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# The tumor microenvironment at a glance

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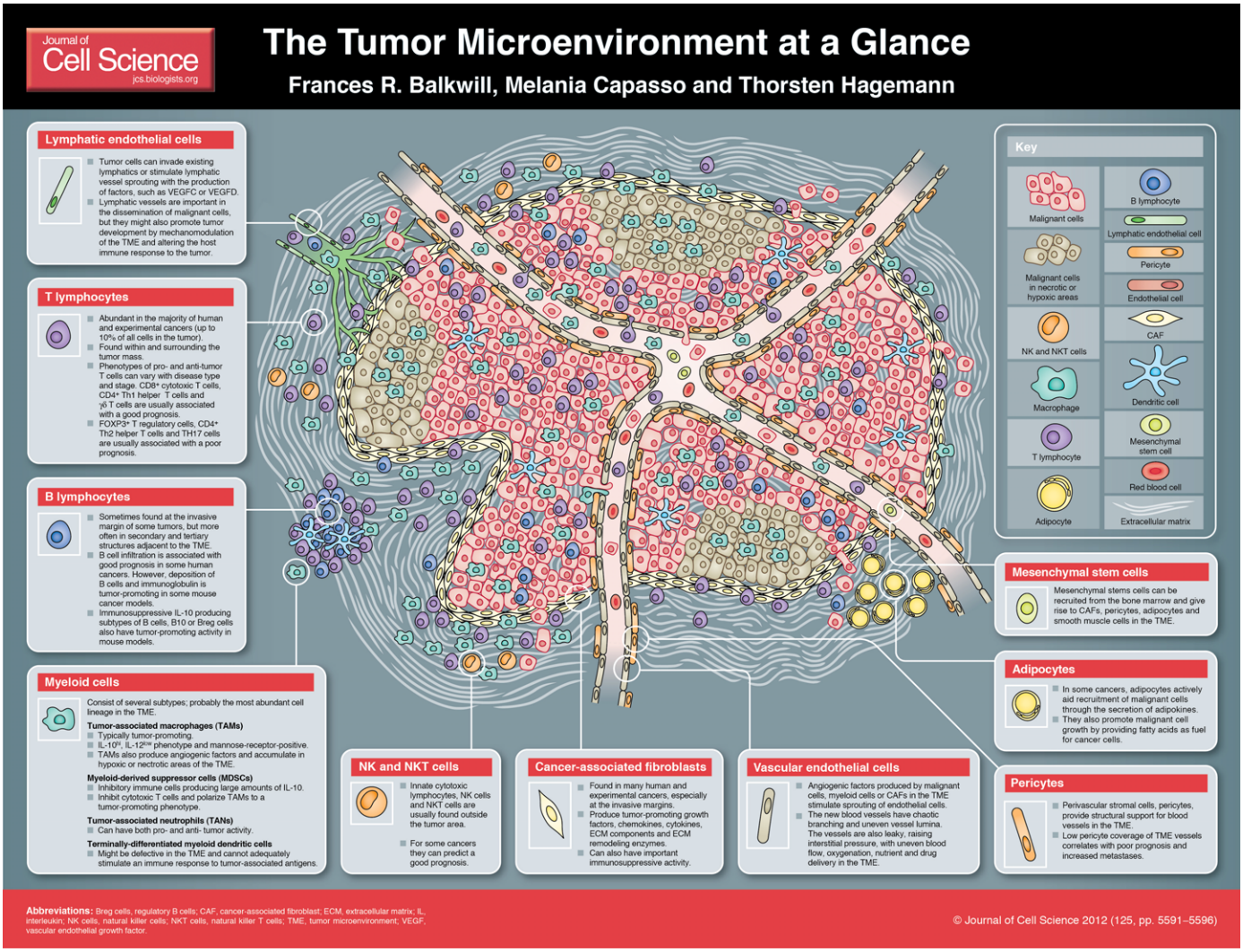
Cancers are not just masses of malignant cells but complex 'rogue' organs, to which many other cells are recruited and can be corrupted by the transformed cells. Interactions between malignant and non-transformed cells create the tumor microenvironment (TME). The non-malignant cells of the TME have a dynamic and often tumor-promoting function at all stages of carcinogenesis (Hanahan and Coussens, 2012). Intercellular communication is driven by a complex and dynamic network of cytokines, chemokines, growth factors, and inflammatory and matrix remodeling enzymes against a background of major perturbations to the physical and chemical properties of the tissue. The evolution, structure and activities of the cells in the TME have many parallels with the processes of wound healing and inflammation, but cells such as macrophages are also found in cancers that have no known association with chronic inflammatory conditions (Grivennikov et al., 2010; Hanahan and Weinberg, 2011; Mantovani et al., 2008). One reason for this is that inflammatory and wound-healing processes are activated downstream of oncogenic mutations in the malignant cells (Mantovani et al., 2008). This Cell Science at a Glance article will describe the functions of major non-malignant cell types that are found in the TME of most human and experimental cancers; the cells of the immune system, the tumor vasculature and lymphatics, as well as fibroblasts, pericytes and adipocytes, and will discuss their importance in cancer development, spread and response to treatment (see poster). The common features of many TMEs suggest that targeting the non-malignant cells, or mediators of their communication, have applications across different tumor types and could also complement other treatment options.

## Cells of the tumor microenvironment

Apart from malignant cells, the TME contains cells of the immune system, the tumor vasculature and lymphatics, as well




*(See poster insert)*

as fibroblasts, pericytes and sometimes adipocytes, which are discussed in detail below. These cells are frequently distinguished by cell-type-specific markers, which are often cell surface molecules. An excellent summary of some of these is given by Joyce and Pollard (Joyce and Pollard, 2009), and some of the commonly used and most informative markers will be detailed below.

