Rodrigo Nalio Ramos

"O MICROAMBIENTE SUPPRESSOR NO CÂNCER: EFEITOS LOCAIS E SISTÊMICOS EM MONÓCITOS DE PACIENTES"

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   Supervisor: Prof. José Alexandre Marzagão Barbuto, MD, PhD.

2) Escola de doutorado em Biologia Molecular, Integrada e Celular da Universidade de Lyon1 - Lyon – França.

   Supervisor: Christophe Caux, PhD.

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ABSTRACT

RAMOS, R. N. **The immunosuppressive microenvironment in cancer: local and systemic effects on patients' monocytes**. 2015. 196 p. Thesis (PhD in Immunology) - Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, 2015.

Cancer development is currently associated with an immune system failure, mainly due to its dysfunction to sense, recognize and eliminate tumor cells efficiently. In that context, two Antigen-Presenting Cells (APCs) that can be derived from monocytes, the Dendritic Cells (DCs) and the Macrophages (MΦ), have a crucial role in the identification of tissue imbalances and in the stimulation of adaptive antitumor immunity. However, tumor-derived factors modulate those APCs avoiding the optimal priming of the immune responses, culminating in the cancer establishment. Thereby, we investigated here how the tumor microenvironment could modulate the differentiation of monocytes into APCs and its systemic effects on circulating monocytes. Our data revealed that in breast and ovarian cancers, Tumor-Associated Macrophages (TAMs) are the most frequent subpopulation within $CD45^{+}MHCII^{+}$ leukocytes and found in variable frequency as either $CD163^{low}$ or $CD163^{high}$ TAMs. The latter ($CD163^{high}$ TAMs) expressed higher PD-L1 levels and produced elevated IL-10 amounts under LPS activation. Furthermore, a retrospective immunohistochemistry study of breast cancer patients (n=283) with 12.5-year of follow-up reveals a strong correlation between high intra-tumor $CD163^{+}$ TAM and poor patient survival. Additionally, the high frequency of $CD163^{high}$ TAMs was correlated with a low $CD3^{+}$ T cell infiltration. In another experiments, tumor-conditioned medium from primary breast tumors skewed the differentiation of healthy monocytes towards a $CD163^{high}IL\text{-}10^{high}$ phenotype *in vitro*, which not only fail to stimulate but also suppressed naïve $CD4^{+}$ T cell expansion and IFN-γ and TNF-α production *via* IL-10. This acquired phenotype of conditioned-monocytes was associated to the elevated presence of CCL22, M-CSF, TGF-β1, TGF-β3, and VEGF in the tumor microenvironment. Importantly, evaluating the systemic effects of tumors, breast cancer patients' circulating monocytes failed to fully differentiate into M1-MΦ in presence of GM-CSF/IFN-γ and maintained an altered $CD163^{+/-}IL\text{-}10^{+}TNF\text{-}\alpha^{+}$ M2-like phenotype. Likewise, immature DCs differentiated from breast cancer patients' monocytes (Mo-iDCs) expressed high levels of PD-L1, induced lower CD25 expression on T cells and about twice as many $Foxp3^{+}$ Tregs than Th1 or Th2 cells, a phenomenon partially reduced in transwell co-cultures. Moreover, blocking of TGF-β1 and PD-L1 with mAb significantly reduced the induction of $CD4^{+}Foxp3^{+}$ Tregs by patients' Mo-iDCs in co-cultures. Furthermore, fresh monocytes isolated from breast cancer patients blood display an anti-inflammatory functional status by producing higher levels of IL-1RA, IL-10, IL-27, M-CSF, sCD40L and VEGF-A under LPS stimulus when compared to healthy donors' monocytes. Altogether our data suggest that the tumor microenvironment favors the local differentiation of suppressive $CD163^{high}IL\text{-}10^{high}$ MΦ and drives systemic blood monocytes to differentiate into biased MΦ and DCs with suppressive abilities. These findings put forward the importance of new strategies to neutralize cancer-derived factors responsible for $CD163^{high}$ TAMs differentiation and for the modulation of blood circulating monocytes, aiming to improve immunotherapy strategies for cancer patients.

Keywords: Breast Cancer. Monocytes. Interleukin 10. Macrophages. Dendritic Cells.

RESUMO

RAMOS, R. N. **O microambiente suppressor no câncer: efeitos locais e sistêmicos em monócitos de pacientes**. 2015. 196 f. Tese (Doutorado em Imunologia) - Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, 2015.

O desenvolvimento do câncer é normalmente associado a desvios no sistema imune, principalmente devido a sua falha em perceber, reconhecer e eliminar células neoplásicas de maneira eficiente. Nesse contexto, duas Células Apresentadoras de Antígenos (APCs), Células Dendríticas (DCs) e Macrófagos (MΦ), têm um papel crucial na identificação de alterações nos tecidos e na estimulação da imunidade adaptativa antitumoral. No entanto, fatores derivados de tumores modulam essas APCs, impedindo a iniciação das respostas imunes e culminando no estabelecimento do câncer. Investigamos aqui como o microambiente tumoral poderia modular a diferenciação de monócitos em APCs *in vitro* e de modo sistêmico. Nossos dados revelaram que em cânceres de mama e ovário, Macrófagos-Associados a Tumores (TAMs) são a subpopulação mais frequente em leucócitos $CD45^{+}MHCII^{+}$, e são encontrados em uma frequência variável de TAMs $CD163^{low}$ ou TAMs $CD163^{high}$. O último, (TAMs $CD163^{high}$) expressaram maiores níveis de PD-L1 e elevada produção de IL-10 sob a ativação de LPS. Além disso, a análise retrospectiva por imunohistoquímica revelou uma forte correlação entre a presença de TAMs $CD163^{+}$ e uma baixa taxa de sobrevida em pacientes com câncer de mama. Ainda, a alta frequência de TAMs $CD163^{high}$ foi correlacionada com um baixo infiltrado de células T $CD3^{+}$. Monócitos saudáveis condicionados por sobrenadantes de tumores de mama tiveram sua diferenciação *in vitro* direcionada para um fenótipo $CD163^{high}IL\text{-}10^{high}$, células capazes de suprimir a expansão de células T naive $CD4^{+}$ e a produção de IFN-γ e TNF-α via IL-10. Esse fenótipo adquirido por monócitos condicionados foi associado à presença de altos níveis de CCL22, M-CSF, TGF-β1, TGF-β3, e VEGF no microambiente tumoral. Interessantemente, avaliando os efeitos sistêmicos dos tumores, monócitos circulantes de pacientes com câncer de mama falharam em diferenciar-se em M1- MΦ na presença de GM-CSF/IFN-γ e mantiveram um fenótipo alterado $CD163^{+/-}IL\text{-}10^{+}TNF\text{-}\alpha^{+}$. De modo similar, DCs imaturas (Mo-iDCs) diferenciadas de monócitos de pacientes com câncer de mama expressaram altos níveis de PD-L1, induziram baixa expressão de CD25 em linfócitos T e induziram duas vezes mais células T reguladoras $Foxp3^{+}$ (Tregs) do que células Th1 ou Th2, fenômeno parcialmente reduzido quando em co-culturas de transwell. Ainda, Mo-iDCs de pacientes ativadas por LPS, ou sob o bloqueio de TGF-β1 ou PD-L1 com mAb apresentaram uma capacidade reduzida em induzir Tregs $Foxp3^{+}$ *in vitro*, mas ainda acima do nível observado em Mo-iDCs de doadores saudáveis. Adicionalmente, monócitos isolados do sangue de pacientes com câncer de mama produziram altos níveis de IL-1RA, IL-10, IL-27, M-CSF, sCD40L e VEGF-A sob a ativação por LPS (24h) quando comparados a monócitos de doadores sadios. Em conclusão, nossos dados sugerem que o microambiente tumoral favorece a diferenciação de MΦ supressivos $CD163^{high}IL\text{-}10^{high}$ e atua sistemicamente no potencial de diferenciação de monócitos sanguíneos os direcionando para MΦ e DCs com habilidades supressoras. Esses achados colocam em evidência a importância de novas estratégias que neutralizem os fatores derivados do câncer responsáveis pela diferenciação de TAMs $CD163^{high}$ e pela modulação sistêmica de monócitos sanguíneos, visando o melhoramento de abordagens imunoterapêuticas para a intervenção clínica de pacientes portadores de câncer.

