Yan, L. and Pienta, K.J. (2007) Targeting CCL2 with systemic delivery of neutralizing antibodies induces prostate cancer tumor regression in vivo. *Cancer Res*, 67, 9417-9424.
- Loges, S., Schmidt, T., Tjwa, M., van Geyte, K., Lievens, D., Lutgens, E., Vanhoutte, D., Borgel, D., Plaisance, S., Hoylaerts, M., Luttun, A., Dewerchin, M., Jonckx, B. and Carmeliet, P. (2010) Malignant cells fuel tumor growth by educating infiltrating leukocytes to produce the mitogen Gas6. *Blood*, 115, 2264-2273.
- Ma, G., Pan, P.Y., Eisenstein, S., Divino, C.M., Lowell, C.A., Takai, T. and Chen, S.H. (2011) Paired Immunoglobulin-like Receptor-B Regulates the Suppressive Function and Fate of Myeloid-Derived Suppressor Cells. *Immunity*, 34, 385-395.
- Mahmoud, S.M., Paish, E.C., Powe, D.G., Macmillan, R.D., Grainge, M.J., Lee, A.H., Ellis, I.O. and Green, A.R. (2011) Tumor-infiltrating CD8+ lymphocytes predict clinical outcome in breast cancer. *J Clin Oncol*, 29, 1949-1955.



- Mantovani, A. (2009) The yin-yang of tumor-associated neutrophils. *Cancer Cell*, 16, 173-174.
- Mantovani, A., Allavena, P. and Sica, A. (2004a) Tumour-associated macrophages as a prototypic type II polarised phagocyte population: role in tumour progression. *Eur J Cancer*, 40, 1660-1667.
- Mantovani, A., Allavena, P., Sica, A. and Balkwill, F. (2008) Cancer-related inflammation. *Nature*, 454, 436-444.
- Mantovani, A., Bottazzi, B., Colotta, F., Sozzani, S. and Ruco, L. (1992) The origin and function of tumor-associated macrophages. *Immunol Today*, 13, 265-270.
- Mantovani, A. and Sica, A. (2010) Macrophages, innate immunity and cancer: balance, tolerance, and diversity. *Curr Opin Immunol*, 22, 231-237.
- Mantovani, A., Sica, A., Allavena, P., Garlanda, C. and Locati, M. (2009) Tumor-associated macrophages and the related myeloid-derived suppressor cells as a paradigm of the diversity of macrophage activation. *Hum Immunol*, 70, 325-330.
- Mantovani, A., Sica, A. and Locati, M. (2005) Macrophage polarization comes of age. *Immunity*, 23, 344-346.
- Mantovani, A., Sica, A., Sozzani, S., Allavena, P., Vecchi, A. and Locati, M. (2004b) The chemokine system in diverse forms of macrophage activation and polarization. *Trends Immunol*, 25, 677-686.
- Marigo, I., Bosio, E., Solito, S., Mesa, C., Fernandez, A., Dolcetti, L., Ugel, S., Sonda, N., Biccato, S., Falisi, E., Calabrese, F., Basso, G., Zanovello, P., Cozzi, E., Mandruzzato, S. and Bronte, V. (2010) Tumor-induced tolerance and immune suppression depend on the C/EBPbeta transcription factor. *Immunity*, 32, 790-802.
- Marigo, I., Dolcetti, L., Serafini, P., Zanovello, P. and Bronte, V. (2008) Tumor-induced tolerance and immune suppression by myeloid derived suppressor cells. *Immunol Rev*, 222, 162-179.
- Markiewski, M.M., DeAngelis, R.A., Benencia, F., Ricklin-Lichtsteiner, S.K., Koutoulaki, A., Gerard, C., Coukos, G. and Lambris, J.D. (2008) Modulation of the antitumor immune response by complement. *Nat Immunol*, 9, 1225-1235.
- Martin, C.K., Werbeck, J.L., Thudi, N.K., Lanigan, L.G., Wolfe, T.D., Toribio, R.E. and Rosol, T.J. (2010) Zoledronic acid reduces bone loss and tumor growth in an orthotopic xenograft model of osteolytic oral squamous cell carcinoma. *Cancer Res*, 70, 8607-8616.
- Martinez, F.O., Helming, L. and Gordon, S. (2009) Alternative activation of macrophages: an immunologic functional perspective. *Annu Rev Immunol*, 27, 451-483.
- Mason, S.D. and Joyce, J.A. (2011) Proteolytic networks in cancer. *Trends Cell Biol*, 21, 228-237.
- Mirza, N., Fishman, M., Fricke, I., Dunn, M., Neuger, A.M., Frost, T.J., Lush, R.M., Antonia, S. and Gabrilovich, D.I. (2006) All-trans-retinoic acid improves differentiation of myeloid cells and immune response in cancer patients. *Cancer Res*, 66, 9299-9307.
- Morgan, G.J., Davies, F.E., Gregory, W.M., Cocks, K., Bell, S.E., Szubert, A.J., Navarro-Coy, N., Drayson, M.T., Owen, R.G., Feyler, S., Ashcroft, A.J., Ross, F., Byrne, J., Roddie, H., Rudin, C., Cook, G., Jackson, G.H. and Child, J.A. (2010) First-line treatment with zoledronic acid as compared with clodronic acid in multiple myeloma (MRC Myeloma IX): a randomised controlled trial. *Lancet*, 376, 1989-1999.
- Moussai, D., Mitsui, H., Pettersen, J.S., Pierson, K.C., Shah, K.R., Suarez-Farinas, M., Cardinale, I.R., Bluth, M.J., Krueger, J.G. and Carucci, J.A. (2011) The human

