Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Pharmacology

Leukocyte trafficking in tumor microenvironment

Annalisa Del Prete[1,2], Tiziana Schioppa[1], Laura Tiberio[1], Helena Stabile[3] and Silvano Sozzani[1,2]

The tumor microenvironment consists of both malignant and non-malignant cells and a plethora of soluble mediators. Different types of tumors have specific tumor microenvironments characterized by distinct chemokines and chemotactic factors that influence leukocyte recruitment. The immune cell infiltrate continuously interacts with stroma cells and influence tumor growth. Emerging evidence suggests that the regulation of the composition and the metabolic state of tumor-associated leukocytes may represent a new promising intervention strategy. Here we summarize the current knowledge on the role of tumor-associated immune cells in tumor growth and dissemination, with a specific focus on the nature of the chemotactic factors responsible for their accumulation and activation in tumors.

Addresses
[1] Department of Molecular and Translational Medicine, University of Brescia, Brescia, Italy
[2] IRCCS-Humanitas Clinical and Research Center, Rozzano-Milan, Italy
[3] Department of Molecular Medicine, Istituto Pasteur-Fondazione Cenci Bolognetti, "Sapienza" University of Rome, Italy

Corresponding author: Sozzani, Silvano (silvano.sozzani@unibs.it)

Current Opinion in Pharmacology 2017, **35**:40–47

This review comes from a themed issue on **Cancer**

Edited by **Antonio Sica**

http://dx.doi.org/10.1016/j.coph.2017.04.008

Introduction

Non-resolving inflammation is considered to be one of the hallmarks of cancer [1••]. Tumor microenvironment (TME) includes resident stromal components, such as fibroblasts and endothelial cells, and recruited immune cells, including myeloid and lymphoid cells, as well as bone marrow-derived precursors [2••]. Cancer cells continuously cross talk with stromal cells and TME components do not act as passive bystanders, but are able to orchestrate and shape cancer progression as well as the therapeutic response [3••,4].

Leukocyte recruitment into tumors depends on the local production of chemotactic factors and on the regulation of their cognate receptors expressed by leukocytes [3••,5]. Chemotactic receptors represent a family of G protein-coupled seven transmembrane surface receptors (GPCRs) [6•]. With approximately 50 proteins, chemokines are the largest subset of chemotactic proteins [6•] with many of them actively produced by tumor cells. Infiltrating leukocytes promote tumor growth and sustain chronic inflammation stimulating tumor cell proliferation, angiogenesis and promoting metastases [7••]. Innate and adaptive immune cells also contribute to the formation of tumor leukocyte infiltrate and to a state of immune surveillance [8]. The balance between these pro- versus anti-tumor responses within TME can have a significant impact on clinical outcomes.

This review summarizes the contribution of TME-released chemotactic factors, responsible for leukocyte recruitment and activation, with the belief that the better understanding of the role of these signals will provide insights into the development of new therapeutic approaches.

Macrophages

During tumor progression several types of inflammatory cells are recruited from the blood into the tumor mass. Circulating monocytes can respond to a plethora of chemokines and growth factors produced by the tumor cells and by the components of TME, such as endothelial cells, pericytes, immune cells, fibroblasts, stem cells and progenitor cells. Recognized chemokines active in this process include CCL2, CCL5, CX3CL1 and CXCL12. Recently, the role of other molecules, such as CSF-1/M-CSF, CSF-2/GM-CSF and oxysterols in monocyte recruitment, maturation and polarization has also emerged [9,10]. Although the vast majority of tumor-associated macrophages (TAM) seem to originate from monocytes, the role and the interplay between tissue-resident and monocyte-derived macrophages may deserve further investigation [11,12].

TAM are characterized by a high heterogeneity and plasticity and may interact in a complex bidirectional way with tumor cells, cancer stem cells and stroma cells [7••]. The nomenclature used to define macrophage functional plasticity in response to environmental signals has been recently reviewed and is based on (i) the source of the macrophages; (ii) the definition of the activators and (iii) the markers used to describe macrophage activation [13]. Indeed, when blood-derived monocytes infiltrate tumors, they differentiate into macrophages and

under the influence of the TME, can polarize into M1 or M2 macrophages, which differ in their profile of cytokine secretion (*i.e.*, IL-12^{high}IL-10^{low} vs. IL-12^{low}IL-10^{high}, respectively), chemokine repertoire (*e.g.*, CXCL9/CXCL10 vs. CCL17/CCL22, respectively), iron, glucose and folate metabolism, and scavenger receptor expression [13]. M1- and M2-polarized macrophages, as they are usually referred to in the literature, are extremes of a continuum in a universe of functional different states [13,14]. In tumors additional factors relevant to their functional polarization include IL-4 and IL-13, produced by Th2 cells, IL-10, produced by T regulatory (Treg) and B cells, other cytokines (*e.g.*, TGF-β and CSF-1), immune complexes, hypoxia and metabolic products of cancer cells (*e.g.*, lactic acid) [15–18].

TAM can directly interact with cancer cells leading to epithelial-to-mesenchymal transition, increased cell mobility, intravasation and favoring cancer cell extravasation to metastatic sites [19,20]. In addition, TAM play a crucial role in promoting tumor angiogenesis and tissue remodeling. Distinct types of perivascular macrophages have been associated with metastasis and recently it was shown that in hypoxic TAM, alterations of glucose metabolism can normalize the altered structure of tumor blood vessels and reduce metastasis [21]. Experimental and clinical evidence has shown that TAM have an immunosuppressive phenotype and promote tumor growth. Accordingly, their abundance in the tumor environment is generally, but not always, associated with poor prognosis [1••,3••,7••,16]. These observations have boosted research for therapies aimed at controlling TAM recruitment and activation in TME (Figure 1).

Neutrophils

Neutrophils are the most abundant circulating leukocytes and play a crucial role in host defense [22,23]. As part of the TME, neutrophils display high degree of plasticity, since they can polarize into cytotoxic, antitumor cells (N1) or in cells promoting tumor growth (N2), depending on the local microenvironment [24••,25]. Increased number of circulating neutrophils has been observed both in tumor bearing mice and in advanced cancer patients, where they are associated to poor outcome [3••,25,26]. Therefore, tumor associated neutrophils (TAN) may represent a prognostic factor and a marker of therapeutic responses in solid tumors [26–28].