Palavras-chave: Neoplasias mamárias. Monócitos. Interleucina 10. Macrófagos. Células Dendríticas.

1 INTRODUCTION

1.1 Cancer as a complex disease

Cancer is the name given to a large group of malignant proliferative diseases that nowadays constitute the second cause of death worldwide, which was responsible for circa 8 million deaths in 2012 (WORLD HEALTH ORGANIZATION - WHO). Among the different types of cancer, breast cancer appears as the main cause of death for women in the world, representing the first and second causes of cancer deaths in developing and developed countries (INCA – Brazil), respectively with 521.000 deaths of breast cancer registered in 2012 in the world (WHO). In Brazil, about 57,000 new cases of female breast cancer were diagnosed in 2014, representing around 20% of total cases of cancer (INSTITUTO NACIONAL DO CANCER - INCA - Brazil). Likewise, data from the “Institut National du Cancer” in France, registered about 48,000 new cases of female breast cancer in 2012 (INSTITUT NACIONAL DU CANCER - INCA - France), highlighting the worldwide relevance of this disease.

Malignant neoplasias are multi-factorial disorders, which incidence has been showing an increase year by year in developed countries, suggesting that it might be associated with modern habits (JEMAL et al., 2004; RADICE; REDAELLI, 2003). Most organs and tissues are subjected to the development of neoplasia and several characteristics are used to define the disease (cellular origin, tissue organization, vascularization, local and systemic spread, chromosomal and genetic alterations, but also tumor infiltration by leukocytes). With the advances in our knowledge of the biology of cancer, there is an increasing tendency to reclassify this disease based on its molecular characteristics rather than its morphology (which predominated till recently).

Genetic insults occur throughout the life and, combined with environmental factors, can lead to cancer initiation and/or promotion. Well known external agents like UV radiation, tobacco, alcohol and diet are frequently linked to cancer development, acting directly or indirectly as promoters of the disease (ROSSI et al., 2014; TSAI et al., 2010; WARREN et al., 2014). Thus, cancer is a genetic anomaly characterized by the abnormal differentiation of cells that lose their proliferation control, frequently have defects in their mechanisms of apoptosis and a high genetic instability. In order to generate a tumor, however, the neoplastic cell has to acquire

the ability to induce angiogenesis, a process that can be considered as a turning point in carcinogenesis (FOLKMAN et al., 1989). From that point, those genetically unstable cells, proliferating independently from tissue regulation, may acquire the definitive hallmark of cancer: the ability to invade other tissues. The “final” step in the malignant differentiation of the neoplastic cell is the acquisition of the metastatic potential that will allow its growth at distant sites and organs (review by HANAHAN; WEINBERG, 2000).

Within this general pathway, specific genomic alterations have been associated with cancer development. For breast cancer, BRCA1, p53, and Her2/neu expression have been described as the most important genomic targets of alterations in patients and are useful molecules to predict tumor development and the choice of treatment (MA et al., 2014; SONG et al., 2014).

1.2 Cancer Immunosurveillance

It is necessary to note that carcinogenesis is a silent phenomenon, which happens slowly, but not only in the neoplastic cells: tissues surrounding the tumor are also gradually modified during the process. Throughout oncogenesis, a very complex and typical microenvironment is formed, characterized by local pH alterations; zones of hypoxia; angiogenesis; inflammation with recruitment/accumulation of a distinct profile of immune cells. Besides that, several mechanisms of cancer control probably are turned on, and one of the most important is the presence of an efficient immune system, able to survey and eliminate the newly formed neoplastic cells (BURNET, 1957; DUNN et al., 2002). Although the first idea of immunosurveillance was conceived by Paul Ehrlich in 1909, only later in the 1950s the official hypothesis was postulated by Macfarlane Burnet (1957) and Lewis Thomas (1959), speculating the participation of lymphocytes as sentinels capable to recognize and destroy tumors. Only later, after the 1970s, when athymic nude mice lineages were used as models (STUTMAN, 1974 and 1979), it emerged the participation of adaptive immunity in tumor responses, however not convincing enough to confirm Ehrlich’s hypothesis. Even mouse models were not well established in that time, these preliminary findings corroborated observations in humans, where individuals with primary immunodeficiencies (GATTI; GOOD, 1971)

and patients treated with immunosuppressive drugs after transplantation (SHEIL, 1986) showed higher risk to develop cancer. The immunosurveillance premise was confirmed later by models showing that IFN-γ and perforin deficient mice and RAG2 knockout mice (KAPLAN et al., 1998; SHANKARAN et al., 2001; STREET et al., 2001) presented increased frequency and growth of chemically-induced or spontaneous tumors. Interestingly, even considering the crucial role of the immune system in the elimination of tumors, the process of inflammation has been lately considered as advantageous for tumor growth, at least in certain tumor models (HANAHAN; WEINBERG, 2011). Several mechanisms of tumor evasion have been described in the past century, but the role of the inflammation and of the immune system in the natural history of tumors has been “reinserted” in the studies just recently (HANAHAN; WEINBERG, 2011). Moreover, differently from infections, the development of malignant neoplasias is normally characterized as a silent and very slow process where non-self antigens are presented in low levels, failing to trigger an immune response.

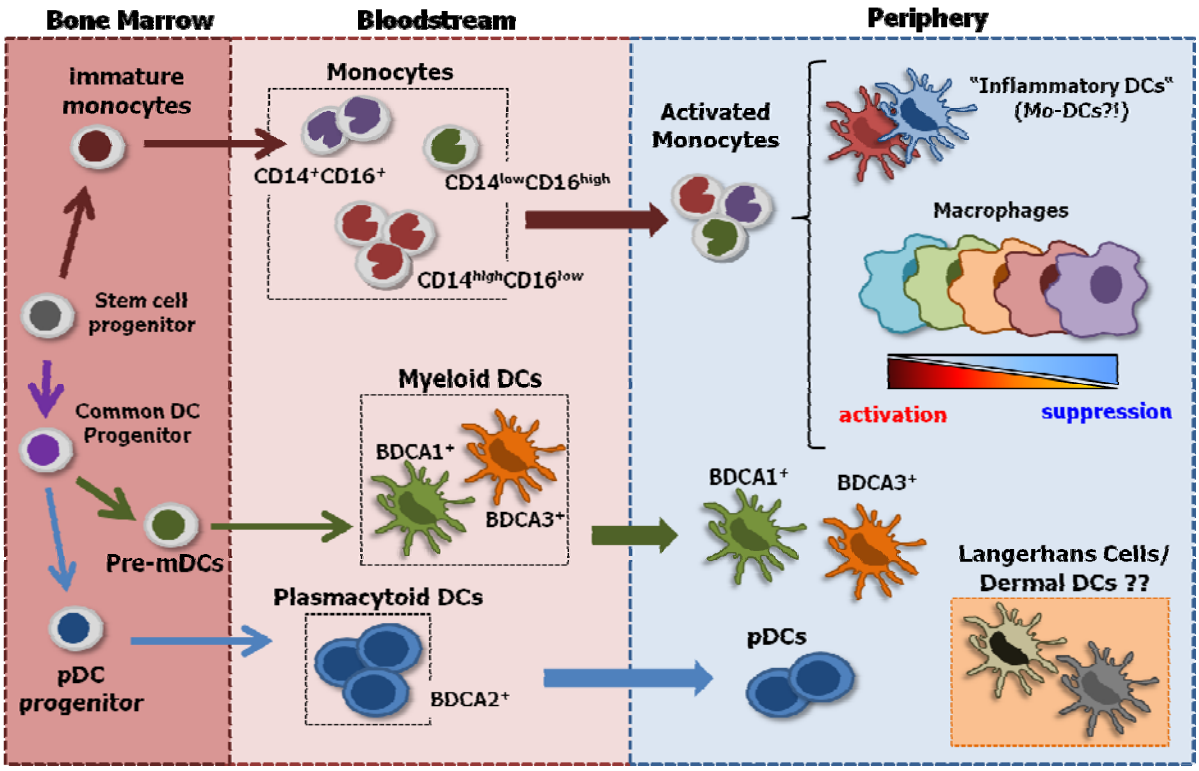
1.3 The Immune System: Human Antigen-Presenting Cells