### T lymphocytes

There are many different T cell populations within the TME that infiltrate the tumor areas, at the invasive tumor margin and in draining lymphoid organs. Among these, cytotoxic CD8+ memory T cells ($CD8^+CD45RO^+$), which are normally antigen 'experienced' and capable of killing tumor cells, are strongly associated with a good prognosis (Fridman et al., 2012). $CD8^+$ T cells are supported by $CD4^+$ T helper 1 (TH1) cells, which are characterized by the production of the cytokines interleukin-2 (IL-2) and interferon gamma (IFN-γ); high numbers of these in the TME also correlate with a good prognosis (Fridman et al., 2012). Other $CD4^+$ cell populations, such as TH2 cells producing IL-4, IL-5 and IL-13, which support B cell responses, or TH17 cells, producing IL-17A, IL-17F, IL-21 and IL-22 that favor anti-microbial tissue inflammation, are generally thought to promote tumor growth (Fridman et al., 2012), although they have also been associated with a favorable outcome, as in the case of TH2 cells in breast cancer (Yoon et al., 2010) and TH17 cells in esophageal cancers (Lv et al., 2011). The $CD4^+$ T cells most often described as tumor promoting are the immunosuppressive T regulatory cells (Tregs), which are characterized by expression of FOXP3 and CD25 (Hsieh et al., 2012). Constitutive and induced Tregs exert an immune suppressive function through the production of IL-10, transforming growth factor beta (TGF-β) and cell-mediated contact through cytotoxic T-lymphocyte antigen 4 (CTLA4), inhibiting recognition and clearance of tumor cells by the immune system (Campbell and Koch, 2011). High numbers of Tregs in the TME correlate with worse prognosis in many types of cancer (Bates et al., 2006; Curiel et al., 2004; Hiraoka et al., 2006). Tregs can also be tumor suppressive as in some B cell cancers; their presence in Hodgkin's Lymphoma correlates with a good prognosis, presumably through a direct suppression of tumor cell growth (Fozza and Longinotti, 2011; Koreishi et al., 2010; Tzankov et al., 2008).

γδ T lymphocytes have some characteristics of innate rather than adaptive immune cells and show potent cytotoxic activity against a wide range of malignant cells, including cancer stem cells (Gomes et al., 2010; Hannani et al., 2012). Although experimental animal cancer studies suggest they exert immune surveillance activity, it is not yet certain whether the presence of γδ T cells in the TME reflects a good or bad prognosis.

### B lymphocytes

B cells can be found at the invasive margin of tumors, but are more common in draining lymph nodes and lymphoid structures adjacent to the TME. B cell infiltration into the TME is associated with good prognosis in some breast and ovarian cancers (Coronella et al., 2001; Milne et al., 2009); however, this is in contrast to mouse models, in which B cells inhibit tumor-specific cytotoxic T cell responses (Qin et al., 1998). More recent data support a tumor-promoting role for B cells and immunoglobulin deposition in a genetic mouse model of skin cancer (Andreu et al., 2010; de Visser et al., 2005). An immunosuppressive population of IL-10-producing B cells, known as regulatory B cells (Bregs) or B10 cells (Mauri and Bosma, 2012), increases tumor burden and inhibits tumor-specific immune responses in inflammation-induced skin cancer (Schioppa et al., 2011), and also appear to favor lung metastasis in a mouse model of breast cancer (Olkhanud et al., 2011). Bregs also inhibit the clearance of tumor cells by anti-CD20 antibodies in a mouse model of lymphoma (Horikawa et al., 2011). However, none of these effects are due to Bregs infiltrating the TME; instead they appear to affect other immune cells in the surrounding lymphoid tissue or in the draining lymph node (Schioppa et al., 2011), as well as modulate the activity of myeloid cells (Andreu et al., 2010). It remains to be established if B cells and Bregs in particular have similar roles in human cancers.

### NK and NKT cells

Innate cytotoxic lymphocytes, natural killer (NK) cells and natural killer T (NKT) cells, also infiltrate the tumor stroma, but are not found in contact with tumor cells. For many cancers, such as colorectal, gastric, lung, renal and liver, they appear to predict a good prognosis (Tachibana et al., 2005). However, although they are present in the TME, NK cells might not be able to exert their tumor-killing function. A number of studies reported that NK cells in the tumor stroma have an anergic phenotype that is induced by malignant cell-derived transforming growth factor beta (TGF-β) (Fridman et al., 2012).

### Tumor-associated macrophages

Tumor-associated macrophages (TAMs) are abundant in most human and experimental murine cancers and their activities are usually pro-tumorigenic (Qian and Pollard, 2010). According to Condeelis and Pollard, TAMs are obligate partners for malignant cell migration, invasion and metastases (Condeelis and Pollard, 2006). Most TAMs have an IL-$10^{high}$, IL-$12^{low}$ phenotype with expression of the mannose receptor and scavenger receptor class A (SR-A, also known as SCARA) (Biswas and Mantovani, 2010; Mantovani, 2011; Mantovani et al., 2002). There is pre-clinical and clinical evidence that an abundance of TAMs in the TME is associated with poor prognosis (Bingle et al., 2002). Additionally, gene array studies in follicular lymphoma demonstrate that the expression of genes that are associated with a strong 'macrophage' signature confers a poor prognosis, independent of other clinical variables (Dave et al., 2004).

Macrophages are major contributors to tumor angiogenesis (Lin et al., 2006; Zumsteg and Christofori, 2009). Transcriptional profiling on high-density oligonucleotide arrays of TAMs shows that they are highly enriched in transcripts that encode angiogenic molecules (Ojalvo et al., 2010). Comparison of TAM transcriptomes with available clinical databases shows that these transcriptional signatures are predictive of survival (Ojalvo et al., 2010; Zabuawala et al., 2010).

The bidirectional interaction between macrophages and the tumor microenvironment shapes their phenotype and response to the environmental conditions. Tumor hypoxia is important, because many TAMs accumulate in hypoxic and/or necrotic areas of tumors. It is thought that these areas attract TAMs by releasing hypoxia-induced chemoattractants, such as vascular endothelial growth factor (VEGF), endothelins and endothelial-monocyte-activating polypeptide II (EMAP2, also known as AIMP1) (Murdoch et al., 2004). A distinct, hypoxia-induced pro-angiogenic

human macrophage phenotype has been identified (Burke et al., 2002; White et al., 2004).