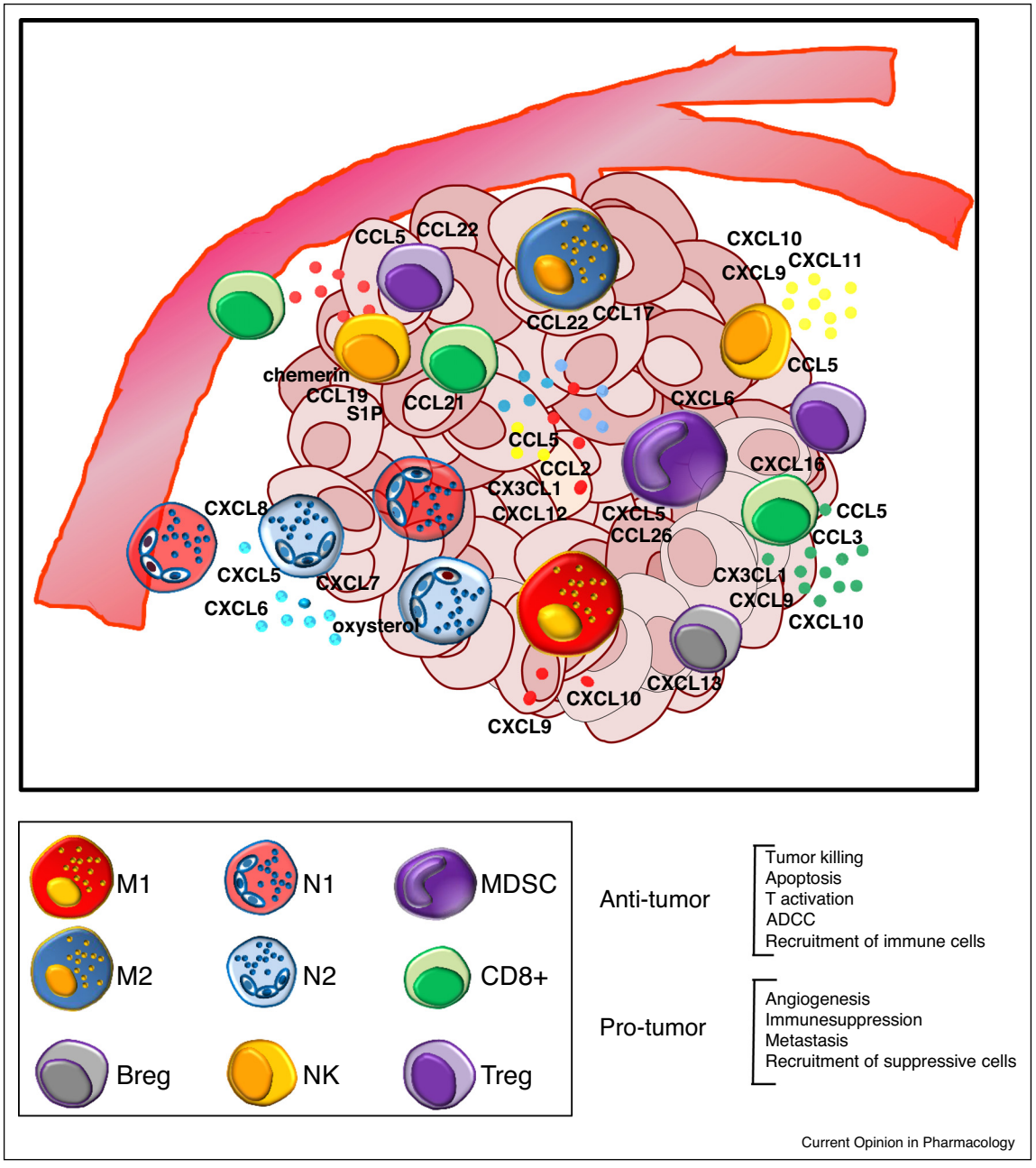
Neutrophils are actively recruited to the TME by chemotactic gradients originating within the tumor mass [29]. The main chemotactic receptors expressed by neutrophils and responsible for their migration into tumors, are CXCR1 and CXCR2, with their ligands (*e.g.*, CXCL8, CXCL5, CXCL6 and CXCL7) being abundantly produced by tumor cells. For instance, CXCL8 was shown to regulate TAN infiltration in Ras-driven cancers [30]. CXCL5 overexpression was observed in hepatocellular carcinoma and contributed to the epithelial-mesenchymal transition in this cancer [31,32]. CXCL6 is important for the recruitment of neutrophils in melanoma tumors [33]. CXCL7 is involved in liver metastasis of colon cancer and is associated to poor survival [34]. A tumor-derived lipid, the liver X receptor ligand oxysterol was recently shown to promote CXCR2-mediated neutrophil recruitment and tumor growth in murine models of lymphoma and lung cancer [35•]. A positive role in tumor growth was also described for other mediators involved in neutrophil recruitment, including the bioactive lipid sphingosine-1 phosphate [36], VEGF and MMP9 (two hypoxia-inducible factor-1 downstream products) [37,38], the myeloid related proteins S100A8 and S100A9 [39] and the cytokine IL-17 [40,41]. The role of neutrophils in the control of tumor growth is further supported by the presence of oncogenic alterations associated with neutrophil infiltration. For instance, the oncogene MET is required for the recruitment of anti-tumor neutrophils [42], while the deficiency of the tumor suppressor LKB1 is responsible for the recruitment of pro-tumor neutrophils in a mouse model of lung adenocarcinoma [43].

Dendritic cells

Infiltration of antigen presenting dendritic cells (DC) in TME is an early sign of tumor-triggered host immune response. DC differentiate from bone marrow precursors under the influence of specific transcription factors that drive the development of different subsets, such as conventional (cDC) and plasmacytoid (pDC) DC [44••]. Different chemokines are apparently involved in DC recruitment. For instance CCL3 was found to be involved in the recruitment of pre-DC from the bone marrow to TME [45]. In hepatocellular carcinoma, tumor infiltrating DC express the chemokine receptors CCR1 and CCR5 [46], whereas the CCR6/CCL20 axis is responsible for the recruitment of pDC in melanoma [47]. Differentiation of DC from precursors, recruited into the TME by a CCL2/CCR2-dependent mechanism, was described to be induced by anticancer chemotherapy [48,49]. The expression of CCR7 by tumor-infiltrating CD141$^+$ DC correlated with a better prognosis [50]. In addition, Flt3L-driven intra-tumor expansion of CD103$^+$ DC, the mouse counterpart of human CD141$^+$ DC, was recently shown to enhance tumor response to anti-PD-L1 immunotherapy [51].

Although DC subsets with strong antigen-presenting activity have been identified in cancer [52], DC with immunosuppressive regulatory activity are mostly found in advanced solid tumors of different histological origin [53,54]. DC with a suppressive phenotype were identified in different ovarian cancer models [55] and a pro-tumor phenotype was recently described in lung DC by transcriptome analysis [56]. Finally, in different tumors tolerogenic pDC were also described and shown to

Figure 1



Leukocyte recruitment in the tumor microenvironment.
Representation of the main chemotactic factors responsible for the recruitment of tumor infiltrating cells. The composition of immune cells in the tumor microenvironment can affect the outcome of tumor development and therapy. These cells can drive both pro- and anti-tumor activities as indicated.

promote tumor progression through the induction of regulatory T cells [57].