The immune system is made up of diverse cells and specialized tissues responsible for the homeostasis in a well-orchestrated function. Specialized cells, the Antigen-Presenting Cells (APCs), are strategically distributed in tissues and organs, where they are able to quickly sense and identify the environmental imbalances, identify pathogens or damage, and stimulate immunity. Several subpopulations of cells have been described in humans, including Monocytes, Dendritic Cells, and Macrophages, which build very heterogeneous scenery of antigen presentation (scheme 1).

The APCs’ sensitivity to environmental modifications is critical for the initiation of immune responses, and is possible due to their large repertoire of pattern recognition receptors (PRR), extracellular and intracellular, which are able to identify molecular patterns associated with pathogens and/or tissue damage (PAMPs and DAMPs, respectively). Continuously, APCs internalize and process large molecules into smaller ones that will be presented to T lymphocytes in the context of specialized molecules – belonging to the Major Histocompatibility Complex products, when the

presented molecules are proteins, and to the CD1 family, when they are lipids. The consequence of this presentation will depend on the signals received from the environment by the APCs via their PRR. When the tissue, where the APC captured the potential antigens, contains enough molecular patterns signaling damage/danger, the APCs undergo a process of maturation that allows them to trigger an adaptive immune response.

Scheme 1 – Monocytes, DCs and Macrophages subsets in humans



1.3.1 Dendritic Cells

Dendritic cells (DCs) are considered the most important subpopulation of APCs with unique abilities to activate and stimulate naïve T lymphocytes (BANCHEREAU et al., 2000). Diverse DC subsets have been identified in mouse and humans during the past decades, and their dual role in the balance between immunity versus tolerance is increasingly recognized. In healthy tissues, immature DCs capture and process antigens, which, presented to T cells will lead to tolerance;

however, when DCs recognize a tissue imbalance, they acquire a mature phenotype during their migration to the draining lymph node, where they can stimulate (naïve) T cells, thus triggering the adaptive response to the antigens they present (MELLMAN; STEINMAN, 2001). During the maturation process DCs show an increased expression of the CCR7 chemokine receptor (GUERMONPREZ et al., 2002; YANAGIHARA et al., 1998) and up-regulate the expression of co-stimulatory (CD80, CD86 e CD40) and MHC molecules (class I and II), crucial signals that will directly regulate the quality and the intensity of T cell responses (BANCHEREAU et al., 2000; CAUX et al., 1994a; CAUX et al., 1994b). DCs consist of a very heterogeneous group of cells in mice and humans that may share similar functions but are not completely defined. In the literature, human DCs were characterized and divided in two major populations in peripheral blood: the plasmacytoid DCs (defined as $BDCA2^+$) and myeloid/conventional DCs (defined as $BDCA1^+$ or $BDCA3^+$).

Human plasmacytoid DCs (pDCs), further characterized by the expression of the BDCA2 marker (CD303), have their origin in the bone marrow and can be found in the circulation and in several tissues, where they respond to viral infections with the production of high levels of IFN-α (reviewed by MATHAN, 2013). Some authors have described a role for pDCs in the induction and proliferation of regulatory T cells *in vivo* and *in vitro* (OCHANDO et al., 2006; OUABED et al., 2008; SHARMA et al., 2007; TAKAGI et al., 2011) and, also, in the activation of Th17 responses in experimental autoimmune encephalomyelitis (ISAKSSON et al., 2009) and in mouse models of cancer (GUERY et al., 2014)

Human myeloid/conventional DCs (mDCs) are also derived from the bone marrow and found at low concentrations in the blood, lymphoid organs, and other tissues. These cells are further subdivided into two distinct subsets: $BDCA1^+$ ($CD1c^+$) cells are apparently the best inducers of T $CD4^+$ and cytotoxic responses, whereas $BDCA3^+$ ($CD141^+$) cells, have been described as more efficient to cross-present antigens. Recent studies have shown that human $BDCA3^+$ mDCs, though present in lymphoid tissues at very low frequencies, are highly effective in the cross-presentation of tumor and necrotic antigens for the induction of T $CD8^+$ activation (BACHEM et al., 2010; JONGBLOED et al., 2010; SEGURA et al., 2013a). In turn, $BDCA1^+$ mDCs may be considered as the better equipped DC subset to sense tissue imbalances, mainly due to their wide expression of Toll-like receptors (HÉMONT et al., 2013). These, when engaged, lead to an efficient maturation of mDCs, the

production of IL-12 and the expression of high levels of co-stimulatory molecules, favoring the differentiation of T cells towards the Th1 profile (NIZZOLI et al., 2013).

Additionally, diverse strategies allowed the differentiation *in vitro* of myeloid DCs from circulating precursors, like $CD34^+$ cells - in presence of GM-CSF and TNF-α (CAUX et al., 1996) - or blood monocytes - with GM-CSF and IL-4 (SALLUSTO; LANZAVECCHIA, 1994) - generating monocyte-derived DCs (Mo-DCs). The possibility of Mo-DCs generation has opened a large spectrum of possibilities to study and exploit DCs in immunotherapeutic protocols for infections and cancer (BANCHEREAU et al., 2005; BARBUTO et al., 2004). It is worth noting that some researchers do not consider Mo-DCs as an *in vivo* existing population in humans (NAIK, 2008). However, more recently, an elegant study based on gene signature revealed that human Mo-DCs generated *in vitro* may, indeed, be equivalent to the inflammatory DCs *in vivo*, a DC subset that arises in inflammatory conditions. Inflammatory DCs, defined as $CD14^+BDCA1^+FC\varepsilon RI^+$, were found in synovial and ovarian ascites fluids and share some functional abilities with monocyte/macrophages, but were uniquely able to expand Th17 lymphocytes *ex-vivo* (SEGURA et al., 2013b). All in all, one can say that DCs are extremely important in the activation and modulation of immunity, mainly by their ability to prime naïve T cells, but their origin and development, in humans, is only starting to be unraveled (BRETON et al., 2015; LEE et al., 2015).

1.3.2 Macrophages

Though DCs are the major inducers of naïve T cell responses, other well-known APCs, the macrophages (MΦ), are equally critical for lymphocyte activation in tissues. Macrophages have an essential role in the modulation of tissue microenvironment, fundamentally by their ability of phagocytosis and clearance, by the large quantity of cytokine they secrete and by their spectral plasticity. During an inflammatory process, newly arrived monocytes can be rapidly recruited to tissues, where they differentiate into macrophages, contributing to local immunity, while resident macrophages can live long in tissues, up to decades, and are deeply committed to maintain tissue equilibrium, regulating the intensity of inflammation, and acting in tissue remodeling (GORDON; MARTINEZ, 2010). Thus, macrophages in

tissues may derive from two distinct differentiation pathways: one giving rise to the resident MΦ, which, in mice at least, seem to emerge at the fetal stage, from hematopoietic precursors in the liver and have a low rate of renewal (reaching up to 30 years in humans); the other pathway is detected during infections or inflammatory processes, when blood monocytes migrate into tissues and differentiated into MΦ. Though heterogeneous, MΦ share some functional characteristics, even when localized in distinct tissues, where they receive different names: Alveolar Macrophages, Peritoneal Macrophages, Kupffer cells, Microglia, Osteoclast, etc (Reviwed by EPELMAN et al., 2014). These cells are involved in the control of infections (GORDON, 2003; RUSSEL et al., 2009), in the resolution of acute inflammation (SERHAN; SAVILL, 2005), and in the regulation of the metabolic responses to tissue stress (HOTAMISLIGIL; ERBAY, 2008). Through their broad range of functions and dynamic plasticity, macrophages are also implicated in several chronic pathological conditions including diabetes and atherosclerosis (MEDZHITOV, 2008; TABAS, 2010).