- cutaneous squamous cell carcinoma microenvironment is characterized by increased lymphatic density and enhanced expression of macrophage-derived VEGF-C. *J Invest Dermatol*, 131, 229-236.
- Movahedi, K., Guillems, M., Van den Bossche, J., Van den Bergh, R., Gysemans, C., Beschin, A., De Baetselier, P. and Van Ginderachter, J.A. (2008) Identification of discrete tumor-induced myeloid-derived suppressor cell subpopulations with distinct T cell-suppressive activity. *Blood*, 111, 4233-4244.
- Movahedi, K., Laoui, D., Gysemans, C., Baeten, M., Stange, G., Van den Bossche, J., Mack, M., Pipeleers, D., In't Veld, P., De Baetselier, P. and Van Ginderachter, J.A. (2010) Different tumor microenvironments contain functionally distinct subsets of macrophages derived from Ly6C(high) monocytes. *Cancer Res*, 70, 5728-5739.
- Murdoch, C., Muthana, M., Coffelt, S.B. and Lewis, C.E. (2008) The role of myeloid cells in the promotion of tumour angiogenesis. *Nat Rev Cancer*, 8, 618-631.
- Murdoch, C., Tazzyman, S., Webster, S. and Lewis, C.E. (2007) Expression of Tie-2 by human monocytes and their responses to angiopoietin-2. *J Immunol*, 178, 7405-7411.
- Murray, P.J. and Wynn, T.A. (2011) Obstacles and opportunities for understanding macrophage polarization. *J Leukoc Biol*, 89, 557-563.
- Nagaraj, S., Gupta, K., Pisarev, V., Kinarsky, L., Sherman, S., Kang, L., Herber, D.L., Schneck, J. and Gabrilovich, D.I. (2007) Altered recognition of antigen is a mechanism of CD8+ T cell tolerance in cancer. *Nat Med*, 13, 828-835.
- Ohno, S., Inagawa, H., Dhar, D.K., Fujii, T., Ueda, S., Tachibana, M., Suzuki, N., Inoue, M., Soma, G. and Nagasue, N. (2003) The degree of macrophage infiltration into the cancer cell nest is a significant predictor of survival in gastric cancer patients. *Anticancer Res*, 23, 5015-5022.
- Ohtaki, Y., Ishii, G., Nagai, K., Ashimine, S., Kuwata, T., Hishida, T., Nishimura, M., Yoshida, J., Takeyoshi, I. and Ochiai, A. (2010) Stromal macrophage expressing CD204 is associated with tumor aggressiveness in lung adenocarcinoma. *J Thorac Oncol*, 5, 1507-1515.
- Ohtani, H., Naito, Y., Saito, K. and Nagura, H. (1997) Expression of costimulatory molecules B7-1 and B7-2 by macrophages along invasive margin of colon cancer: a possible antitumor immunity? *Lab Invest*, 77, 231-241.
- Ojalvo, L.S., Whittaker, C.A., Condeelis, J.S. and Pollard, J.W. (2010) Gene expression analysis of macrophages that facilitate tumor invasion supports a role for Wnt-signaling in mediating their activity in primary mammary tumors. *J Immunol*, 184, 702-712.
- Olsson, A.K., Larsson, H., Dixelius, J., Johansson, I., Lee, C., Oellig, C., Bjork, I. and Claesson-Welsh, L. (2004) A fragment of histidine-rich glycoprotein is a potent inhibitor of tumor vascularization. *Cancer Res*, 64, 599-605.
- Ostrand-Rosenberg, S. and Sinha, P. (2009) Myeloid-derived suppressor cells: linking inflammation and cancer. *J Immunol*, 182, 4499-4506.
- Pages, F., Berger, A., Camus, M., Sanchez-Cabo, F., Costes, A., Molidor, R., Mlecnik, B., Kirilovsky, A., Nilsson, M., Damotte, D., Meatchi, T., Bruneval, P., Cugnenc, P.H., Trajanoski, Z., Fridman, W.H. and Galon, J. (2005) Effector memory T cells, early metastasis, and survival in colorectal cancer. *N Engl J Med*, 353, 2654-2666.
- Pahler, J.C., Tazzyman, S., Erez, N., Chen, Y.Y., Murdoch, C., Nozawa, H., Lewis, C.E. and Hanahan, D. (2008) Plasticity in tumor-promoting inflammation: impairment of