Natural killer cells

Natural killer (NK) cells are part of the emerging population of innate lymphocytes (ILC). NK cells act both as immunoregulators and cytotoxic effector cells against virus-infected and transformed cells [58,59]. Circulating human NK cells can be divided in two major subsets: the high cytokine producers $CD56^{high}CD16^{+/-}$ NK cells and the main cytotoxic $CD56^{low}CD16^{high}$ NK cell population [60••,61]. These two subsets are endowed with distinct chemokine receptors and adhesion molecules [62]. In particular $CD56^{low}CD16^{high}$ NK cells express chemokines and homing receptors (*i.e.*, CXCR1, $CX3CR1^{high}$ and CD62L, CXCR2 and $CXCR3^{low}$)

driving their preferential recruitment to inflamed peripheral tissues, while $CD56^{high}CD16^{+/-}$ NK cells express high levels of CXCR3, CCR7, CCR5 and CD62L that are all responsible for their preferential homing to secondary lymphoid organs [63]. In addition to these, other chemotactic factors, such as chemerin and the sphingosine 1-phosphate (S1P) can direct NK cells during inflammation and in steady-state conditions [64,65]. In solid tumors an enrichment of the $CD56^{high}CD16^{+/-}$ subset is usually observed. This is the case in lung cancer, where CXCL9, CXCL10 and CXCL11, the CXCR3 ligands, a main chemokine receptor expressed by $CD56^{high}CD16^{+/-}$ NK cells, were also found upregulated [66,67]. Conversely, in the same tumor, CXCL2, a chemokine active on the recruitment of $CD56^{low}$ NK cells, was downregulated. Similarly, a higher frequency of $CD56^{high}CD16^{+/-}$ NK cells is found in breast cancer and correlates with increased local levels of CCL5 and CCL19. In patients with melanoma, metastatic lymph nodes are characterized by the prevalence of a population of $CD56^{low}CD57^{+}KIRs^{+}CD69^{+}$ NK cells expressing CCR7, the lymph node homing chemokine receptor [68]. Haematological malignancies mainly originate in the BM, also the main site of NK cell generation. CXCL12/CXCR4 and CXCL10/CXCR3 represents two important chemokine axes guiding ingress/egress of NK cells from the BM. In BM plasma samples from patients with active multiple myeloma a decreased concentration of CXCL12 and an up-regulation of CXCR3 ligands (CXCL9, CXCL10 and CXCL11) was correlated with the altered distribution of BM NK cell subsets [69]. The role of tumor-infiltrating NK cells in cancer progression has not been fully elucidated. Several studies have demonstrated a pro-tumorigenic phenotype of cancer infiltrating NK cells due to their ability to release angiogenic factors and immunosuppressive cytokines [70]. In tumor microenvironment both cancer cells and other tumor-resident immune cells may alter the NK cell phenotype and impair their cytotoxic activity thus promoting tumor evasion [71]. Notably in this context the membrane expression of TGF-β by Treg cells seems to be the principal mechanism of NK cell suppression [72,73]. Moreover T regulatory cells can affect NK cell proliferation and activation by depriving IL-2 released by activated $CD4^{+}$ T cells [74].

Adaptive immune effectors cells

During the past ten years, the quantification of lymphocytes in large histopathological collections coupled to bioinformatics analyses has demonstrated, in most solid cancers, a positive correlation between the number of infiltrating effector T lymphocytes ($CD8^{+}$ cytotoxic lymphocytes and Th1 cells) and patient survival [2••,75]. Poor lymphocyte infiltration represents one main reason for the immune failure in the eradication of cancer cells [76]. More complex is the understanding of the role in tumor progression of tumor-infiltrating B cells. Though, the presence of B cells and tumour-reactive antibodies correlates with extended patient survival in many human cancers and in preclinical experimental models [77].

Tumor-specific cytotoxic T cells are recruited in response to the pro-inflammatory chemokines CCL3 and CCL5 [78,79]. However, these chemokines also attract immunosuppressive cells, such as TAM and Tregs and this may explain why their role in the antitumor response remains controversial [80]. Several studies demonstrated that the intratumor expression of CXCR3 ligands (*e.g.*, CXCL9 and CXCL10) correlates with the infiltration of effector T cells and increase of patient survival [81–83]. CXCL9 and CXCL10 are the main inflammatory chemokines attracting Th1, $CD8^{+}$ T cells and NK cells. Of relevance, these chemokines also exert anti-angiogenic functions. In colorectal cancer, high levels of CX3CL1 and CXCL16 in the tumor correlated with higher cytotoxic T cells, tumor infiltration and better prognosis [84].

In preclinical studies, two homeostatic chemokines, namely CCL19 and CCL21, were shown to reduce tumor burden and were proposed as immunotherapeutic agents [85]. In this case, the antitumor effect might be mediated by the concomitant attraction of dendritic cells and naïve T cells in tertiary lymphoid structures. Indeed CCL19 and CCL21, together with CXCL13 (a chemokine active on naïve B cells), are overexpressed in tumor-associated lymphoid tertiary structures as compared to tumor areas [86]. Tertiary lymphoid structures were recently demonstrated to represent niches where local antitumor T- and B-cell response is initiated and maintained [87••].

Myeloid and lymphoid regulatory cells

Over the last decade, much progress has been made to characterize the cells responsible for tumor-induced immunosuppression. Myeloid-derived suppressor cells (MDSCs) are a heterogeneous mixture of immature myeloid cells with either monocytic or granulocytic morphology [88,89]. MDSCs have a tumor-promoting action by suppressing antitumor effector cells and by the production of iNOS and arginase [90]. The heterogeneity of this cell population is mirrored by the complex variety of chemokines responsible for their recruitment into tumors. In colorectal cancer, MDSCs are recruited by CCR2-, CXCR2- and CXCR4-dependent mechanisms to promote tumor progression and metastases [91]. In prostate tumors, MDSC recruitment is mediated by the production of CXCL5 by cancer cells; accordingly, blockage of the CXCL5/CXCR2 interaction elicited antitumor response and increased host survival [92]. The CXCR5/CXCL13 axis was reported to be crucial for the recruitment of MDSCs in gastric cancer [93], while the hypoxia-inducible chemokine CCL26 and its low affinity receptor CX3CR1 were reported to regulate the recruitment of MDSCs in primary hepatocellular carcinoma [94].