### Myeloid-derived suppressor cells

Myeloid-derived suppressor cells (MDSCs) are currently defined as a population of inhibitory immune cells that are increased in numbers in a variety of mouse and human cancers (Gabrilovich et al., 2012; Sica and Bronte, 2007). The characterization of human MDSCs is difficult as their phenotype is quite variable. Indeed, they can even differentiate into TAMs (Kusmartsev et al., 2005; Kusmartsev et al., 2004). Murine and human MDSCs inhibit $CD8^+$ T cell activation through the expression of nitric oxide synthase 2 (NOS2) and arginase (ARG1) (Bronte et al., 2003). They also induce the development of Tregs (Huang et al., 2006) and the polarization of macrophages to a TAM-like phenotype (Sinha et al., 2007).

### Dendritic cells

Dendritic cells (DCs) have important functions in antigen processing and presentation (Gabrilovich et al., 2012). The DCs that are found in the TME are thought to be defective, that is, they cannot adequately stimulate an immune response to tumor-associated antigens. The hypoxic and inflammatory microenvironment of the TME further impairs DC function to activate immune function, and some DCs have been found to suppress T cell responses at the tumor site. Two recent studies designate ZBTB46 as a new transcription factor that is specifically expressed in all classical human and murine DCs (Meredith et al., 2012; Satpathy et al., 2012). This work suggests that DCs are a unique immune cell lineage and will help in our understanding of DCs in the TME.

### Tumor-associated neutrophils

The contribution of tumor-associated neutrophils (TANs) to primary tumor growth and metastasis is somewhat controversial. There is evidence that neutrophils promote primary tumor growth in mouse cancer models (Pekarek et al., 1995) and have a pro-tumorigenic effects by enhancing angiogenesis (Nozawa et al., 2006; Shojaei et al., 2008), increasing degradation of the extracellular matrix (ECM) (De Larco et al., 2004) and immune suppression (Youn and Gabrilovich, 2010). Furthermore, CD11b+ bone-marrow-derived cells, a heterogeneous myeloid cell population, have been associated with priming of the premetastatic lung and enhanced seeding of circulating tumor cells (Erler et al., 2009; Yan et al., 2010). By contrast, an antitumor function of these cells has been observed following immunological (Hicks et al., 2006) or cytokine activation (Colombo et al., 1992). Under these conditions, neutrophils can actively eliminate disseminated tumor cells (Granot et al., 2011), as well as indirectly through inhibition of TGF-β (Fridlender et al., 2009).

### Cancer-associated fibroblasts

When tissues are injured, residential fibroblasts differentiate into myofibroblasts in response to paracrine signals (Li and Wang, 2011). The induction of myofibroblasts can also cause organ fibrosis, which enhances the risk of cancer development (Desmoulière et al., 2004; Radisky et al., 2007). Myofibroblasts are abundant in many TMEs and are also called cancer-associated fibroblasts (CAFs) (Sugimoto et al., 2006). CAFs can derive from multiple resident precursors, such as endothelial cells, smooth muscle cells and myoepithelial cells, or mesenchymal stem cells (Brittan et al., 2002; Spaeth et al., 2009; Tomasek et al., 2002; Willis et al., 2006).

CAFs secrete growth factors, such as the EGF family members hepatocyte growth factor (HGF), fibroblast growth factor (FGFs) and insulin-like growth factor 1 (IGF1), which are mitogenic for malignant cells (Brittan et al., 2002; Spaeth et al., 2009; Tomasek et al., 2002; Willis et al., 2006). TGF-β from fibroblasts induces epithelial–mesenchymal transition (EMT) in malignant cells and contributes to the immune-suppressive microenvironment (Erez et al., 2010). Fibroblast-produced CXCL12 chemokine can promote growth and survival of malignant cells and also has chemoattractant properties that stimulate the migration of other stromal cell types and their progenitors into the TME (Orimo et al., 2005). In mouse models of skin, breast and pancreatic tumors, CAFs express a pro-inflammatory gene signature, which contributes to the support of tumor growth by enhancing neovascularisation and the recruitment of immune cells (Orimo et al., 2005). These tumor-promoting effects are abolished upon inhibition of the transcription factor NF-κB, suggesting that, in stromal cells, this inflammatory signaling pathway has an important function in tumor progression (Erez et al., 2010). Another major contribution of fibroblasts to the composition of the TME is their secretion of ECM components and of ECM remodeling enzymes (Erez et al., 2010).

In some cancers, CAFs are arranged in fibrovascular cores that branch throughout the tumor mass, whereas in others, they surround the malignant cells with dense desmoplastic stroma that can occupy the majority of the space and thus restrict the ability of anti-cancer drugs to reach the malignant cell target. An increased density of CAFs is often seen at the invasive front of a tumor (Erez et al., 2010).

A recent study investigated the impact of deleting cells that are positive for the fibroblast marker fibroblast activation protein-α (FAP) in tumor-bearing mice (Kraman et al., 2010). Depletion of these cells induced tumor necrosis that was mediated by IFN-γ and TNF-α, and the authors also showed that FAP-positive TME cells are important mediators of immune suppression (Kraman et al., 2010).

### Adipocytes

In some cancers, for instance intra-abdominal tumors that metastasize to the omentum, adipocytes actively aid the recruitment of malignant cells through the secretion of adipokines and also promote the growth of malignant cells by providing fatty acids as fuel for the cancer cells (Nieman et al., 2011).

### Vascular endothelial cells

Many soluble factors present in the TME, such as VEGFs, FGFs, platelet-derived growth factors (PDGFs) and chemokines stimulate endothelial cells and their associated pericytes during the neovascularization that is needed for cancer growth (Carmeliet and Jain, 2011). When a quiescent blood vessel senses an angiogenic signal from malignant or inflammatory cells, or owing to hypoxic conditions in the TME, angiogenesis is stimulated and new vessels sprout from the existing vasculature (Carmeliet and Jain, 2011). The tumor vasculature is abnormal in almost every aspect of its structure and function (Jain, 2005). For example, blood vessels are heterogeneous with chaotic branching structures and an uneven vessel lumen, and are leaky. The leakiness of the vessels raises the interstitial fluid pressure causing unevenness of blood flow, oxygenation, nutrient and drug distribution in the TME. This, in turn, increases hypoxia and facilitates metastasis. VEGF (also known as VEGFA) is the predominant angiogenic factor in the TME and is produced by

both malignant cells and inflammatory leukocytes; however, advanced tumors can produce a range of other angiogenic factors that can substitute for VEGF (Carmeliet and Jain, 2011).