MΦ seem to be weak inducers of naïve T cell activation, a phenomenon that, *in vivo*, could be due to their poor competence to migrate to lymph nodes for antigen presentation, in contrast to DCs. On the other hand, MΦ present a large spectrum of morphological and functional plasticity, which is affected by local tissue conditions and by their cell-to-cell interactions during the immune responses. Diverse authors have described MΦ as a bi-functional population that can be classified as M1-MΦ (inflammatory) or M2-MΦ (anti-inflammatory), assuming similar parameters to those used to define Th1 and Th2 responses. However, this classification may be an oversimplification of their biology. To define the two polarized subtypes, tissue localization, surface markers, and the profile of produced cytokines are used (SICA; MANTOVANI, 2012). Human M1 macrophages show high expression of CD86 and HLA-DR, and produce diverse pro-inflammatory molecules as IL-12, TNF-α, CXCL9 and iNOS. On the other side, M2 anti-inflammatory macrophages are usually defined by their elevated expression of the scavenger receptor CD163 and by the production of typical anti-inflammatory cytokines, as IL-10 and TGF-beta, and the angiogenic factor VEGF (SICA; MANTOVANI, 2012). Nonetheless, it is important to highlight that this clearly bipolar behavior is observed when MΦ are differentiated *in vitro*, under well-defined conditions (JAGUIN et al., 2013; LACEY et al., 2012). The plasticity of MΦ *in vivo* is much more complex. It must be fine tuned to fit the needs of tissues

subjected, for example, to chronic infections or tumor development (MOSSER; EDWARDS, 2008), as the present work will demonstrate.

1.3.3 Monocytes

As the previous paragraphs have demonstrated, monocytes are an important blood cell, generated in the bone marrow and present in peripheral blood with a half-life of 1-2 days. Though monocyte recruitment to the tissues occurs during infections or inflammatory diseases, their contribution to the homeostatic tissue population (e.g. resident Macrophages) without diseases is minimal after birth (reviewed by AUFFRAY et al., 2009). These cells are heterogeneous and can be divided in three distinct subpopulations: one major subset, defined as CD14 positive but with low CD16 expression ($CD14^{++}CD16^{neg/low}$, called classical monocytes); one minor subset that express low or no CD14, but high CD16 ($CD14^{low}CD16^{++}$, called non-classical monocytes); and one transient or intermediate subpopulation identified by the double expression of intermediate levels of CD14 and CD16 ($CD14^{+}CD16^{+}$) (PASSLICK et al., 1989).

The $CD14^{++}CD16^{neg/low}$ monocytes represent about 90% of total blood monocytes, express high levels of the chemokine receptor 2 (CCR2) and low levels of the chemokine receptor CX3CR1. It is the only subset able to produce IL-10 rather than TNF-α after LPS activation *in vitro* (SKRZECZYŃSKA-MONCZNIK et al., 2008; WEBER et al., 2000; ZIEGLER-HEITBROCK et al., 1992). In contrast, human $CD14^{low}CD16^{++}$ monocytes secrete high levels of TNF-α in response to LPS (actually, they are the highest producers when compared to the other subpopulations), a characteristic that gave them the name of inflammatory monocytes (BELGE et al., 2002; SKRZECZYŃSKA-MONCZNIK et al., 2008). Transient monocytes ($CD14^{++}CD16^{+}$), on the other hand, secrete intermediate levels of both IL-10 and TNF-α, depending on the stimulus. Furthermore, these cells express the Fc gamma receptors CD64 and CD32 and have high phagocytic activity (GRAGE-GRIEBENOW et al., 2001). Studies in literature have reported that monocytes expressing high levels of CD16 are increased in the peripheral blood of patients with acute inflammation (MIZUNO et al., 2005) and infectious diseases (HORELT et al., 2002), but are dramatically reduced in subjects submitted to

glucocorticoid treatment (FINGERLE-ROWSON et al., 1998). It is interesting to mention that the complete absence of $CD16^+$ monocytes from the circulation is not necessarily associated with disease (FRANKENBERGER et al., 2013). Thus, a dynamical plasticity among subsets of monocytes is readily detectable (ZIEGLER-HEITBROCK; HOFER, 2013), but their contribution to tissue Macrophage/DC subpopulations in the time-course of human diseases remains poorly understood.

1.4 The Immune System: Stimulation of T lymphocyte subsets

Besides the role these three cell populations (DCs, MΦ, and monocytes) play in the inflammatory process, they are also critical for the generation and evolution of adaptive immune responses. It is well known that APCs, through a series of signals, generate a combinatory "code" that primes T cells and starts the adaptive immune response. The activation of naïve T cells depends on the engagement of its T cell receptor (TCR), interacting with the MHC class I or II molecules plus antigenic peptide complexes, and a combination of co-stimulatory signals (frequently termed "second signal"). This activation can be further modulated by the various cytokines in the microenvironment, and significantly by those produced by DCs, resulting on lymphocyte polarization and expansion. These interactions occur in the secondary lymphoid organs and are essential for the conversion of naïve $CD4^+$ T lymphocytes into function-committed T cells, which coordinate the overall immune response, through the stimulation of other immune cells. Actually, $CD4^+$ T lymphocytes may acquire different cytokine secretion profiles and, thus, be separated into four major subsets: T helper (Th) 1 cells, Th2, Th17 and regulatory T cells (Tregs).

Th1 cells are usually induced by the combination of signals delivered by high levels of CD80/CD86 on the APCs and IL-12, are characterized by the expression of the transcription factor T-bet, and secrete high levels of IFN-γ. This subset is frequently associated with effective responses to intracellular bacteria and pathogen destruction. It also induces the activation of T $CD8^+$ lymphocytes, Natural Killer (NK) cells and pro-inflammatory macrophages (OESTREICH; WEINMANN, 2012).

Th2 cells, on the other hand, are mainly induced by IL-4 signaling and typically express the intra-nuclear factor GATA-3. Th2 lymphocytes secrete IL-4, IL-5 and IL-13, cytokines that are usually involved in allergic responses and in the elimination of

helminths, phenomena that involve the activation of mast cells and eosinophils (HO, 2009).

Th17 were described more recently and seem to be induced by TGF-β plus IL-6, in cooperation with IL-23 and IL-1β signaling. The transcription factor that characterizes these cells is the ROR-γt and their most typical product is IL-17 (A through F isoforms). These cells seem to be needed for effective immune responses against extracellular pathogens and fungi (ZIELINSKI et al., 2012). Some authors further correlate Th17 cells with chronic tissue inflammation, sometimes cooperating with Th1 cells during the development of several autoimmune diseases (ANNUNZIATO et al., 2012).