Lymphocyte subsets endowed with immunosuppressive activity are known to regulate both innate and tumor-specific immune responses. Treg cells are T cell subsets that can be identified by the expression of CD4, CD25 and by the expression of the transcription factor FoxP3. Because of their chemokine receptor repertoire (*i.e.*, CCR4, CCR6, CCR5), Treg cells tend to accumulate in tumor-enriched with the cognate ligands (*i.e.*, CCL5, CCL22) [95,96]. The accumulation of Tregs is a poor prognostic factor in many tumors including melanoma, non-small lung cancer, pancreatic, ovarian, cervical carcinoma and glioblastoma [2••,97] and therapeutic Treg depletion promotes anti-tumor immunity [98]. However, it should be noted that this correlation does not apply to all types of cancers and in certain tumors, such as colorectal cancer, Treg infiltration correlates with a better prognosis [2••]. In these situations, Treg cells are thought to inhibit the inflammatory process that promotes cell transformation and tumor growth. Similar to Treg cells, regulatory B (Breg) cells are a B cell subset able to inhibit effector T cells and innate immune responses and to promote the generation of Treg cells. Their immunosuppressive activity resides in the ability to produce anti-inflammatory mediators and cytokines, such as IL-10 and TGF-β [99••]. Bregs express the chemokine receptor CXCR5 and respond to the ligand CXCL13 to migrate to lymph nodes and to infiltrate tumors (*e.g.*, prostate cancer) [100]. Similar to Tregs, the prognostic value of Breg cells is controversial. Many studies have reported that a high number of Breg cells is associated with longer survival of patients with colorectal or ovarian cancers. In contrast, in other types of tumors, such as lung, esophageal and liver cancers, the presence of Breg cells is indicative of shorter survival [101]. Interestingly, in human lymphoid malignancies, such as chronic lymphocytic leukemia (CLL), malignant B cells share immunoregulatory functions and IL-10 production with Bregs. These characteristics may contribute to the immunosuppression state observed in CLL patients [102].

Concluding remarks

Emerging evidence indicates that tumor infiltrating leukocytes endowed with an immunosuppressive phenotype may represent a promising target of immunotherapy to unleash immune responses [3••]. In the past few years, this strategy has started to be explored. For instance, novel therapies directed against myeloid tumor infiltrate are emerging and include multiple strategies, such as the inhibition of the recruitment and/or proliferation of monocytes/macrophages; the selective ablation of mononuclear phagocytes; the re-education of TAM, from tumor-promoting cells to cells endowed with tumoricidal functions, and the pharmacologically modulation of TAM metabolism responsible for tumor-promoting functions [3••,103]. For instance, alteration of macrophage polarization was obtained after CSF-1R inhibition with the consequent block of glioma progression [104]. Pharmacological or genetic inhibition of oxysterols synthesis by cells of a pancreatic neuroendocrine tumor blocked the recruitment of proangiogenic neutrophils and tumor growth [105]. Transient Treg depletion induced regression of metastatic lesions in advanced stage melanoma patients [98]. In breast cancer patients undergoing tumor resection and radiotherapy, Treg depletion, prior to treatment, is associated with an anti-tumor immune response and improved clinical outcomes [98]. Additionally, Treg depletion followed by cancer antigen vaccination generated effective anti-tumor $CD4^+$ and $CD8^+$ T-cell responses in metastatic breast cancer patients [98]. All these approaches have been exploited with promising results at the preclinical level and some of them are currently undergoing clinical trials [3••,103]. Future studies will clarify the clinical impact of leukocyte-directed therapeutic strategies used alone or in combination with cytoreductive therapies.

Conflict of interest statement

Nothing declared.

Acknowledgements

This work was supported by AIRC (Associazione Italiana Ricerca sul Cancro); Fondazione Berlucchi. We thank Annunciata Vecchi, Daniela Bosisio and Angela Gismondi for their suggestions and critical reading of the manuscript.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. •• Hanahan D, Weinberg RA: **Hallmarks of cancer: the next generation**. *Cell* 2011, **144**:646-674.
This review represents a milestone in the understanding of the multistep development of human tumors and the emerging hallmarks of cancer.

2. •• Fridman WH, Pages F, Sautes-Fridman C, Galon J: **The immune contexture in human tumours: impact on clinical outcome**. *Nat. Rev. Cancer* 2012, **12**:298-306.
Important article on the context-specific nature of infiltrating immune cells that can affect the prognosis of patients.

3. •• Mantovani A, Marchesi F, Malesci A, Laghi L, Allavena P: **Tumour-associated macrophages as treatment targets in oncology**. *Nat. Rev. Clin. Oncol.* 2017. Jan 24-Epub ahead of print.
A recent review on tumor-associated macrophages, focused on therapeutic strategies that can complement and synergize with chemotherapy and immunotherapy.

4. Klemm F, Joyce JA: **Microenvironmental regulation of therapeutic response in cancer**. *Trends Cell. Biol.* 2015, **25**:198-213.

5. Balkwill FR: **The chemokine system and cancer**. *J. Pathol.* 2012, **226**:148-157.

6. • Bachelerie F, Ben-Baruch A, Burkhardt AM, Combadiere C, Farber JM, Graham GJ, Horuk R, Sparre-Ulrich AH, Locati M, Luster AD *et al.*: **International Union of Basic and Clinical Pharmacology. LXXXIX. Update on the extended family of chemokine receptors and introducing a new nomenclature for atypical chemokine receptors**. *Pharmacol. Rev.* 2014, **66**:1-79.
An updated review on chemokines and chemokine receptors containing the new nomenclature for atypical chemokine receptors.

7. •• Mantovani A, Allavena P, Sica A, Balkwill F: **Cancer-related inflammation**. *Nature* 2008, **454**:436-444.

One of the most important work describing the role of soluble and cellular mediators of inflammation in cancer development and progression.

8. Jinushi M: **Yin and yang of tumor inflammation: how innate immune suppressors shape the tumor microenvironments**. *Int. J. Cancer* 2014, **135**:1277-1285.

9. Van Overmeire E, Stijlemans B, Heymann F, Keirsse J, Morias Y, Elkrim Y, Brys L, Abels C, Lahmar Q, Ergen C *et al.*: **M-CSF and GM-CSF receptor signaling differentially regulate monocyte maturation and macrophage polarization in the tumor microenvironment**. *Cancer Res.* 2016, **76**:35-42.

10. Traversari C, Sozzani S, Steffensen KR, Russo V: **LXR-dependent and -independent effects of oxysterols on immunity and tumor growth**. *Eur. J. Immunol.* 2014, **44**:1896-1903.

11. Franklin RA, Liao W, Sarkar A, Kim MV, Bivona MR, Liu K, Pamer EG, Li MO: **The cellular and molecular origin of tumor-associated macrophages**. *Science* 2014, **344**:921-925.

12. Shand FH, Ueha S, Otsuji M, Koid SS, Shichino S, Tsukui T, Kosugi-Kanaya M, Abe J, Tomura M, Ziogas J *et al.*: **Tracking of intertissue migration reveals the origins of tumor-infiltrating monocytes**. *Proc. Natl. Acad. Sci. U. S. A.* 2014, **111**:7771-7776.