### Pericytes

Perivascular stromal cells, known as pericytes, are an integral component of the tumor vasculature that provide structural support to blood vessels (Armulik et al., 2011). Clinical studies, for example in bladder and colorectal cancer (O'Keeffe et al., 2008; Yonenaga et al., 2005), suggest that low pericyte coverage of the vasculature correlates with poor prognosis and increased metastases. An explanation for the association of pericyte coverage with poor prognosis has come from a recent study, in which pericyte depletion in mouse genetic models suppressed primary tumor growth, but increased hypoxia, EMT and MET receptor activation (Cooke et al., 2012). Pericyte depletion in these mouse experiments also enhanced metastasis, the authors further showed that low pericyte coverage coupled with activation of the MET receptor correlated with a poor prognosis in women with invasive breast cancer (Cooke et al., 2012). Hence 'normal' pericyte coverage of the tumor vasculature might act as a key negative regulator of metastases.

### Lymphatic endothelial cells

Tumors drive lymphangiogenesis or lymphatic hyperplasia through the production of VEGFC or VEGFD (Alitalo, 2011). Although tumor cells can invade existing lymphatic vessels, if malignant cells or macrophages secrete high levels of VEGFC or VEGFD, the TME will have extensive lymphatic vessel sprouting, enlargement of collecting lymph vessels and lymph node lymphangiogenesis. Lymphatic endothelial cells in the TME and lymphatic vessels formed by them have an important function in the dissemination of malignant cells, there is emerging evidence that they also affect the progression of cancer by mechanically modulating the TME and by altering the host immune response to the tumor (Swartz and Lund, 2012).

## The ECM of the tumor microenvironment

The ECM provides not only a physical scaffold for all cells in the TME but also has a dynamic role in the evolution and spread of cancers, especially as the adhesion of a cell to the ECM is key to its movement out of and into the TME. The ECM also contains key growth factors, such as angiogenic factors and chemokines, that interact with cell surface receptors and give each tissue its tensile and compressive strength and elasticity [see the recent poster article on the ECM (Frantz et al., 2010)]. Tumors are typically stiffer than the surrounding normal tissues owing to an increased ECM deposition by CAFs (Weigelt and Bissell, 2008). Collagen and elastin fibers are reorientated and cross-linked by lysyl oxidase (LOX) and transglutaminase present in the TME, resulting, for example, in larger, more rigid fibrils (Levental et al., 2009). Matrix metalloproteases (MMPs) that degrade ECM proteins are secreted and activated by malignant cells, TAMs and CAFs. MMPs further remodel the ECM, thereby also releasing chemokines and growth and angiogenic factors. Other proteases that are upregulated in the cells of the TME include a large family of cysteine proteases, the cathepsins. Cathepsin L, for instance, processes and activates heparanase, thereby aiding metastasis, angiogenesis and inflammation (Edovitsky et al., 2004; Lerner et al., 2011).

## Targeting the tumor microenvironment

Although there might be heterogeneity in the composition of the TME as discussed above, the common features of many TMEs suggest that targeting the cells that are present, or mediators of their communication have applications across different tumor types and could also complement other treatment options. Indeed, clinical trials with anti-CTLA4 antibodies and other immunotherapy approaches, for instance, are being conducted in several types of advanced cancer (Mellman et al., 2011). Angiogenesis inhibitors, as well as multi-targeted tyrosine kinase inhibitors that impact on VEGF signaling pathways, have been approved for clinical use in a variety of human cancers (Carmeliet and Jain, 2011).

There is a wide range of pre-clinical and clinical approaches aimed at eliminating or reprogramming myeloid cells in the TME (reviewed by Gabrilovich et al., 2012). The tumor ECM might also be a target, especially to increase the response of a tumor to chemotherapy by increasing the access of drugs to the tumor (Provenzano et al., 2012). Finally, our understanding of cancer-related inflammation has reached a point where knowledge is being translated into clinical trials, sometimes using agents that are already under investigation in inflammatory diseases, such as therapeutic antibodies (Balkwill and Mantovani, 2010; Hanahan and Coussens, 2012; Swartz et al., 2012).

## Perspectives

The non-malignant cells of the TME can comprise >50% of the mass of primary tumors and their metastases, but there are still many unanswered questions regarding their biology and function. We know little about evolution of the TME during cancer progression and treatment. It is now clear that in both hematological cancers and solid tumors there is Darwinian evolution of malignant cells, leading to heterogeneous mutations within single tumors and at different sites of metastasis (Yap et al., 2012). This raises important questions, such as is there a similar heterogeneity in the other TME constituents? Is the TME of metastases different from that of matched primary tumors? Can the composition of the TME be modulated by oncogenic mutations? Certainly in breast cancers there is heterogeneity in both malignant and stromal gene-expression signatures (Bertos and Park, 2011). It is also still not clear whether tumors at different sites in the body have a different TME composition, but we do know that there are important differences in the TME between different cancers, both murine and human (e.g. Bertos and Park, 2011). In addition, an aging immune system could have a more tumor-promoting phenotype.

Although VEGF inhibitors, such as bevacizumab, extend disease-free interval in a variety of advanced human cancers, it has been difficult to determine their impact on overall survival, mainly because the hypoxia that is induced by VEGF blockade switches on a more invasive and metastatic program in the malignant cells. In addition, the TME might also evolve to produce other angiogenic factors. Hence, targeting the TME could also stimulate further evolution of the cancer and its resistance to treatment.