- macrophage recruitment evokes a compensatory neutrophil response. *Neoplasia*, 10, 329-340.
- Palucka, K., Ueno, H., Zurawski, G., Fay, J. and Banchereau, J. (2010) Building on dendritic cell subsets to improve cancer vaccines. *Curr Opin Immunol*, 22, 258-263.
- Pekarek, L.A., Starr, B.A., Toledano, A.Y. and Schreiber, H. (1995) Inhibition of tumor growth by elimination of granulocytes. *J Exp Med*, 181, 435-440.
- Pelegri, P. and Surprenant, A. (2009) Dynamics of macrophage polarization reveal new mechanism to inhibit IL-1 $\beta$  release through pyrophosphates. *Embo J*, 28, 2114-2127.
- Piccard, H., Muschel, R.J. and Opdenakker, G. (2011) On the dual roles and polarized phenotypes of neutrophils in tumor development and progression. *Crit Rev Oncol Hematol*.
- Pollard, J.W. (2004) Tumour-educated macrophages promote tumour progression and metastasis. *Nat Rev Cancer*, 4, 71-78.
- Pollard, J.W. (2008) Macrophages define the invasive microenvironment in breast cancer. *J Leukoc Biol*, 84, 623-630.
- Pollard, J.W. (2009) Trophic macrophages in development and disease. *Nat Rev Immunol*, 9, 259-270.
- Popivanova, B.K., Kostadinova, F.I., Furuichi, K., Shamekh, M.M., Kondo, T., Wada, T., Egashira, K. and Mukaida, N. (2009) Blockade of a chemokine, CCL2, reduces chronic colitis-associated carcinogenesis in mice. *Cancer Res*, 69, 7884-7892.
- Porta, C., Rimoldi, M., Raes, G., Brys, L., Ghezzi, P., Di Liberto, D., Dieli, F., Ghisletti, S., Natoli, G., De Baetselier, P., Mantovani, A. and Sica, A. (2009) Tolerance and M2 (alternative) macrophage polarization are related processes orchestrated by p50 nuclear factor kappaB. *Proc Natl Acad Sci U S A*, 106, 14978-14983.
- Pucci, F., Venneri, M.A., Biziato, D., Nonis, A., Moi, D., Sica, A., Di Serio, C., Naldini, L. and De Palma, M. (2009) A distinguishing gene signature shared by tumor-infiltrating Tie2-expressing monocytes, blood "resident" monocytes, and embryonic macrophages suggests common functions and developmental relationships. *Blood*, 114, 901-914.
- Qian, B.Z. and Pollard, J.W. (2010) Macrophage diversity enhances tumor progression and metastasis. *Cell*, 141, 39-51.
- Rauh, M.J., Ho, V., Pereira, C., Sham, A., Sly, L.M., Lam, V., Huxham, L., Minchinton, A.I., Mui, A. and Krystal, G. (2005) SHIP represses the generation of alternatively activated macrophages. *Immunity*, 23, 361-374.
- Ravasi, T., Wells, C., Forest, A., Underhill, D.M., Wainwright, B.J., Aderem, A., Grimmond, S. and Hume, D.A. (2002) Generation of diversity in the innate immune system: macrophage heterogeneity arises from gene-autonomous transcriptional probability of individual inducible genes. *J Immunol*, 168, 44-50.
- Ribatti, D. and Vacca, A. (2009) The role of monocytes-macrophages in vasculogenesis in multiple myeloma. *Leukemia*, 23, 1535-1536.
- Riches, D.W. (1995) Signalling heterogeneity as a contributing factor in macrophage functional diversity. *Semin Cell Biol*, 6, 377-384.
- Rolny, C., Mazzone, M., Tugues, S., Laoui, D., Johansson, I., Coulon, C., Squadrito, M.L., Segura, I., Li, X., Knevels, E., Costa, S., Vinckier, S., Dresselaer, T., Akerud, P., De Mol, M., Salomaki, H., Phillipson, M., Wyns, S., Larsson, E., Buysschaert, I., Botling,