13. Murray PJ, Allen JE, Biswas SK, Fisher EA, Gilroy DW, Goerdt S, Gordon S, Hamilton JA, Ivashkiv LB, Lawrence T *et al.*: **Macrophage activation and polarization: nomenclature and experimental guidelines**. *Immunity* 2014, **41**:14-20.

14. Mantovani A, Biswas SK, Galdiero MR, Sica A, Locati M: **Macrophage plasticity and polarization in tissue repair and remodelling**. *J. Pathol.* 2013, **229**:176-185.

15. Colegio OR, Chu NQ, Szabo AL, Chu T, Rhebergen AM, Jairam V, Cyrus N, Brokowski CE, Eisenbarth SC, Phillips GM *et al.*: **Functional polarization of tumour-associated macrophages by tumour-derived lactic acid**. *Nature* 2014, **513**:559-563.

16. Coussens LM, Zitvogel L, Palucka AK: **Neutralizing tumor-promoting chronic inflammation: a magic bullet?** *Science* 2013, **339**:286-291.

17. De Palma M, Lewis CE: **Macrophage regulation of tumor responses to anticancer therapies**. *Cancer Cell* 2013, **23**: 277-286.

18. Noy R, Pollard JW: **Tumor-associated macrophages: from mechanisms to therapy**. *Immunity* 2014, **41**:49-61.

19. Su S, Liu Q, Chen J, Chen F, He C, Huang D, Wu W, Lin L, Huang W, Zhang J *et al.*: **A positive feedback loop between mesenchymal-like cancer cells and macrophages is essential to breast cancer metastasis**. *Cancer Cell* 2014, **25**:605-620.

20. Kitamura T, Qian BZ, Soong D, Cassetta L, Noy R, Sugano G, Kato Y, Li J, Pollard JW: **CCL2-induced chemokine cascade promotes breast cancer metastasis by enhancing retention of metastasis-associated macrophages**. *J. Exp. Med.* 2015, **212**:1043-1059.

21. Wenes M, Shang M, Di Matteo M, Goveia J, Martin-Perez R, Serneels J, Prenen H, Ghesquiere B, Carmeliet P, Mazzone M: **Macrophage metabolism controls tumor blood vessel morphogenesis and metastasis**. *Cell Metab.* 2016, **24**:701-715.

22. Mayadas TN, Cullere X, Lowell CA: **The multifaceted functions of neutrophils**. *Annu. Rev. Pathol.* 2014, **9**:181-218.

23. Kruger P, Saffarzadeh M, Weber AN, Rieber N, Radsak M, von Bernuth H, Benarafa C, Roos D, Skokowa J, Hartl D: **Neutrophils: between host defence, immune modulation, and tissue injury**. *PLoS Pathog.* 2015, **11**:e1004651.

24. •• Fridlender ZG, Sun J, Kim S, Kapoor V, Cheng G, Ling L, Worthen GS, Albelda SM: **Polarization of tumor-associated neutrophil phenotype by TGF-beta: "N1" versus "N2" TAN**. *Cancer Cell* 2009, **16**:183-194.

Important article describing the polarization of neutrophils in the tumor microenvironment which can show protumor or antitumor phenotype.

25. Sionov RV, Fridlender ZG, Granot Z: **The multifaceted roles neutrophils play in the tumor microenvironment**. *Cancer Microenviron.* 2015, **8**:125-158.

26. Dumitru CA, Moses K, Trellakis S, Lang S, Brandau S: **Neutrophils and granulocytic myeloid-derived suppressor cells: immunophenotyping, cell biology and clinical relevance in human oncology**. *Cancer Immunol. Immunother.* 2012, **61**: 1155-1167.

27. Templeton AJ, McNamara MG, Seruga B, Vera-Badillo FE, Aneja P, Ocana A, Leibowitz-Amit R, Sonpavde G, Knox JJ, Tran B *et al.*: **Prognostic role of neutrophil-to-lymphocyte ratio in solid tumors: a systematic review and meta-analysis**. *J. Natl. Cancer Inst.* 2014, **106**:dju124.

28. Galdiero MR, Bianchi P, Grizzi F, Di Caro G, Basso G, Ponzetta A, Bonavita E, Barbagallo M, Tartari S, Polentarutti N *et al.*: **Occurrence and significance of tumor-associated neutrophils in patients with colorectal cancer**. *Int. J. Cancer* 2016, **139**:446-456.

29. Powell DR, Huttenlocher A: **Neutrophils in the tumor microenvironment**. *Trends Immunol.* 2016, **37**:41-52.

30. Sparmann A, Bar-Sagi D: **Ras-induced interleukin-8 expression plays a critical role in tumor growth and angiogenesis**. *Cancer Cell* 2004, **6**:447-458.

31. Zhou SL, Dai Z, Zhou ZJ, Wang XY, Yang GH, Wang Z, Huang XW, Fan J, Zhou J: **Overexpression of CXCL5 mediates neutrophil infiltration and indicates poor prognosis for hepatocellular carcinoma**. *Hepatology* 2012, **56**:2242-2254.

32. Zhou SL, Zhou ZJ, Hu ZQ, Li X, Huang XW, Wang Z, Fan J, Dai Z, Zhou J: **CXCR2/CXCL5 axis contributes to epithelial-mesenchymal transition of HCC cells through activating PI3K/Akt/GSK-3beta/Snail signaling**. *Cancer Lett.* 2015, **358**:124-135.

33. Verbeke H, Struyf S, Berghmans N, Van Coillie E, Opdenakker G, Uyttenhove C, Van Snick J, Van Damme J: **Isotypic neutralizing antibodies against mouse GCP-2/CXCL6 inhibit melanoma growth and metastasis**. *Cancer Lett.* 2011, **302**:54-62.

34. Desurmont T, Skrypek N, Duhamel A, Jonckheere N, Millet G, Leteurtre E, Gosset P, Duchene B, Ramdane N, Hebbar M *et al.*: **Overexpression of chemokine receptor CXCR2 and ligand CXCL7 in liver metastases from colon cancer is correlated to shorter disease-free and overall survival**. *Cancer Sci.* 2015, **106**:262-269.

35. • Raccosta L, Fontana R, Maggioni D, Lanterna C, Villablanca EJ, Paniccia A, Musumeci A, Chiricozzi E, Trincavelli ML, Daniele S *et al.*: **The oxysterol-CXCR2 axis plays a key role in the recruitment of tumor-promoting neutrophils**. *J. Exp. Med.* 2013, **210**:1711-1728.

Original article describing the role of oxysterols in the recruitment of tumor-promoting neutrophils.