Not all T cell subsets, however, are involved in antigen elimination - a fundamental T cell subpopulation is that of the regulatory T cells (Tregs). These are characterized by the expression of the nuclear transcriptional factor Foxp3 and can be further divided into natural Tregs (nTregs), which are generated in the thymus and those induced in the periphery, the induced Tregs (iTregs). nTregs represent about 5-10% of total $CD4^+$ circulating T lymphocytes in humans and are the consequence of an alternative differentiation pathway for thymocytes with a high affinity for self-peptide-MHC complexes. This differentiation pathway seems to rely, in humans, upon migratory DCs activated by the thymic stromal lymphopoietin (TSLP), which create a microenvironment supportive for the induction of Foxp3 in immature $CD4^+CD8^-$ thymocytes, contributing to their positive selection (SAKAGUCHI et al., 2010). In addition, nTregs are extremely important for the maintenance of self-tolerance and immune homeostasis, since individuals with IPEX, a syndrome characterized by Foxp3 deficiency, present serious autoimmune disorders (BARZAGHI et al., 2012). Though iTregs also express Foxp3 and, thus, should be absent in these patients, the role of this latter subpopulation could be, at least in part, overtook by other peripherally induced T cells, like Tr-1 and Th3 cells, which also have suppressive abilities, due to the production of IL-10 and TGF-beta, respectively (FARIA; WEINER, 2005; RONCAROLO et al., 2006).

On the other hand, iTregs are generated in the periphery by the conversion of conventional $CD4^+$ T cells into $CD127^{low}CD25^{high}Foxp3^+$ regulatory T cells. For this conversion it seems that stimulatory signals, from the TCR engagement, added to inhibitory signals, like those delivered by TGF-β and/or IL-10, and, very likely, many others, derived from local APCs, combine, driving the cells through a still

incompletely understood pathway. As mentioned before, other subpopulations of T cells with suppressive abilities have been described in literature.

Actually, other subsets of $CD4^+$ T helper cells are likely to be identified as the investigation of specific conditions progresses, a situation that can be exemplified by the recent description of Th9 and Th22 cells involved in patients with ulcerative colitis (GERLACH et al., 2014) and multiple sclerosis (ROLLA et al., 2014), respectively. Indeed, it is important to point that the profiles of T cell responses are not static in the course of infections or inflammation, but represent a dynamical and cooperative balance between innate and adaptive elements that can lead to immunity or disease. In this dynamical balance, the functional status of DCs, MΦ, and Monocytes is essential, since they are very effective sensors of tissue homeostasis and disequilibrium and able "translators" of the microenvironment to the adaptive immunity.

Even if not addressed in our present work, another important aspect of the immune system is its ability to develop humoral responses. Besides their obvious role in the production of antibodies, whose roles in tumor immunity are not negligible, B lymphocytes are also able to present antigens via MHC-II and, thus, might affect more closely the issues addressed in the present work. Nevertheless, these possible roles will not be further discussed, but should be, eventually integrated in a view that would lead to the full comprehension of tumor-immune system interactions.

1.5 The Immune System under tumor development: new players for a new game

So, the immune system is an effective participant in the maintenance of physiological equilibrium in the organism. When this is disrupted by an infection, immune sentinels, in general, are fast to identify the situation and to trigger immunity. However, the development of tumors is normally recognized late, probably due to its low immunogenicity and high capacity to hide the tissue microenvironment changes induced by its presence. This is most evident in the analysis of DCs and MΦ within tumors, whose functional alterations resulting in ineffective anti-tumor immune responses, contributing not only for the persistence but also for the growth and tumor metastasis. Actually, during cancer development, tumor and stromal cells promote

the migration/expansion of immunosuppressive regulatory T lymphocytes (Treg) (FAGET et al., 2011; GOBERT et al., 2009), the accumulation of anti-inflammatory Tumor-Associated Macrophages (TAMs) (BISWAS; MANTOVANI, 2010; POLLARD, 2004), and cause alterations in DC biology at the activation and functional levels (BALEEIRO et al., 2008; SISIRAK; FAGET et al., 2012).

Several studies have associated Tregs accumulation with tumor immune escape mechanism in cancer (CURIEL, 2007; ZOU, 2005; ZOU, 2006). Some authors consider this fact as a major obstacle in the development of cancer immunotherapy (DUNN; OLD; SCHREIBER, 2004; SAKAGUCHI, 2005; ZOU, 2005). Coherently, other authors during the past decades have described the profile of infiltrating immune cells in different human tumors as an important predictive factor for disease progression (FRIDMAN et al., 2013). Indeed, the profile of tumor-infiltrating lymphocytes may be, according to some authors, the most important characteristic in the pathological analysis of tumors, for prognosis evaluation (GALON et al., 2012). On the other hand, the induction of an immune response able to eliminate tumor cells is crucially dependent on the ability of APCs to recognize tissue disequilibrium, capture/process and present tumor antigens. However, during its establishment, the tumor microenvironment affects profoundly this recognition, thus changing the possible immune reactivity to the tumor.

1.5.1 Tumor-Associated Macrophages and Dendritic Cells

In clinical studies, the increase of TAMs in tissues has been directly correlated with tumor growth (BINGLE et al., 2002) and also with a worse clinical outcome in several types of human cancer, including ovarian, breast, non-small cell lung cancer, and Hodgkin's lymphoma (CAMPBELL et al., 2010; POLLARD, 2009; STEIDL et al., 2010). Indeed, in the tumor context, macrophages are usually associated to a range of pro-tumor actions, such as: the production of angiogenic (VEGF) and survival factors for malignant cells, the promotion of tissue remodeling and the production of immunosuppressive cytokines (e.g. IL-10, TGF-beta) that block T cell effector functions in the microenvironment (reviewed by QIAN; POLLARD, 2011). It is relevant to notice that polarization/modulation of macrophages is not exclusively due to tumor factors, but driven by reciprocal interactions with, both, malignant and

stromal cells in the microenvironment (LEWIS; POLLARD, 2006; LEWIS; HUGHES, 2007). One example of such participation of stromal cells was shown by Sharma and colleagues (2010), who demonstrated that tumor-associated fibroblasts specific molecular signatures were strongly associated with different stages of breast cancer development, and also with TAMs functional profiles.

Nevertheless, TAMs, themselves, seem to contribute for tumor growth, since their presence was associated with an increase in the tumor vasculature density in several human carcinomas, including breast (BOLAT et al., 2006; UZZAN et al., 2004). TAMs also regulate the composition and structure of extracellular matrix (ECM) through their deposition of components, which consequently may regulate tumor and stromal cell migration/invasion. As examples, we can mention the production of diverse types of collagens; the release of matrix metalloproteinases (MMPs), the production of serine proteases and cathepsins (KESSENBROCK et al., 2010). Furthermore, several studies have associated TAMs function with an increased ability of tumors to invade and metastasize, as shown in melanoma (VARNEY et al., 2005), breast (BECK et al., 2009; ROBINSON et al., 2009), ovarian (KAWAMURA et al., 2009), and colorectal (BAILEY et al., 2007) cancers.

TAMs have been detected in human tumors, mainly in retrospective studies using immunohistochemistry, by different markers. Though CD68 has been used for that, over a long period, to that purpose, CD163 has been more recently recognized as superior (HEUSINKVELD; VAN DER BURG, 2011) since subsets of dermal DCs (PETZELBAUER et al. 1993) and fibroblasts can express CD68 (RUFFELL et al., 2012). Furthermore, CD163 has been extensively associated to a M2-like profile, both for *in vitro* differentiated cells and *ex-vivo* obtained TAMs (HEUSINKVELD; VAN DER BURG, 2011), reveling a superior specificity than CD68. However, CD163 functions *per se* have not been directly associated to M2-MΦ functions. CD163 is a scavenger receptor able to capture free-hemoglobin, resulting from the rupture of red cells, as it could be expected in uncontrolled strong inflammation (FABRIEK et al., 2005) but some authors also showed its function on bacteria binding to human MΦ triggering the production of cytokines (FABRIEK et al., 2009).