We now recognize that cancer chemotherapy and radiotherapy do not just target malignant cells, but that their actions on the TME contribute to success or failure of the treatment. In mouse cancers and clinical trials, there is evidence that chemotherapy is most successful when it causes a form of cell death that stimulates an anti-tumor immune response (Galluzzi et al., 2012). Conversely, chemotherapy stimulates a rapid increase in the infiltration of innate cells into the damaged TME. The addition of chemokine and cytokine receptor

antagonists, or of matrix metalloprotease inhibitors might therefore increase the effectiveness and toxicity profile of traditional chemotherapy (DeNardo et al., 2011; Nakasone et al., 2012).

The importance of the TME in designing new cancer treatment regimes is now apparent. Targeting several different aspects of the TME during cancer treatment might allow us to reach a 'tipping point' where its tumor-promoting and suppressive immune system is disabled or reprogrammed, its chaotic blood supply is normalized or destroyed, and as malignant cells are destroyed, new antigens are uncovered that are recognized by the reawakened immune system.

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A high-resolution version of the poster is available for downloading in the online version of this article at jcs.biologists.org.

## References

**Alitalo, K.** (2011). The lymphatic vasculature in disease. *Nat. Med.* **17**, 1371-1380.

**Andreu, P., Johansson, M., Affara, N. I., Pucci, F., Tan, T., Junankar, S., Korets, L., Lam, J., Tawfik, D., DeNardo, D. G. et al.** (2010). FcRgamma activation regulates inflammation-associated squamous carcinogenesis. *Cancer Cell* **17**, 121-134.

**Armulik, A., Genové, G. and Betsholtz, C.** (2011). Pericytes: developmental, physiological, and pathological perspectives, problems, and promises. *Dev. Cell* **21**, 193-215.

**Balkwill, F. and Mantovani, A.** (2010). Cancer and inflammation: implications for pharmacology and therapeutics. *Clin. Pharmacol. Ther.* **87**, 401-406.

**Bates, G. J., Fox, S. B., Han, C., Leek, R. D., Garcia, J. F., Harris, A. L. and Banham, A. H.** (2006). Quantification of regulatory T cells enables the identification of high-risk breast cancer patients and those at risk of late relapse. *J. Clin. Oncol.* **24**, 5373-5380.

**Bertos, N. R. and Park, M.** (2011). Breast cancer – one term, many entities? *J. Clin. Invest.* **121**, 3789-3796.

**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. and Mantovani, A.** (2010). Macrophage plasticity and interaction with lymphocyte subsets: cancer as a paradigm. *Nat. Immunol.* **11**, 889-896.

**Brittan, M., Hunt, T., Jeffery, R., Poulsom, R., Forbes, S. J., Hodivala-Dilke, K., Goldman, J., Alison, M. R. and Wright, N. A.** (2002). Bone marrow derivation of pericryptal myofibroblasts in the mouse and human small intestine and colon. *Gut* **50**, 752-757.

**Bronte, V., Serafini, P., Mazzoni, A., Segal, D. M. and Zanovello, P.** (2003). L-arginine metabolism in myeloid cells controls T-lymphocyte functions. *Trends Immunol.* **24**, 301-305.

**Burke, B., Tang, N., Corke, K. P., Tazzyman, D., Ameri, K., Wells, M. and Lewis, C. E.** (2002). Expression of HIF-1alpha by human macrophages: implications for the use of macrophages in hypoxia-regulated cancer gene therapy. *J. Pathol.* **196**, 204-212.

**Campbell, D. J. and Koch, M. A.** (2011). Treg cells: patrolling a dangerous neighborhood. *Nat. Med.* **17**, 929-930.

**Carmeliet, P. and Jain, R. K.** (2011). Molecular mechanisms and clinical applications of angiogenesis. *Nature* **473**, 298-307.

**Colombo, M. P., Modesti, A., Parmiani, G. and Forni, G.** (1992). Local cytokine availability elicits tumor rejection and systemic immunity through granulocyte-T-lymphocyte cross-talk. *Cancer Res.* **52**, 4853-4857.

**Condeelis, J. and Pollard, J. W.** (2006). Macrophages: obligate partners for tumor cell migration, invasion, and metastasis. *Cell* **124**, 263-266.

**Cooke, V. G., LeBleu, V. S., Keskin, D., Khan, Z., O'Connell, J. T., Teng, Y., Duncan, M. B., Xie, L., Maeda, G., Vong, S. et al.** (2012). Pericyte depletion results in hypoxia-associated epithelial-to-mesenchymal transition and metastasis mediated by met signaling pathway. *Cancer Cell* **21**, 66-81.

**Coronella, J. A., Telleman, P., Kingsbury, G. A., Truong, T. D., Hays, S. and Junghans, R. P.** (2001). Evidence for an antigen-driven humoral immune response in medullary ductal breast cancer. *Cancer Res.* **61**, 7889-7899.

**Curiel, T. J., Coukos, G., Zou, L., Alvarez, X., Cheng, P., Mottram, P., Evdemon-Hogan, M., Conejo-Garcia, J. R., Zhang, L., Burow, M. et al.** (2004). Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. *Nat. Med.* **10**, 942-949.

**Dave, S. S., Wright, G., Tan, B., Rosenwald, A., Gascoyne, R. D., Chan, W. C., Fisher, R. I., Braziel, R. M., Rimsza, L. M., Grogan, T. M. et al.** (2004). Prediction of survival in follicular lymphoma based on molecular features of tumor-infiltrating immune cells. *N. Engl. J. Med.* **351**, 2159-2169.

**De Larco, J. E., Wuertz, B. R. and Furcht, L. T.** (2004). The potential role of neutrophils in promoting the metastatic phenotype of tumors releasing interleukin-8. *Clin. Cancer Res.* **10**, 4895-4900.

**de Visser, K. E., Korets, L. V. and Coussens, L. M.** (2005). De novo carcinogenesis promoted by chronic inflammation is B lymphocyte dependent. *Cancer Cell* **7**, 411-423.

**DeNardo, D. G., Brennan, D. J., Rexhepaj, E., Ruffell, B., Shiao, S. L., Madden, S. F., Gallagher, W. M., Wadhwani, N., Keil, S. D., Junaid, S. A. et al.** (2011). Leukocyte complexity predicts breast cancer survival and functionally regulates response to chemotherapy. *Cancer Discovery* **1**, 54-67.