36. Florey O, Haskard DO: **Sphingosine 1-phosphate enhances Fc gamma receptor-mediated neutrophil activation and recruitment under flow conditions**. *J. Immunol.* 2009, **183**:2330-2336.

37. Dumitru CA, Fechner MK, Hoffmann TK, Lang S, Brandau S: **A novel p38-MAPK signaling axis modulates neutrophil biology in head and neck cancer**. *J. Leukoc. Biol.* 2012, **91**:591-598.

38. Song J, Wu C, Zhang X, Sorokin LM: **In vivo processing of CXCL5 (LIX) by matrix metalloproteinase (MMP)-2 and MMP-9 promotes early neutrophil recruitment in IL-1beta-induced peritonitis**. *J. Immunol.* 2013, **190**:401-410.

39. Hiratsuka S, Watanabe A, Aburatani H, Maru Y: **Tumour-mediated upregulation of chemoattractants and recruitment of myeloid cells predetermines lung metastasis**. *Nat. Cell Biol.* 2006, **8**:1369-1375.

40. Coffelt SB, Kersten K, Doornebal CW, Weiden J, Vrijland K, Hau CS, Verstegen NJ, Ciampricotti M, Hawinkels LJ, Jonkers J *et al.*: **IL-17-producing gammadelta T cells and neutrophils conspire to promote breast cancer metastasis**. *Nature* 2015, **522**:345-348.

41. Benevides L, da Fonseca DM, Donate PB, Tiezzi DG, De Carvalho DD, de Andrade JM, Martins GA, Silva JS: **IL17 promotes mammary tumor progression by changing the behavior of tumor cells and eliciting tumorigenic neutrophils recruitment**. *Cancer Res.* 2015, **75**:3788-3799.

42. Finisguerra V, Di Conza G, Di Matteo M, Serneels J, Costa S, Thompson AA, Wauters E, Walmsley S, Prenen H, Granot Z *et al.*: **MET is required for the recruitment of anti-tumoural neutrophils**. *Nature* 2015, **522**:349-353.

43. Koyama S, Akbay EA, Li YY, Aref AR, Skoulidis F, Herter-Sprie GS, Buczkowski KA, Liu Y, Awad MM, Denning WL *et al.*: **STK11/LKB1 deficiency promotes neutrophil recruitment and proinflammatory cytokine production to suppress T-cell activity in the lung tumor microenvironment**. *Cancer Res.* 2016, **76**:999-1008.

44. •• Murphy TL, Grajales-Reyes GE, Wu X, Tussiwand R, Briseno CG, Iwata A, Kretzer NM, Durai V, Murphy KM: **Transcriptional control of dendritic cell development**. *Annu. Rev. Immunol.* 2016, **34**:93-119.
Recent work describing the trascription drivers that controls the development and diversification of DCs from common progenitors in the bone marrow.

45. Diao J, Zhao J, Winter E, Cattral MS: **Recruitment and differentiation of conventional dendritic cell precursors in tumors**. *J. Immunol.* 2010, **184**:1261-1267.

46. Iida N, Nakamoto Y, Baba T, Kakinoki K, Li YY, Wu Y, Matsushima K, Kaneko S, Mukaida N: **Tumor cell apoptosis induces tumor-specific immunity in a CC chemokine receptor 1- and 5-dependent manner in mice**. *J. Leukoc. Biol.* 2008, **84**:1001-1010.

47. Charles J, Di Domizio J, Salameire D, Bendriss-Vermare N, Aspord C, Muhammad R, Lefebvre C, Plumas J, Leccia MT, Chaperot L: **Characterization of circulating dendritic cells in melanoma: role of CCR6 in plasmacytoid dendritic cell recruitment to the tumor**. *J. Invest. Dermatol.* 2010, **130**: 1646-1656.

48. Ma Y, Adjemian S, Mattarollo SR, Yamazaki T, Aymeric L, Yang H, Portela Catani JP, Hannani D, Duret H, Steegh K *et al.*: **Anticancer chemotherapy-induced intratumoral recruitment and differentiation of antigen-presenting cells**. *Immunity* 2013, **38**:729-741.

49. Ma Y, Mattarollo SR, Adjemian S, Yang H, Aymeric L, Hannani D, Portela Catani JP, Duret H, Teng MW, Kepp O *et al.*: **CCL2/CCR2-dependent recruitment of functional antigen-presenting cells into tumors upon chemotherapy**. *Cancer Res.* 2014, **74**: 436-445.

50. Roberts EW, Broz ML, Binnewies M, Headley MB, Nelson AE, Wolf DM, Kaisho T, Bogunovic D, Bhardwaj N, Krummel MF: **Critical role for CD103(+)/CD141(+) dendritic cells bearing CCR7 for tumor antigen trafficking and priming of T cell immunity in melanoma**. *Cancer Cell* 2016, **30**:324-336.

51. Salmon H, Idoyaga J, Rahman A, Leboeuf M, Remark R, Jordan S, Casanova-Acebes M, Khudoynazarova M, Agudo J, Tung N *et al.*: **Expansion and activation of CD103(+) dendritic cell progenitors at the tumor site enhances tumor responses to therapeutic PD-L1 and BRAF inhibition**. *Immunity* 2016, **44**: 924-938.

52. Broz ML, Binnewies M, Boldajipour B, Nelson AE, Pollack JL, Erle DJ, Barczak A, Rosenblum MD, Daud A, Barber DL *et al.*: **Dissecting the tumor myeloid compartment reveals rare activating antigen-presenting cells critical for T cell immunity**. *Cancer Cell* 2014, **26**:638-652.

53. Veglia F, Gabrilovich DI: **Dendritic cells in cancer: the role revisited**. *Curr. Opin. Immunol.* 2017, **45**:43-51.

54. Conejo-Garcia JR, Rutkowski MR, Cubillos-Ruiz JR: **State-of-the-art of regulatory dendritic cells in cancer**. *Pharmacol. Ther.* 2016, **164**:97-104.

55. Tesone AJ, Rutkowski MR, Brencicova E, Svoronos N, Perales-Puchalt A, Stephen TL, Allegrezza MJ, Payne KK, Nguyen JM, Wickramasinghe J *et al.*: **Satb1 overexpression drives tumor-promoting activities in cancer-associated dendritic cells**. *Cell Rep.* 2016, **14**:1774-1786.

56. Pyfferoen L, Brabants E, Everaert C, De Cabooter N, Heyns K, Deswarte K, Vanheerswynghels M, De Prijck S, Waegemans G, Dullaers M *et al.*: **The transcriptome of lung tumor-infiltrating dendritic cells reveals a tumor-supporting phenotype and a microRNA signature with negative impact on clinical outcome**. *Oncoimmunology* 2017, **6**:e1253655.

57. Lombardi VC, Khaiboullina SF, Rizvanov AA: **Plasmacytoid dendritic cells, a role in neoplastic prevention and progression**. *Eur. J. Clin. Invest.* 2015, **45(Suppl. 1)**:1-8.