- J., Himmelreich, U., Van Ginderachter, J.A., De Palma, M., Dewerchin, M., Claesson-Welsh, L. and Carmeliet, P. (2011) HRG inhibits tumor growth and metastasis by inducing macrophage polarization and vessel normalization through downregulation of PlGF. *Cancer Cell*, 19, 31-44.
- Saccani, A., Schioppa, T., Porta, C., Biswas, S.K., Nebuloni, M., Vago, L., Bottazzi, B., Colombo, M.P., Mantovani, A. and Sica, A. (2006) p50 nuclear factor-kappaB overexpression in tumor-associated macrophages inhibits M1 inflammatory responses and antitumor resistance. *Cancer Res*, 66, 11432-11440.
- Sawanobori, Y., Ueha, S., Kurachi, M., Shimaoka, T., Talmadge, J.E., Abe, J., Shono, Y., Kitabatake, M., Kakimi, K., Mukaida, N. and Matsushima, K. (2008) Chemokine-mediated rapid turnover of myeloid-derived suppressor cells in tumor-bearing mice. *Blood*, 111, 5457-5466.
- Schioppa, T., Uranchimeg, B., Saccani, A., Biswas, S.K., Doni, A., Rapisarda, A., Bernasconi, S., Saccani, S., Nebuloni, M., Vago, L., Mantovani, A., Melillo, G. and Sica, A. (2003) Regulation of the chemokine receptor CXCR4 by hypoxia. *J Exp Med*, 198, 1391-1402.
- Schor, S.L., Ellis, I.R., Jones, S.J., Baillie, R., Seneviratne, K., Clausen, J., Motegi, K., Vojtesek, B., Kankova, K., Furrie, E., Sales, M.J., Schor, A.M. and Kay, R.A. (2003) Migration-stimulating factor: a genetically truncated onco-fetal fibronectin isoform expressed by carcinoma and tumor-associated stromal cells. *Cancer Res*, 63, 8827-8836.
- Sconocchia, G., Zlobec, I., Lugli, A., Calabrese, D., Iezzi, G., Karamitopoulou, E., Patsouris, E.S., Peros, G., Horcic, M., Tornillo, L., Zuber, M., Droeser, R., Muraro, M.G., Mengus, C., Oertli, D., Ferrone, S., Terracciano, L. and Spagnoli, G.C. (2011) Tumor infiltration by FcγRIII (CD16)+ myeloid cells is associated with improved survival in patients with colorectal carcinoma. *Int J Cancer*, 128, 2663-2672.
- Shojaei, F. and Ferrara, N. (2008) Refractoriness to antivascular endothelial growth factor treatment: role of myeloid cells. *Cancer Res*, 68, 5501-5504.
- Shojaei, F., Wu, X., Malik, A.K., Zhong, C., Baldwin, M.E., Schanz, S., Fuh, G., Gerber, H.P. and Ferrara, N. (2007a) Tumor refractoriness to anti-VEGF treatment is mediated by CD11b+Gr1+ myeloid cells. *Nat Biotechnol*, 25, 911-920.
- Shojaei, F., Wu, X., Zhong, C., Yu, L., Liang, X.H., Yao, J., Blanchard, D., Bais, C., Peale, F.V., van Bruggen, N., Ho, C., Ross, J., Tan, M., Carano, R.A., Meng, Y.G. and Ferrara, N. (2007b) Bv8 regulates myeloid-cell-dependent tumour angiogenesis. *Nature*, 450, 825-831.
- Sica, A. (2010) Role of tumour-associated macrophages in cancer-related inflammation. *Exp Oncol*, 32, 153-158.
- Sica, A. and Bronte, V. (2007) Altered macrophage differentiation and immune dysfunction in tumor development. *J Clin Invest*, 117, 1155-1166.
- Sica, A., Larghi, P., Mancino, A., Rubino, L., Porta, C., Totaro, M.G., Rimoldi, M., Biswas, S.K., Allavena, P. and Mantovani, A. (2008) Macrophage polarization in tumour progression. *Semin Cancer Biol*, 18, 349-355.
- Sica, A., Melillo, G. and Varesio, L. (2011) Hypoxia: a double-edged sword of immunity. *J Mol Med*.
- Sica, A., Saccani, A., Bottazzi, B., Polentarutti, N., Vecchi, A., van Damme, J. and Mantovani, A. (2000) Autocrine production of IL-10 mediates defective IL-12 production and NF-kappa B activation in tumor-associated macrophages. *J Immunol*, 164, 762-767.