**Desmoulière, A., Guyot, C. and Gabbiani, G.** (2004). The stroma reaction myofibroblast: a key player in the control of tumor cell behavior. *Int. J. Dev. Biol.* **48**, 509-517.

**Edovitsky, E., Elkin, M., Zcharia, E., Peretz, T. and Vlodavsky, I.** (2004). Heparanase gene silencing, tumor invasiveness, angiogenesis, and metastasis. *J. Natl. Cancer Inst.* **96**, 1219-1230.

**Erez, N., Truitt, M., Olson, P., Arron, S. T. and Hanahan, D.** (2010). Cancer-associated fibroblasts are activated in incipient neoplasia to orchestrate tumor-promoting inflammation in an NF-kappaB-dependent manner. *Cancer Cell* **17**, 135-147.

**Erler, J. T., Bennewith, K. L., Cox, T. R., Lang, G., Bird, D., Koong, A., Le, Q. T. and Giaccia, A. J.** (2009). Hypoxia-induced lysyl oxidase is a critical mediator of bone marrow cell recruitment to form the premetastatic niche. *Cancer Cell* **15**, 35-44.

**Fozza, C. and Longinotti, M.** (2011). T-cell traffic jam in Hodgkin's lymphoma: pathogenetic and therapeutic implications. *Adv. Hematol.* **2011**, 501659.

**Frantz, C., Stewart, K. M. and Weaver, V. M.** (2010). The extracellular matrix at a glance. *J. Cell Sci.* **123**, 4195-4200.

**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.

**Fridman, W. H., Pagès, F., Sautès-Fridman, C. and Galon, J.** (2012). The immune contexture in human tumours: impact on clinical outcome. *Nat. Rev. Cancer* **12**, 298-306.

**Gabrilovich, D. I., Ostrand-Rosenberg, S. and Bronte, V.** (2012). Coordinated regulation of myeloid cells by tumours. *Nat. Rev. Immunol.* **12**, 253-268.

**Galluzzi, L., Senovilla, L., Zitvogel, L. and Kroemer, G.** (2012). The secret ally: immunostimulation by anticancer drugs. *Nat. Rev. Drug Discov.* **11**, 215-233.

**Gomes, A. Q., Martins, D. S. and Silva-Santos, B.** (2010). Targeting γδ T lymphocytes for cancer immunotherapy: from novel mechanistic insight to clinical application. *Cancer Res.* **70**, 10024-10027.

**Granot, Z., Henke, E., Comen, E. A., King, T. A., Norton, L. and Benezra, R.** (2011). Tumor entrained neutrophils inhibit seeding in the premetastatic lung. *Cancer Cell* **20**, 300-314.

**Grivennikov, S. I., Greten, F. R. and Karin, M.** (2010). Immunity, inflammation, and cancer. *Cell* **140**, 883-899.

**Hanahan, D. and Coussens, L. M.** (2012). Accessories to the crime: functions of cells recruited to the tumor microenvironment. *Cancer Cell* **21**, 309-322.

**Hanahan, D. and Weinberg, R. A.** (2011). Hallmarks of cancer: the next generation. *Cell* **144**, 646-674.

**Hannani, D., Ma, Y., Yamazaki, T., Déchanet-Merville, J., Kroemer, G. and Zitvogel, L.** (2012). Harnessing γδ T cells in anticancer immunotherapy. *Trends Immunol.* **33**, 199-206.

**Hicks, A. M., Riedlinger, G., Willingham, M. C., Alexander-Miller, M. A., Von Kap-Herr, C., Pettenati, M. J., Sanders, A. M., Weir, H. M., Du, W., Kim, J. et al.** (2006). Transferable anticancer innate immunity in spontaneous regression/complete resistance mice. *Proc. Natl. Acad. Sci. USA* **103**, 7753-7758.

**Hiraoka, N., Onozato, K., Kosuge, T. and Hirohashi, S.** (2006). Prevalence of FOXP3+ regulatory T cells increases during the progression of pancreatic ductal adenocarcinoma and its premalignant lesions. *Clin. Cancer Res.* **12**, 5423-5434.

**Horikawa, M., Minard-Colin, V., Matsushita, T. and Tedder, T. F.** (2011). Regulatory B cell production of IL-10 inhibits lymphoma depletion during CD20 immunotherapy in mice. *J. Clin. Invest.* **121**, 4268-4280.

**Hsieh, C. S., Lee, H. M. and Lio, C. W.** (2012). Selection of regulatory T cells in the thymus. *Nat. Rev. Immunol.* **12**, 157-167.

**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.

**Jain, R. K.** (2005). Normalization of tumor vasculature: an emerging concept in antiangiogenic therapy. *Science* **307**, 58-62.

**Joyce, J. A. and Pollard, J. W.** (2009). Microenvironmental regulation of metastasis. *Nat. Rev. Cancer* **9**, 239-252.

**Koreishi, A. F., Saenz, A. J., Persky, D. O., Cui, H., Moskowitz, A., Moskowitz, C. H. and Teruya-Feldstein, J.** (2010). The role of cytotoxic and regulatory T cells in relapsed/refractory Hodgkin lymphoma. *Appl. Immunohistochem. Mol. Morphol.* **18**, 206-211.

**Kraman, M., Bambrough, P. J., Arnold, J. N., Roberts, E. W., Magiera, L., Jones, J. O., Gopinathan, A., Tuveson, D. A. and Fearon, D. T.** (2010). Suppression of antitumor immunity by stromal cells expressing fibroblast activation protein-alpha. *Science* **330**, 827-830.

**Kusmartsev, S., Nefedova, Y., Yoder, D. and Gabrilovich, D. I.** (2004). Antigen-specific inhibition of CD8+ T cell response by immature myeloid cells in cancer is mediated by reactive oxygen species. *J. Immunol.* **172**, 989-999.

**Kusmartsev, S., Nagaraj, S. and Gabrilovich, D. I.** (2005). Tumor-associated CD8+ T cell tolerance induced

by bone marrow-derived immature myeloid cells. *J. Immunol.* **175**, 4583-4592.