58. Artis D, Spits H: **The biology of innate lymphoid cells**. *Nature* 2015, **517**:293-301.

59. Vivier E, Raulet DH, Moretta A, Caligiuri MA, Zitvogel L, Lanier LL, Yokoyama WM, Ugolini S: **Innate or adaptive immunity? The example of natural killer cells**. *Science* 2011, **331**:44-49.

60. •• Horowitz A, Strauss-Albee DM, Leipold M, Kubo J, Nemat-Gorgani N, Dogan OC, Dekker CL, Mackey S, Maecker H, Swan GE *et al.*: **Genetic and environmental determinants of human NK cell diversity revealed by mass cytometry**. *Sci. Transl. Med.* 2013, **5**:208ra145.
This study is the most comprehensive analysis of human NK cell heterogeneity.

61. Cooper MA, Fehniger TA, Caligiuri MA: **The biology of human natural killer-cell subsets**. *Trends Immunol.* 2001, **22**:633-640.

62. Carrega P, Ferlazzo G: **Natural killer cell distribution and trafficking in human tissues**. *Front. Immunol.* 2012, **3**:347.

63. Bernardini G, Gismondi A, Santoni A: **Chemokines and NK cells: regulators of development, trafficking and functions**. *Immunol. Lett.* 2012, **145**:39-46.

64. Parolini S, Santoro A, Marcenaro E, Luini W, Massardi L, Facchetti F, Communi D, Parmentier M, Majorana A, Sironi M *et al.*: **The role of chemerin in the colocalization of NK and dendritic cell subsets into inflamed tissues**. *Blood* 2007, **109**:3625-3632.

65. Walzer T, Chiossone L, Chaix J, Calver A, Carozzo C, Garrigue-Antar L, Jacques Y, Baratin M, Tomasello E, Vivier E: **Natural killer cell trafficking in vivo requires a dedicated sphingosine 1-phosphate receptor**. *Nat. Immunol.* 2007, **8**:1337-1344.

66. Wennerberg E, Kremer V, Childs R, Lundqvist A: **CXCL10-induced migration of adoptively transferred human natural killer cells toward solid tumors causes regression of tumor growth in vivo**. *Cancer Immunol. Immunother.* 2015, **64**:225-235.

67. Carrega P, Morandi B, Costa R, Frumento G, Forte G, Altavilla G, Ratto GB, Mingari MC, Moretta L, Ferlazzo G: **Natural killer cells infiltrating human nonsmall-cell lung cancer are enriched in CD56 bright CD16(−) cells and display an impaired capability to kill tumor cells**. *Cancer* 2008, **112**:863-875.

68. Ali TH, Pisanti S, Ciaglia E, Mortarini R, Anichini A, Garofalo C, Tallerico R, Santinami M, Gulletta E, Ietto C *et al.*: **Enrichment of CD56(dim)KIR + CD57 + highly cytotoxic NK cells in tumour-infiltrated lymph nodes of melanoma patients**. *Nat. Commun.* 2014, **5**:5639.

69. Bernardini G, Antonangeli F, Bonanni V, Santoni A: **Dysregulation of chemokine/chemokine receptor axes and NK cell tissue localization during diseases**. *Front. Immunol.* 2016, **7**:402.

70. Bruno A, Ferlazzo G, Albini A, Noonan DM: **A think tank of TINK/TANKs: tumor-infiltrating/tumor-associated natural killer cells in tumor progression and angiogenesis**. *J. Natl. Cancer Inst.* 2014, **106**:dju200.

71. Stabile H, Fionda C, Gismondi A, Santoni A: **Role of distinct natural killer cell subsets in anticancer response**. *Front. Immunol.* 2017, **8**:293.

72. Ghiringhelli F, Menard C, Terme M, Flament C, Taieb J, Chaput N, Puig PE, Novault S, Escudier B, Vivier E *et al.*: **CD4+CD25+ regulatory T cells inhibit natural killer cell functions in a transforming growth factor-beta-dependent manner**. *J. Exp. Med.* 2005, **202**:1075-1085.

73. Pedroza-Pacheco I, Madrigal A, Saudemont A: **Interaction between natural killer cells and regulatory T cells: perspectives for immunotherapy**. *Cell Mol. Immunol.* 2013, **10**:222-229.

74. Kerdiles Y, Ugolini S, Vivier E: **T cell regulation of natural killer cells**. *J. Exp. Med.* 2013, **210**:1065-1068.

75. Giraldo NA, Becht E, Pages F, Skliris G, Verkarre V, Vano Y, Mejean A, Saint-Aubert N, Lacroix L, Natario I *et al.*: **Orchestration and prognostic significance of immune checkpoints in the microenvironment of primary and metastatic renal cell cancer**. *Clin. Cancer Res.* 2015, **21**:3031-3040.

76. Gajewski TF, Fuertes M, Spaapen R, Zheng Y, Kline J: **Molecular profiling to identify relevant immune resistance mechanisms in the tumor microenvironment**. *Curr. Opin. Immunol.* 2011, **23**:286-292.

77. Guy TV, Terry AM, Bolton HA, Hancock DG, Shklovskaya E, Fazekas de St. Groth B: **Pro- and anti-tumour effects of B cells and antibodies in cancer: a comparison of clinical studies and preclinical models**. *Cancer Immunol. Immunother.* 2016, **65**: 885-896.

78. Maric M, Liu Y: **Strong cytotoxic T lymphocyte responses to a macrophage inflammatory protein 1alpha-expressing tumor: linkage between inflammation and specific immunity**. *Cancer Res.* 1999, **59**:5549-5553.

79. Mule JJ, Custer M, Averbook B, Yang JC, Weber JS, Goeddel DV, Rosenberg SA, Schall TJ: **RANTES secretion by gene-modified tumor cells results in loss of tumorigenicity in vivo: role of immune cell subpopulations**. *Hum. Gene Ther.* 1996, **7**: 1545-1553.

80. Moran CJ, Arenberg DA, Huang CC, Giordano TJ, Thomas DG, Misek DE, Chen G, Iannettoni MD, Orringer MB, Hanash S *et al.*: **RANTES expression is a predictor of survival in stage I lung adenocarcinoma**. *Clin. Cancer Res.* 2002, **8**:3803-3812.

81. Luster AD, Leder P: **IP-10, a -C-X-C- chemokine, elicits a potent thymus-dependent antitumor response in vivo**. *J. Exp. Med.* 1993, **178**:1057-1065.

82. Kondo T, Ito F, Nakazawa H, Horita S, Osaka Y, Toma H: **High expression of chemokine gene as a favorable prognostic factor in renal cell carcinoma**. *J. Urol.* 2004, **171**:2171-2175.