- Sierra, J.R., Corso, S., Caione, L., Cepero, V., Conrotto, P., Cignetti, A., Piacibello, W., Kumanogoh, A., Kikutani, H., Comoglio, P.M., Tamagnone, L. and Giordano, S. (2008) Tumor angiogenesis and progression are enhanced by Sema4D produced by tumor-associated macrophages. *J Exp Med*, 205, 1673-1685.
- Sinha, P., Clements, V.K., Bunt, S.K., Albelda, S.M. and Ostrand-Rosenberg, S. (2007) Cross-talk between myeloid-derived suppressor cells and macrophages subverts tumor immunity toward a type 2 response. *J Immunol*, 179, 977-983.
- Sinha, P., Okoro, C., Foell, D., Freeze, H.H., Ostrand-Rosenberg, S. and Srikrishna, G. (2008) Proinflammatory S100 proteins regulate the accumulation of myeloid-derived suppressor cells. *J Immunol*, 181, 4666-4675.
- Solinas, G., Schiarea, S., Liguori, M., Fabbri, M., Pesce, S., Zammataro, L., Pasqualini, F., Nebuloni, M., Chiabrando, C., Mantovani, A. and Allavena, P. (2010) Tumor-conditioned macrophages secrete migration-stimulating factor: a new marker for M2-polarization, influencing tumor cell motility. *J Immunol*, 185, 642-652.
- Srivastava, M.K., Sinha, P., Clements, V.K., Rodriguez, P. and Ostrand-Rosenberg, S. (2010) Myeloid-derived suppressor cells inhibit T-cell activation by depleting cystine and cysteine. *Cancer Res*, 70, 68-77.
- Steidl, C., Lee, T., Shah, S.P., Farinha, P., Han, G., Nayar, T., Delaney, A., Jones, S.J., Iqbal, J., Weisenburger, D.D., Bast, M.A., Rosenwald, A., Muller-Hermelink, H.K., Rimsza, L.M., Campo, E., Delabie, J., Braziel, R.M., Cook, J.R., Tubbs, R.R., Jaffe, E.S., Lenz, G., Connors, J.M., Staudt, L.M., Chan, W.C. and Gascoyne, R.D. (2010) Tumor-associated macrophages and survival in classic Hodgkin's lymphoma. *N Engl J Med*, 362, 875-885.
- Stein, M., Keshav, S., Harris, N. and Gordon, S. (1992) Interleukin 4 potently enhances murine macrophage mannose receptor activity: a marker of alternative immunologic macrophage activation. *J Exp Med*, 176, 287-292.
- Stout, R.D., Jiang, C., Matta, B., Tietzel, I., Watkins, S.K. and Suttles, J. (2005) Macrophages sequentially change their functional phenotype in response to changes in microenvironmental influences. *J Immunol*, 175, 342-349.
- Stout, R.D. and Suttles, J. (2005) Immunosenescence and macrophage functional plasticity: dysregulation of macrophage function by age-associated microenvironmental changes. *Immunol Rev*, 205, 60-71.
- Sugita, J., Ohtani, H., Mizoi, T., Saito, K., Shiiba, K., Sasaki, I., Matsuno, S., Yagita, H., Miyazawa, M. and Nagura, H. (2002) Close association between Fas ligand (FasL; CD95L)-positive tumor-associated macrophages and apoptotic cancer cells along invasive margin of colorectal carcinoma: a proposal on tumor-host interactions. *Jpn J Cancer Res*, 93, 320-328.
- Suzuki, E., Kapoor, V., Jassar, A.S., Kaiser, L.R. and Albelda, S.M. (2005) Gemcitabine selectively eliminates splenic Gr-1<sup>+</sup>/CD11b<sup>+</sup> myeloid suppressor cells in tumor-bearing animals and enhances antitumor immune activity. *Clin Cancer Res*, 11, 6713-6721.
- Talmadge, J.E., Donkor, M. and Scholar, E. (2007) Inflammatory cell infiltration of tumors: Jekyll or Hyde. *Cancer Metastasis Rev*, 26, 373-400.
- Tannenbaum, C.S., Koerner, T.J., Jansen, M.M. and Hamilton, T.A. (1988) Characterization of lipopolysaccharide-induced macrophage gene expression. *J Immunol*, 140, 3640-3645.