**Lerner, I., Hermano, E., Zcharia, E., Rodkin, D., Bulvik, R., Doviner, V., Rubinstein, A. M., Ishai-Michaeli, R., Atzmon, R., Sherman, Y. et al.** (2011). Heparanase powers a chronic inflammatory circuit that promotes colitis-associated tumorigenesis in mice. *J. Clin. Invest.* **121**, 1709-1721.

**Levental, K. R., Yu, H., Kass, L., Lakins, J. N., Egeblad, M., Erler, J. T., Fong, S. F., Csiszar, K., Giaccia, A., Weninger, W. et al.** (2009). Matrix crosslinking forces tumor progression by enhancing integrin signaling. *Cell* **139**, 891-906.

**Li, B. and Wang, J. H.** (2011). Fibroblasts and myofibroblasts in wound healing: force generation and measurement. *J. Tissue Viability* **20**, 108-120.

**Lin, E. Y., Li, J.-F., Gnatovskiy, L., Deng, Y., Zhu, L., Grzesik, D. A., Qian, H., Xue, X.-N. and Pollard, J. W.** (2006). Macrophages regulate the angiogenic switch in a mouse model of breast cancer. *Cancer Res.* **66**, 11238-11246.

**Lv, L., Pan, K., Li, X. D., She, K. L., Zhao, J. J., Wang, W., Chen, J. G., Chen, Y. B., Yun, J. P. and Xia, J. C.** (2011). The accumulation and prognosis value of tumor infiltrating IL-17 producing cells in esophageal squamous cell carcinoma. *PLoS ONE* **6**, e18219.

**Mantovani, A.** (2011). B cells and macrophages in cancer: yin and yang. *Nat. Med.* **17**, 285-286.

**Mantovani, A., Sozzani, S., Locati, M., Allavena, P. and Sica, A.** (2002). Macrophage polarization: tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. *Trends Immunol.* **23**, 549-555.

**Mantovani, A., Allavena, P., Sica, A. and Balkwill, F.** (2008). Cancer-related inflammation. *Nature* **454**, 436-444.

**Mauri, C. and Bosma, A.** (2012). Immune regulatory function of B cells. *Annu. Rev. Immunol.* **30**, 221-241.

**Mellman, I., Coukos, G. and Dranoff, G.** (2011). Cancer immunotherapy comes of age. *Nature* **480**, 480-489.

**Meredith, M. M., Liu, K., Darrasse-Jeze, G., Kamphorst, A. O., Schreiber, H. A., Guermonprez, P., Idoyaga, J., Cheong, C., Yao, K. H., Niec, R. E. et al.** (2012). Expression of the zinc finger transcription factor zDC (Zbtb46, Btbd4) defines the classical dendritic cell lineage. *J. Exp. Med.* **209**, 1153-1165.

**Milne, K., Köbel, M., Kalloger, S. E., Barnes, R. O., Gao, D., Gilks, C. B., Watson, P. H. and Nelson, B. H.** (2009). Systematic analysis of immune infiltrates in high-grade serous ovarian cancer reveals CD20, FoxP3 and TIA-1 as positive prognostic factors. *PLoS ONE* **4**, e6412.

**Murdoch, C., Giannoudis, A. and Lewis, C. E.** (2004). Mechanisms regulating the recruitment of macrophages into hypoxic areas of tumors and other ischemic tissues. *Blood* **104**, 2224-2234.

**Nakasone, E. S., Askautrud, H. A., Kees, T., Park, J. H., Plaks, V., Ewald, A. J., Fein, M., Rasch, M. G., Tan, Y. X., Qiu, J. et al.** (2012). Imaging tumor-stroma interactions during chemotherapy reveals contributions of the microenvironment to resistance. *Cancer Cell* **21**, 488-503.

**Nieman, K. M., Kenny, H. A., Penicka, C. V., Ladanyi, A., Buell-Gutbrod, R., Zillhardt, M. R., Romero, I. L., Carey, M. S., Mills, G. B., Hotamisligil, G. S. et al.** (2011). Adipocytes promote ovarian cancer metastasis and provide energy for rapid tumor growth. *Nat. Med.* **17**, 1498-1503.

**Nozawa, H., Chiu, C. and Hanahan, D.** (2006). Infiltrating neutrophils mediate the initial angiogenic switch in a mouse model of multistage carcinogenesis. *Proc. Natl. Acad. Sci. USA* **103**, 12493-12498.

**O'Keeffe, M. B., Devlin, A. H., Burns, A. J., Gardiner, T. A., Logan, I. D., Hirst, D. G. and McKeown, S. R.** (2008). Investigation of pericytes, hypoxia, and vascularity in bladder tumors: association with clinical outcomes. *Oncol. Res.* **17**, 93-101.

**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.

**Olkhanud, P. B., Damdinsuren, B., Bodogai, M., Gress, R. E., Sen, R., Wejksza, K., Malchinkhuu, E., Wersto, R. P. and Biragyn, A.** (2011). Tumor-evoked regulatory B cells promote breast cancer metastasis by converting resting $CD4^+$ T cells to T-regulatory cells. *Cancer Res.* **71**, 3505-3515.

**Orimo, A., Gupta, P. B., Sgroi, D. C., Arenzana-Seisdedos, F., Delaunay, T., Naeem, R., Carey, V. J., Richardson, A. L. and Weinberg, R. A.** (2005). Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion. *Cell* **121**, 335-348.

**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.

**Provenzano, P. P., Cuevas, C., Chang, A. E., Goel, V. K., Von Hoff, D. D. and Hingorani, S. R.** (2012). Enzymatic targeting of the stroma ablates physical barriers to treatment of pancreatic ductal adenocarcinoma. *Cancer Cell* **21**, 418-429.

**Qian, B. Z. and Pollard, J. W.** (2010). Macrophage diversity enhances tumor progression and metastasis. *Cell* **141**, 39-51.

**Qin, Z., Richter, G., Schüler, T., Ibe, S., Cao, X. and Blankenstein, T.** (1998). B cells inhibit induction of T cell-dependent tumor immunity. *Nat. Med.* **4**, 627-630.