Other study revealed a decrease in tumor-infiltrating DCs frequency in tumor areas when compared to normal adjacent tissues (RUFFELL et al., 2012). Besides that, the investigation of their functional status *in situ* revealed tumor-infiltrating DCs as immature in tumor bed, in contrast to activated DCs found in tumor periphery

(BALEEIRO et al., 2008; BELL et al., 1999; TREILLEUX et al., 2004). Interestingly, a recent study published by Goc and collaborators (2014) correlated a lower risk of death in lung cancer patients with the presence of mature DCs and Th1 lymphocytes in peritumoral tertiary lymphoid structures.

Even though the phenotypic and functional characterization of APCs in tumors is well established in murine models as a prognostic factor, in humans, this characterization and its relevance still represent a challenge. Thus, here, we will analyze the phenotypic and functional features of MΦ recovered from human tumors and attempt to correlate their frequency to other infiltrating immune cells and patients' survival, as comparing their characteristics with the "typical" MΦ differentiated *in vitro* from monocyte precursors.

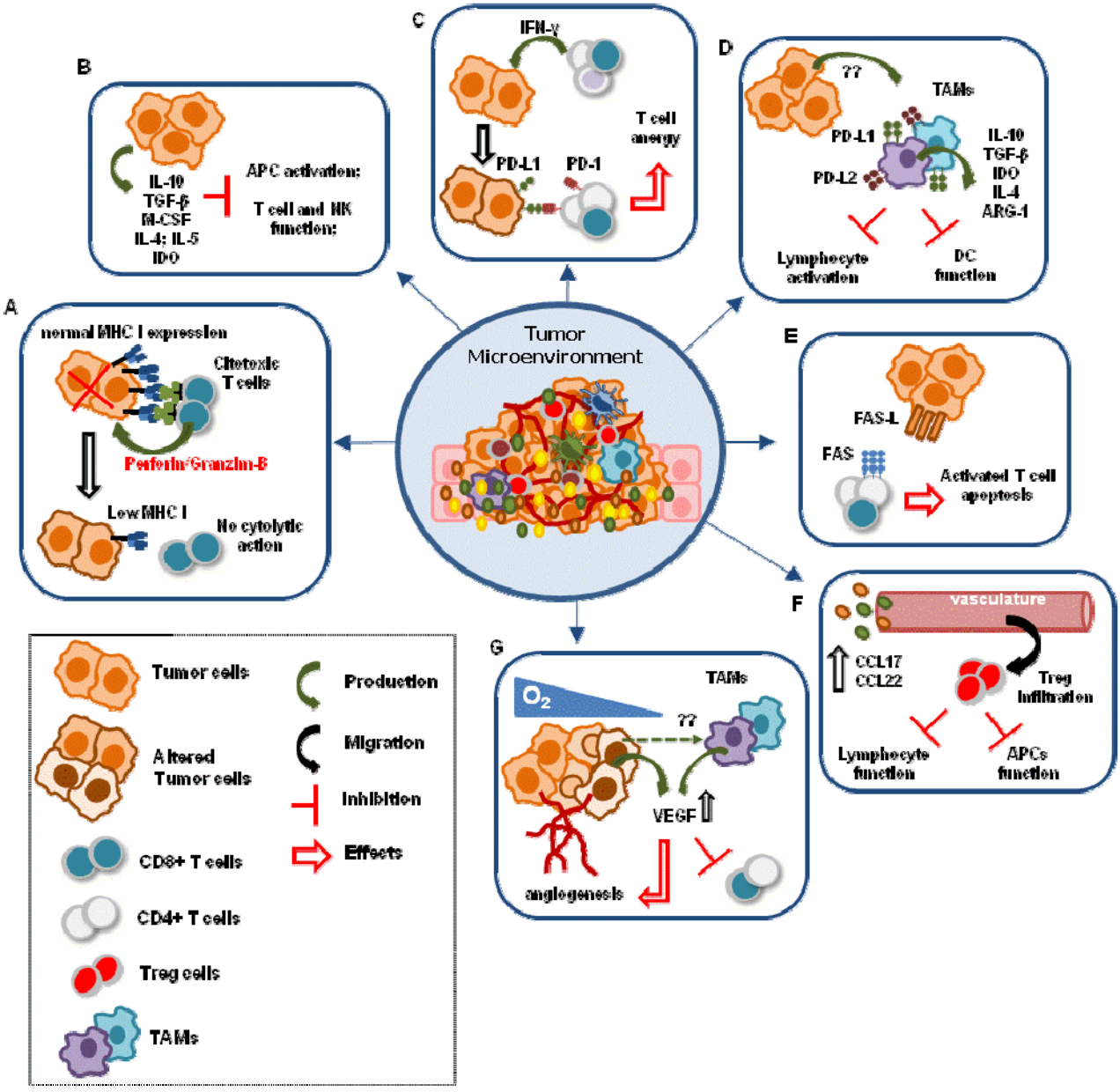
1.6 Mechanisms of tumor escape from the Immune System

It is important to consider that, as mentioned before, tumor cells are able not just to grow, invade and generate metastasis, but also present "smart strategies" to escape from the immune system (Scheme 2). The exact time point where the fine tuned adjustment, where anti-tumor immunity can avoid cancer growth, fails is difficult to define in humans. Contributes to that, surely, the genetic instability that can, eventually generate tumor cells able to evade immunity. This might occur because tumor cells reduce their "visibility", inhibit the immune cells in their environment or recruit specific cell populations.

Indeed, numerous tumor escape mechanisms have been described among which we can highlight: MHC class I down-regulation (SELIGER et al., 1996) (Scheme 2A); the production of anti-inflammatory cytokines as IL-10, IL-4 and IL-5 (YAMAMURA et al., 1993) or TGF-β (TADA et al., 1991) (Scheme 2B); the expression of negative co-stimulatory molecules as PD-L1/PD-L2 by tumor or infiltrating myeloid cells (BLANK; MACKENSEN, 2007; KUANG et al., 2009) (Scheme 2C and D), and apoptosis inducer Fas-L (GORDON; KLEINERMAN, 2009) (Scheme 2E). Actually, signals derived from tumors, not only act directly upon immune effector cells but also induce the conversion and/or the recruitment of cells with suppressive functions to the tissues, as CCL22/CCL17 do, recruiting regulatory T cells to tumor sites (GOBERT et al., 2009) (Scheme 2F). Additionally, VEGF, a well-known

angiogenesis factor, produced in the tumor microenvironment by malignant cells and/or TAMs, thus increasing nutrients's access to tumors cells, can also act as a potent inhibitor of T cell function (VORON et al., 2015) (Scheme 2G).

Scheme 2 – Tumor escape Mechanisms from the Immune System



Despite intense investigation, the precise mechanisms that lead to tumor escape are still poorly defined, but it is clear that, among these mechanisms, the functional modification of APCs should play a relevant role. For the investigation of this issue, it is relevant to note that myeloid DCs and MΦ can be differentiated from the same precursor, the blood monocytes. In inflammatory conditions or in well-defined *in vitro* conditions, this has been well established, but very few studies have investigated monocyte differentiation under the pressure of the tumor microenvironment in human systems. In fact, tumors may generate an anti-inflammatory *milieu*, rich in cytokines secreted by malignant cells, like IL-4, IL-6, VEGF, TGF-β and IL-10, which are able to promote monocytes/macrophages re-education towards an anti-inflammatory M2 profile and to block DCs functional maturation (GABRILOVICH, 2004; MANTOVANI et al., 2002; RABINOVICH et al., 2007).

Data obtained by Ménétrier-Caux and collaborators in 1998, revealed that breast tumor cell lines were able to skew healthy monocytes differentiation into macrophages through combined IL-6 and M-CSF signaling. Additionally, Thomachot and colleagues (2004) also showed that breast carcinoma cell lines were able to block DC maturation. However, the effects of the "complete" tumor microenvironment, as found *in vivo*, upon monocytes have not been explored yet. Nevertheless, we can hypothesize that, indeed, MΦ and DCs found in tumors may derive from "newly arrived" blood monocytes that, receiving the anti-inflammatory signals from the microenvironment during their differentiation, become skewed cells that favor tumor escape and growth.