- Tazawa, H., Okada, F., Kobayashi, T., Tada, M., Mori, Y., Une, Y., Sendo, F., Kobayashi, M. and Hosokawa, M. (2003) Infiltration of neutrophils is required for acquisition of metastatic phenotype of benign murine fibrosarcoma cells: implication of inflammation-associated carcinogenesis and tumor progression. *Am J Pathol*, 163, 2221-2232.
- Tazzyman, S., Lewis, C.E. and Murdoch, C. (2009) Neutrophils: key mediators of tumour angiogenesis. *Int J Exp Pathol*, 90, 222-231.
- Terabe, M., Matsui, S., Park, J.M., Mamura, M., Noben-Trauth, N., Donaldson, D.D., Chen, W., Wahl, S.M., Ledbetter, S., Pratt, B., Letterio, J.J., Paul, W.E. and Berzofsky, J.A. (2003) Transforming growth factor-beta production and myeloid cells are an effector mechanism through which CD1d-restricted T cells block cytotoxic T lymphocyte-mediated tumor immunosurveillance: abrogation prevents tumor recurrence. *J Exp Med*, 198, 1741-1752.
- Venneri, M.A., De Palma, M., Ponzoni, M., Pucci, F., Scielzo, C., Zonari, E., Mazzieri, R., Doglioni, C. and Naldini, L. (2007) Identification of proangiogenic TIE2-expressing monocytes (TEMs) in human peripheral blood and cancer. *Blood*, 109, 5276-5285.
- Vesely, M.D., Kershaw, M.H., Schreiber, R.D. and Smyth, M.J. (2011) Natural innate and adaptive immunity to cancer. *Annu Rev Immunol*, 29, 235-271.
- Wang, H.W. and Joyce, J.A. (2010) Alternative activation of tumor-associated macrophages by IL-4: priming for protumoral functions. *Cell Cycle*, 9, 4824-4835.
- Welford, A.F., Biziato, D., Coffelt, S.B., Nucera, S., Fisher, M., Pucci, F., Di Serio, C., Naldini, L., De Palma, M., Tozer, G.M. and Lewis, C.E. (2011a) TIE2-expressing macrophages limit the therapeutic efficacy of the vascular-disrupting agent combretastatin A4 phosphate in mice. *J Clin Invest*, 121, 1969-1973.
- Welford, A.F., Biziato, D., Coffelt, S.B., Nucera, S., Fisher, M., Pucci, F., Serio, C.D., Naldini, L., Palma, M.D., Tozer, G.M. and Lewis, C.E. (2011b) TIE2-expressing macrophages limit the therapeutic efficacy of the vascular-disrupting agent combretastatin A4 phosphate in mice. *The Journal of Clinical Investigation*, 0, 0-0.
- Wyckoff, J.B., Wang, Y., Lin, E.Y., Li, J.F., Goswami, S., Stanley, E.R., Segall, J.E., Pollard, J.W. and Condeelis, J. (2007) Direct visualization of macrophage-assisted tumor cell intravasation in mammary tumors. *Cancer Res*, 67, 2649-2656.
- Yang, L., DeBusk, L.M., Fukuda, K., Fingleton, B., Green-Jarvis, B., Shyr, Y., Matrisian, L.M., Carbone, D.P. and Lin, P.C. (2004) Expansion of myeloid immune suppressor Gr<sup>+</sup>CD11b<sup>+</sup> cells in tumor-bearing host directly promotes tumor angiogenesis. *Cancer Cell*, 6, 409-421.
- Yang, P., Bamlet, W.R., Sun, Z., Ebbert, J.O., Aubry, M.C., Krowka, M.J., Taylor, W.R., Marks, R.S., Deschamps, C., Swensen, S.J., Wieben, E.D., Cunningham, J.M., Melton, L.J. and de Andrade, M. (2005) Alpha1-antitrypsin and neutrophil elastase imbalance and lung cancer risk. *Chest*, 128, 445-452.
- Yang, R., Cai, Z., Zhang, Y., Yutzy, W.H.t., Roby, K.F. and Roden, R.B. (2006) CD80 in immune suppression by mouse ovarian carcinoma-associated Gr-1<sup>+</sup>CD11b<sup>+</sup> myeloid cells. *Cancer Res*, 66, 6807-6815.
- Youn, J.I. and Gabrilovich, D.I. (2010) The biology of myeloid-derived suppressor cells: the blessing and the curse of morphological and functional heterogeneity. *Eur J Immunol*, 40, 2969-2975.