**Radisky, D. C., Kenny, P. A. and Bissell, M. J.** (2007). Fibrosis and cancer: do myofibroblasts come also from epithelial cells via EMT? *J. Cell. Biochem.* **101**, 830-839.

**Satpathy, A. T., Kc, W., Albring, J. C., Edelson, B. T., Kretzer, N. M., Bhattacharya, D., Murphy, T. L. and Murphy, K. M.** (2012). Zbtb46 expression distinguishes classical dendritic cells and their committed progenitors from other immune lineages. *J. Exp. Med.* **209**, 1135-1152.

**Schioppa, T., Moore, R., Thompson, R. G., Rosser, E. C., Kulbe, H., Nedospasov, S., Mauri, C., Coussens, L. M. and Balkwill, F. R.** (2011). B regulatory cells and the tumor-promoting actions of TNF-α during squamous carcinogenesis. *Proc. Natl. Acad. Sci. USA* **108**, 10662-10667.

**Shojaei, F., Singh, M., Thompson, J. D. and Ferrara, N.** (2008). Role of Bv8 in neutrophil-dependent angiogenesis in a transgenic model of cancer progression. *Proc. Natl. Acad. Sci. USA* **105**, 2640-2645.

**Sica, A. and Bronte, V.** (2007). Altered macrophage differentiation and immune dysfunction in tumor development. *J. Clin. Invest.* **117**, 1155-1166.

**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.

**Spaeth, E. L., Dembinski, J. L., Sasser, A. K., Watson, K., Klopp, A., Hall, B., Andreeff, M. and Marini, F.** (2009). Mesenchymal stem cell transition to tumor-associated fibroblasts contributes to fibrovascular network expansion and tumor progression. *PLoS ONE* **4**, e4992.

**Sugimoto, H., Mundel, T. M., Kieran, M. W. and Kalluri, R.** (2006). Identification of fibroblast heterogeneity in the tumor microenvironment. *Cancer Biol. Ther.* **5**, 1640-1646.

**Swartz, M. A. and Lund, A. W.** (2012). Lymphatic and interstitial flow in the tumour microenvironment: linking mechanobiology with immunity. *Nat. Rev. Cancer* **12**, 210-219.

**Swartz, M. A., Iida, N., Roberts, E. W., Sangaletti, S., Wong, M. H., Yull, F. E., Coussens, L. M. and DeClerck, Y. A.** (2012). Tumor microenvironment complexity: emerging roles in cancer therapy. *Cancer Res.* **72**, 2473-2480.

**Tachibana, T., Onodera, H., Tsuruyama, T., Mori, A., Nagayama, S., Hiai, H. and Imamura, M.** (2005). Increased intratumor Valpha24-positive natural killer T cells: a prognostic factor for primary colorectal carcinomas. *Clin. Cancer Res.* **11**, 7322-7327.

**Tomasek, J. J., Gabbiani, G., Hinz, B., Chaponnier, C. and Brown, R. A.** (2002). Myofibroblasts and mechano-regulation of connective tissue remodelling. *Nat. Rev. Mol. Cell Biol.* **3**, 349-363.

**Tzankov, A., Meier, C., Hirschmann, P., Went, P., Pileri, S. A. and Dirnhofer, S.** (2008). Correlation of high numbers of intratumoral FOXP3+ regulatory T cells with improved survival in germinal center-like diffuse large B-cell lymphoma, follicular lymphoma and classical Hodgkin's lymphoma. *Haematologica* **93**, 193-200.

**Weigelt, B. and Bissell, M. J.** (2008). Unraveling the microenvironmental influences on the normal mammary gland and breast cancer. *Semin. Cancer Biol.* **18**, 311-321.

**White, J. R., Harris, R. A., Lee, S. R., Craigon, M. H., Binley, K., Price, T., Beard, G. L., Mundy, C. R. and Naylor, S.** (2004). Genetic amplification of the transcriptional response to hypoxia as a novel means of identifying regulators of angiogenesis. *Genomics* **83**, 1-8.

**Willis, B. C., duBois, R. M. and Borok, Z.** (2006). Epithelial origin of myofibroblasts during fibrosis in the lung. *Proc. Am. Thorac. Soc.* **3**, 377-382.

**Yan, H. H., Pickup, M., Pang, Y., Gorska, A. E., Li, Z., Chytil, A., Geng, Y., Gray, J. W., Moses, H. L. and Yang, L.** (2010). Gr-1+CD11b+ myeloid cells tip the balance of immune protection to tumor promotion in the premetastatic lung. *Cancer Res.* **70**, 6139-6149.

**Yap, T. A., Gerlinger, M., Futreal, P. A., Pusztai, L. and Swanton, C.** (2012). Intratumor heterogeneity: seeing the wood for the trees. *Sci. Transl. Med.* **4**, 127.

**Yonenaga, Y., Mori, A., Onodera, H., Yasuda, S., Oe, H., Fujimoto, A., Tachibana, T. and Imamura, M.** (2005). Absence of smooth muscle actin-positive pericyte coverage of tumor vessels correlates with hematogenous metastasis and prognosis of colorectal cancer patients. *Oncology* **69**, 159-166.

**Yoon, N. K., Maresh, E. L., Shen, D., Elshimali, Y., Apple, S., Horvath, S., Mah, V., Bose, S., Chia, D., Chang, H. R. et al.** (2010). Higher levels of GATA3 predict better survival in women with breast cancer. *Hum. Pathol.* **41**, 1794-1801.

**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.

**Zabuawala, T., Taffany, D. A., Sharma, S. M., Merchant, A., Adair, B., Srinivasan, R., Rosol, T. J., Fernandez, S., Huang, K., Leone, G. et al.** (2010). An ets2-driven transcriptional program in tumor-associated macrophages promotes tumor metastasis. *Cancer Res.* **70**, 1323-1333.

**Zumsteg, A. and Christofori, G.** (2009). Corrupt policemen: inflammatory cells promote tumor angiogenesis. *Curr. Opin. Oncol.* **21**, 60-70.