83. Mullins IM, Slingluff CL, Lee JK, Garbee CF, Shu J, Anderson SG, Mayer ME, Knaus WA, Mullins DW: **CXC chemokine receptor 3 expression by activated CD8+ T cells is associated with survival in melanoma patients with stage III disease**. *Cancer Res.* 2004, **64**:7697-7701.

84. Ohta M, Tanaka F, Yamaguchi H, Sadanaga N, Inoue H, Mori M: **The high expression of Fractalkine results in a better prognosis for colorectal cancer patients**. *Int. J. Oncol.* 2005, **26**:41-47.

85. Lin Y, Sharma S, John MS: **CCL21 cancer immunotherapy**. *Cancers (Basel)* 2014, **6**:1098-1110.

86. de Chaisemartin L, Goc J, Damotte D, Validire P, Magdeleinat P, Alifano M, Cremer I, Fridman WH, Sautes-Fridman C, Dieu-Nosjean MC: **Characterization of chemokines and adhesion molecules associated with T cell presence in tertiary lymphoid structures in human lung cancer**. *Cancer Res.* 2011, **71**: 6391-6399.

87. •• Dieu-Nosjean MC, Giraldo NA, Kaplon H, Germain C, Fridman WH, Sautes-Fridman C: **Tertiary lymphoid structures, drivers of the anti-tumor responses in human cancers**. *Immunol. Rev.* 2016, **271**:260-275.

Recent review focused on the description of tertiary lymphoid structures, which represent not only sites of lymphoid neogenesis developed in most solid cancers, but also novel biomarkers for statification of overall survival risk for cancer patients.

88. Talmadge JE, Gabrilovich DI: **History of myeloid-derived suppressor cells**. *Nat. Rev. Cancer* 2013, **13**:739-752.

89. Bronte V, Brandau S, Chen SH, Colombo MP, Frey AB, Greten TF, Mandruzzato S, Murray PJ, Ochoa A, Ostrand-Rosenberg S *et al.*: **Recommendations for myeloid-derived suppressor cell nomenclature and characterization standards**. *Nat. Commun.* 2016, **7**:12150.

90. Fan Q, Gu D, Liu H, Yang L, Zhang X, Yoder MC, Kaplan MH, Xie J: **Defective TGF-beta signaling in bone marrow-derived cells prevents hedgehog-induced skin tumors**. *Cancer Res.* 2014, **74**:471-483.

91. Zhao L, Lim SY, Gordon-Weeks AN, Tapmeier TT, Im JH, Cao Y, Beech J, Allen D, Smart S, Muschel RJ: **Recruitment of a myeloid cell subset (CD11b/Gr1 mid) via CCL2/CCR2 promotes the development of colorectal cancer liver metastasis**. *Hepatology* 2013, **57**:829-839.

92. Wang G, Lu X, Dey P, Deng P, Wu CC, Jiang S, Fang Z, Zhao K, Konaparthi R, Hua S *et al.*: **Targeting YAP-dependent MDSC infiltration impairs tumor progression**. *Cancer Discov.* 2016, **6**:80-95.

93. Ding Y, Shen J, Zhang G, Chen X, Wu J, Chen W: **CD40 controls CXCR5-induced recruitment of myeloid-derived suppressor cells to gastric cancer**. *Oncotarget* 2015, **6**:38901-38911.

94. Chiu DK, Xu IM, Lai RK, Tse AP, Wei LL, Koh HY, Li LL, Lee D, Lo RC, Wong CM *et al.*: **Hypoxia induces myeloid-derived suppressor cell recruitment to hepatocellular carcinoma through chemokine (C-C motif) ligand 26**. *Hepatology* 2016, **64**:797-813.

95. Svensson H, Olofsson V, Lundin S, Yakkala C, Bjorck S, Borjesson L, Gustavsson B, Quiding-Jarbrink M: **Accumulation of CCR4(+)CTLA-4 FOXP3(+)CD25(hi) regulatory T cells in colon adenocarcinomas correlate to reduced activation of conventional T cells**. *PLoS One* 2012, **7**:e30695.

96. Tan MC, Goedegebuure PS, Belt BA, Flaherty B, Sankpal N, Gillanders WE, Eberlein TJ, Hsieh CS, Linehan DC: **Disruption of CCR5-dependent homing of regulatory T cells inhibits tumor growth in a murine model of pancreatic cancer**. *J. Immunol.* 2009, **182**:1746-1755.

97. Nishikawa H, Sakaguchi S: **Regulatory T cells in cancer immunotherapy**. *Curr. Opin. Immunol.* 2014, **27**:1-7.

98. Chaudhary B, Elkord E: **Regulatory T Cells in the tumor microenvironment and cancer progression: role and therapeutic targeting**. *Vaccines (Basel)* 2016, **4**:28.

99. •• Rosser EC, Mauri C: **Regulatory B cells: origin, phenotype, and function**. *Immunity* 2015, **42**:607-612.

Recent work discussing the advances in understanding the biology of regulatory B cells.

100. Gorosito Serran M, Fiocca Vernengo F, Beccaria CG, Acosta Rodriguez EV, Montes CL, Gruppi A: **The regulatory role of B cells in autoimmunity, infections and cancer: perspectives beyond IL10 production**. *FEBS Lett.* 2015, **589**:3362-3369.

101. Schwartz M, Zhang Y, Rosenblatt JD: **B cell regulation of the anti-tumor response and role in carcinogenesis**. *J. Immunother. Cancer* 2016, **4**:40.

102. DiLillo DJ, Weinberg JB, Yoshizaki A, Horikawa M, Bryant JM, Iwata Y, Matsushita T, Matta KM, Chen Y, Venturi GM *et al.*: **Chronic lymphocytic leukemia and regulatory B cells share IL-10 competence and immunosuppressive function**. *Leukemia* 2013, **27**:170-182.

103. Zhu J, Zhi Q, Zhou BP, Tao M, Liu J, Li W: **The role of tumor associated macrophages in the tumor microenvironment: mechanism and functions**. *Anticancer Agents Med. Chem.* 2016, **16**:1133-1141.

104. Pyonteck SM, Akkari L, Schuhmacher AJ, Bowman RL, Sevenich L, Quail DF, Olson OC, Quick ML, Huse JT, Teijeiro V *et al.*: **CSF-1R inhibition alters macrophage polarization and blocks glioma progression**. *Nat. Med.* 2013, **19**:1264-1272.

105. Soncini M, Corna G, Moresco M, Coltella N, Restuccia U, Maggioni D, Raccosta L, Lin CY, Invernizzi F, Crocchiolo R *et al.*: **24-Hydroxycholesterol participates in pancreatic neuroendocrine tumor development**. *Proc. Natl. Acad. Sci. U. S. A.* 2016, **113**:E6219-E6227.