- Youn, J.I., Nagaraj, S., Collazo, M. and Gabrilovich, D.I. (2008) Subsets of myeloid-derived suppressor cells in tumor-bearing mice. *J Immunol*, 181, 5791-5802.
- Zachariae, C.O., Anderson, A.O., Thompson, H.L., Appella, E., Mantovani, A., Oppenheim, J.J. and Matsushima, K. (1990) Properties of monocyte chemotactic and activating factor (MCAF) purified from a human fibrosarcoma cell line. *J Exp Med*, 171, 2177-2182.
- Zeisberger, S.M., Odermatt, B., Marty, C., Zehnder-Fjallman, A.H., Ballmer-Hofer, K. and Schwendener, R.A. (2006) Clodronate-liposome-mediated depletion of tumour-associated macrophages: a new and highly effective antiangiogenic therapy approach. *Br J Cancer*, 95, 272-281.
- Zhang, J., Sud, S., Mizutani, K., Gyetko, M.R. and Pienta, K.J. (2011) Activation of urokinase plasminogen activator and its receptor axis is essential for macrophage infiltration in a prostate cancer mouse model. *Neoplasia*, 13, 23-30.
- Zhang, L., Conejo-Garcia, J.R., Katsaros, D., Gimotty, P.A., Massobrio, M., Regnani, G., Makrigiannakis, A., Gray, H., Schlienger, K., Liebman, M.N., Rubin, S.C. and Coukos, G. (2003) Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer. *N Engl J Med*, 348, 203-213.
- Zhang, W., Zhu, X.D., Sun, H.C., Xiong, Y.Q., Zhuang, P.Y., Xu, H.X., Kong, L.Q., Wang, L., Wu, W.Z. and Tang, Z.Y. (2010) Depletion of tumor-associated macrophages enhances the effect of sorafenib in metastatic liver cancer models by antimetastatic and antiangiogenic effects. *Clin Cancer Res*, 16, 3420-3430.
- Zhang, X., Majlessi, L., Deriaud, E., Leclerc, C. and Lo-Man, R. (2009) Coactivation of Syk kinase and MyD88 adaptor protein pathways by bacteria promotes regulatory properties of neutrophils. *Immunity*, 31, 761-771.
- Zumsteg, A., Baeriswyl, V., Imaizumi, N., Schwendener, R., Ruegg, C. and Christofori, G. (2009) Myeloid cells contribute to tumor lymphangiogenesis. *PLoS One*, 4, e7067.