1.7 Immunotherapy as a way to treat cancer patients

It is clear the crucial role of immune system in the surveillance of tissues and organs in the maintaining of homeostasis, avoiding the success of pathogens, infections and tumors. Conversely, it has become more acceptable for scientists that cancer modulates immunity in a singular way, and, thus, therapeutic interventions need to consider not only the cancer cells *per se* but also their ability to "cheat" immune control mechanisms as well. For example, one of such phenomena is the increase in PD-L1 expression by tumor cells in response to IFN-γ produced by

infiltrating T lymphocytes (BLANK et al., 2004). As a consequence, the newly expanded PD-L1+ tumors cells can inhibit infiltrating lymphocytes via PD-1, and escape from immune elimination.

Hence, it's now clear that cancer and the immune system are in close relationship whose fine-tuning may bring benefit for patients. This understanding led to several studies, pre-clinical and clinical, investigating the potential of immunotherapy against cancer in the last years (MCNUTT, 2013). Among these, the success of anti-CTLA-4 and PD-L1 monoclonal antibodies in cancer treatment clearly reinforces the point (HERBST et al., 2014; HODI et al., 2010; ROBERT et al., 2011).

1.8 Systemic effects on immune cells during tumor development

Certainly, several characteristics of malignant and stromal cells acquired during carcinogenesis can add to the establishment of a very complex microenvironment, able to support cancer growth and metastasis, and to promote its escape from the immune system. In this context, our present study will focus on the tumor microenvironment and its potential ability to affect the immune infiltrate, mainly investigating the effects of soluble factors from the microenvironment in the modulation of blood monocytes' differentiation and function. Though the direct effects of tumor derived-factors in the local inhibition of immune cells have been addressed by other studies, very few have called attention to the distant effects of tumors upon monocytes, MΦ, and DC derived from them. Such systemic effects may have profound effects upon the anti-tumor immune responses and have been reported previously in thesis and dissertations from our group, showing that circulating monocytes obtained from breast cancer patients fail to differentiate into functional DCs (Mo-DCs) *in vitro* (AZEVEDO-SANTOS, 2010; RAMOS, 2011). In these studies we described that Mo-DCs derived from breast cancer patients present an altered phenotype, produce high levels of IL-10 and fail to induce T lymphocyte proliferation. Here we will explore additional functional aspects of patients' Mo-DC, investigate the potential of patients' monocytes to differentiate into functional MΦ and we elucidate what are the characteristics of blood monocytes freshly isolated from breast cancer patients.

We expect that the characterization of the unique microenvironment generated by tumor development in humans, able to modulate the immune system and more particularly the monocytes and MΦ axis, at local and systemic levels, may provide insights for the improvement of current immunotherapeutic approaches against cancer, and, possibly, help design new ones targeting monocytes/MΦ.

6 CONCLUSION

Main findings obtained in our study:

- Breast and ovarian tumors shown high infiltration by Tumor-associated Macrophage (TAMs) with very heterogeneous CD163 expression;

- Higher presence of TAMs $CD163^{high}$ is correlated to lower infiltration of T $CD3^{+}$ lymphocytes in breast cancer tissues;

- $CD64^{+}CD163^{high}$ TAMs express high PD-L1 levels and up-regulate its expression and IL-10 production under LPS stimulation *ex-vivo;*

- Higher CD163 expression *in situ* is correlated with poor breast cancer patients' outcome within 12,5 years of retrospective analysis;

- Primary tumor microenvironment derived factors can induce SNDil-MΦ $CD163^{high}$PD-$L1^{high}CD86^{low}$IL-10^{high} phenotype on conditioned monocytes;

- SNDil-MΦ $CD163^{high}$ suppress $CD4^{+}$ T cell expansion via partially role of IL-10;

- The increased presence of IL-8, CCL19, CCL21, VEGF, M-CSF, TGF-β3 TGF-β1 and CCL22 molecules in tumor microenvironment is associated to SNDil-MΦ $CD163^{high}$IL-10^{high} phenotype;

- Breast cancer patients' monocytes originate biased dendritic cells that induce higher frequency of $CD4^{+}CD25^{+}Foxp3^{+}$ regulatory T cells with TGF-β1 and PD-L1 participation;

- Breast cancer patients' monocytes fail to fully differentiate into M1-MΦ, maintaining partial CD163 expression and producing high amounts of IL-10 cytokine;

- Circulating blood monocytes from breast cancer patients display a different profile of cytokine production in comparison to healthy donors, by secreting higher amounts of IL-10, VEGF-A, IL-27, sCD40L, IL-21, IL-1RA and M-CSF under 24 hours of LPS activation.

REFERENCES*

AAROE, J. et al. Gene expression profiling of peripheral blood cells for early detection of breast cancer. **Breast Cancer Res**., v. 12, n. 1, p. R7, 2010.

ADAMS, J. et al. Vascular endothelial growth factor (VEGF) in breast cancerp. comparison of plasma, serum, and tissue VEGF and microvessel density and effects of tamoxifen. **Cancer Res**., v. 60, n. 11, p. 2898-2905, 2000.

AHN, G. O. et al. Transcriptional activation of hypoxia-inducible factor-1 (HIF-1) in myeloid cells promotes angiogenesis through VEGF and S100A8. **Proc. Natl. Acad. Sci. U S A.,** v. 111, n. 7, p. 2698-2703, 2014.

AMARNATH, S. et al. Regulatory T Cells and human myeloid dendritic cells promote tolerance via programmed death ligand-1. **PLoS Biology**, v. 8, p. 1-13, 2010.

ANNUNZIATO, F.; COSMI, L.; LIOTTA, F.; MAGGI, E.; ROMAGNANI, S. Defining the human T helper 17 cell phenotype. **Trends Immunol.,** v. 33, p. 505-512, 2012.

AUFFRAY, C.; SIEWEKE, M. H.; GEISSMANN, F. Blood monocytesp. development, heterogeneity, and relationship with dendritic cells. **Annu. Rev. Immunol**., v. 27, p. 669-692, 2009.

AVRAHAM-DAVIDI, I. et al. On-site education of VEGF-recruited monocytes improves their performance as angiogenic and arteriogenic accessory cells. **J. Exp. Med.,** v. 210, n. 12, p. 2611-2625, 2013.

AZEVEDO-SANTOS, A. P. S. **Efeito do microambiente tumoral sobre as características funcionais e fenotípicas de células dendríticas geradas *in vitro* a partir de monócitos do sangue periférico de voluntarias saudáveis e de pacientes com câncer de mama.** 2010. 115 f. Tese (Doutorado em Imunologia) – Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, 2010.

BACHEM, A. et al. Superior antigen cross-presentation and XCR1 expression define human CD11c+CD141+ cells as homologues of mouse CD8+ dendritic cells. **J. Exp. Med.**, v. 207, p. 1273–1281, 2010.

BAILEY, C. et al. Chemokine expression is associated with the accumulation of tumour associated macrophages (TAMs) and progression in human colorectal cancer. **Clin. Exp. Metastasis**, v. 24, p. 121–130, 2007.

BALEEIRO, R. B.; ANSELMO, L. B.; SOARES, F. A.; PINTO, C. A.; RAMOS, O.; GROSS, J. L.; HADDAD, F.; YOUNES, R. N.; TOMIYOSHI, M. Y.; BERGAMI-SANTOS, P. C.; BARBUTO, J. A. High frequency of immature dendritic cells and altered in situ production of interleukin-4 and tumor necrosis factor-alpha in lung cancer. **Cancer Immunol. Immunother.,** v. 57, p. 1335-1345, 2008.