IntechOpen



## **Tumor Microenvironment and Myelomonocytic Cells**

Edited by Dr. Subhra Biswas

ISBN 978-953-51-0439-1

Hard cover, 298 pages

**Publisher** InTech

**Published online** 30, March, 2012

**Published in print edition** March, 2012

Tumor microenvironment represents an extremely dynamic niche shaped by the interplay of different cell types (e.g. tumor cells, stromal cells), their soluble products (e.g. cytokines, chemokines and growth factors) and varied physico-chemical conditions (e.g. low oxygen concentration or hypoxia). Recent studies have identified myelomonocytic cells as key players in regulating the tumor microenvironment and hence, tumor progression in a variety of cancers. In view of these findings, the present book attempts to provide a comprehensive account of the diversity of tumor microenvironment across different cancers and how myelomonocytic cells have taken the center-stage in regulating this niche to direct cancer progression. A better understanding of the myelomonocytic cells and the mechanisms by which they regulate cancer progression will open new vistas in cancer therapeutics.

### **How to reference**

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Paola Allavena and Antonio Sica (2012). Functions of Diverse Myeloid Cells in the Tumor Micro-Environment, Tumor Microenvironment and Myelomonocytic Cells, Dr. Subhra Biswas (Ed.), ISBN: 978-953-51-0439-1, InTech, Available from: <http://www.intechopen.com/books/tumor-microenvironment-and-myelomonocytic-cells/functions-of-diverse-myeloid-cells-in-the-tumor-micro-environment>

**INTECH**  
open science | open minds

### **InTech Europe**

University Campus STeP Ri  
Slavka Krautzeka 83/A  
51000 Rijeka, Croatia  
Phone: +385 (51) 770 447  
Fax: +385 (51) 686 166  
[www.intechopen.com](http://www.intechopen.com)

### **InTech China**

Unit 405, Office Block, Hotel Equatorial Shanghai  
No.65, Yan An Road (West), Shanghai, 200040, China  
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元  
Phone: +86-21-62489820  
Fax: +86-21-62489821



© 2012 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](https://creativecommons.org/licenses/by/3.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

IntechOpen

IntechOpen