BANCHEREAU, J.; BRIERE, F.; CAUX, C.; DAVOUST, J.; LEBECQUE, S.; LIU, Y. J.; PULENDRAN, B.; PALUCKA, K. Immunobiology of dendritic cells. **Annu. Rev. Immunol.**, v. 18, p. 767-811, 2000.

[*] In accordance to:
ASSOCIAÇÃO BRASILEIRA DE NORMAS TÉCNICAS. **NBR 6023**: information and documentation: references: elaboration. Rio de Janeiro, 2002.

BANCHEREAU, J.; PALUCKA, A. K. Dendritic cells as therapeutic vaccines against cancer. **Nat. Rev. Immunol.**, v. 5, p. 296-306, 2005.

BARBUTO, J. A. Are dysfunctional monocyte-derived dendritic cells in cancer an explanation for cancer vaccine failures? **Immunotherapy,** v. 5, p. 105-107, 2013.

BARBUTO, J. A.; ENSINA, L. F.; NEVES, A. R.; BERGAMI-SANTOS, P.; LEITE, K. R.; MARQUES, R.; COSTA, F.; MARTINS, S. C.; CAMARA-LOPES, L. H.; BUZAID, A. C. Dendritic cell-tumor cell hybrid vaccination for metastatic cancer. **Cancer Immunol. Immunother.**, v. 53, p. 1111-1118, 2004.

BARLEON, B. et al. Migration of human monocytes in response to vascular endothelial growth factor (VEGF) is mediated via the VEGF receptor flt-1. **Blood,** v. 87, p. 3336–3343, 1996.

BARZAGHI, F.; PASSERINI, L.; BACCHETTA, R. Immune dysregulation, polyendocrinopathy, enteropathy, x-linked syndromep. a paradigm of immunodeficiency with autoimmunity. **Front Immunol.,** v. 3, p. 211, eCollection, 2012.

BECK, A. H. et al. The macrophage colony-stimulating factor 1 response signature in breast carcinoma. **Clin. Cancer Res.,** v. 15, p. 778–787, 2009.

BELAI, E. B. et al. PD-1 blockage delays murine squamous cell carcinoma development. **Carcinogenesis,** v. 35, p. 424-431, 2014.

BELGE, K. U. et al. The proinflammatory CD14+CD16+DR++ monocytes are a major source of TNF. **J. Immunol**., v. 168, p. 3536–3542, 2002.

BELKAID, Y.; OLDENHOVE, G. Tuning microenvironmentsp. induction of regulatory T cells by dendritic cells. **Immunity**, v. 29, p. 362–371, 2008.

BELL, D. et al. In breast carcinoma tissue, immature dendritic cells reside within the tumor, whereas mature dendritic cells are located in peritumoral areas. **J. Exp. Med.,** v. 190, p. 1417-1426, 1999.

BINGLE, L.; BROWN, N. J.; LEWIS, C. E. The role of tumour-associated macrophages in tumour progressionp. implications for new anticancer therapies. **J. Pathol.**, v. 196, p. 254-265, 2002.

BENOY, I. et al. Serum interleukin 6, plasma VEGF, serum VEGF, and VEGF platelet load in breast cancer patients. **Clin. Breast Cancer**, v. 2, n. 4, p. 311-315, 2002.

BERGENFELZ, C. et al. Systemic Monocytic-MDSCs are generated from monocytes and correlate with disease progression in breast cancer patients. **PLoS One,** n. 10, n. 5, p. e0127028, 2015.

BISWAS, S. K. et al. A distinct and unique transcriptional program expressed by tumor-associated macrophages (defective NF-kappaB and enhanced IRF-3/STAT1 activation). **Blood,** v. 107, n. 5, p. 2112-2122, 2006.

BISWAS, S. K.; MANTOVANI, A. Macrophage plasticity and interaction with lymphocyte subsetsp. cancer as a paradigm. **Nat. Immunol.,** n. 11, p. 889-896, 2010.

BLANK, C. et al. PD-L1/B7H-1 inhibits the effector phase of tumor rejection by T cell receptor (TCR) transgenic CD8+ T cells. **Cancer Res.,** v. 64, n. 3, p. 1140-1145, 2004.

BLANK, C. et al. Blockade of PD-L1 (B7-H1) augments human tumor-specific T cell responses in vitro. **Int. J. Cancer,** n. 119, p. 317–327, 2006.

BLANK, C. et al. Contribution of the PD-L1/PD-1 pathway to T-cell exhaustionp. an update on implications for chronic infections and tumor evasion. **Cancer Immunol. Immunother.,** v. 56, p. 739–745, 2007.

BOLAT, F. et al. Microvessel density, VEGF expression, and tumor-associated macrophages in breast tumorsp. correlations with prognostic parameters. **J. Exp. Clin. Cancer Res.,** v. 25, p. 365–372, 2006.

BOLPETTI, A.; SILVA, J. S.; VILLA, L. L.; LEPIQUE, A. P. Interleukin-10 production by tumor infiltrating macrophages plays a role in Human Papillomavirus 16 tumor growth. **BMC Immunol**., v. 11, n. 27, p. 2-13, 2010.

BORKOWSKI, T. A. et al. A role for endogenous transforming growth factor β1 in Langerhans cell biologyp. the skin of transforming growth factor β1 null mice is devoid of epidermal Langerhans cells. **J. Exp. Med.,** v. 184, p. 2417–2422, 1996.

BOS, R. et al. Levels of hypoxia-induciblefactor-1 alpha during breast carcinogenesis. **J. Natl. Cancer Inst.,** v. 93, p. 309–314, 2001.

BRAHMER, J. R.; TYKODI, S. S.; CHOW, L.Q. et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. **N. Engl. J. Med.,** v. 366, p. 2455–2465, 2012.

BRAUN, D. A.; FRIBOURG, M.; SEALFON, S. C. Cytokine response is determined by duration of receptor and signal transducers and activators of transcription 3 (STAT3) activation. **J. Biol. Chem.,** v. 288, n. 5, p. 2986-2993, 2013.

BRAUN S. et al. Cytokeratin-positive cells in the bone marrow and survival of patients with stage I, II, or III breast cancer. **N. Engl. J. Med.,** v. 342, p. 325-533, 2000.

BRAUN, S. et al. A pooled analysis of bone marrow micrometastasis in breast cancer. **N. Engl. J. Med.,** v. 353, n. 8, p. 793-802, 2005.

BRETON G. et al. Circulating precursors of human CD1c+ and CD141+ dendritic cells. **J. Exp. Med.,** v. 212, n. 3, p. 401-413, 2015.

BUECHLER, C.; RITTER, M.; ORSÓ, E.; LANGMANN, T.; KLUCKEN, J.; SCHMITZ, G. Regulation of scavenger receptor CD163 expression in human monocytes and macrophages by pro- and antiinflammatory stimuli. **J. Leukoc. Biol.,** v. 67, p. 97-103, 2000.

BUELENS, C.; WILLEMS, F.; DELVAUX, A.; PIÉRARD, G.; DELVILLE, J.P.; VELU, T.; GOLDMAN, M. Interleukin-10 differentially regulates B7-1 (CD80) and B7-2 (CD86) expression on human peripheral blood dendritic cells. **Eur. J. Immunol.,** v. 25, n. 9, p. 2668-2672, 1995.

BURNET M. Cancerp. a biological approach. III. Viruses associated with neoplastic conditions. IV. Practical applications. **Br. Med J.,** v. 5023, n. 1, p. 841-847, 1957.

CABRAL-MARQUES, O. et al. Dendritic cells from X-linked hyper-IgM patients present impaired responses to Candida albicans and Paracoccidioides brasiliensis. **J. Allergy Clin. Immunol.,** v.129, p. 778-786, 2012.

CAMPANELLI, A. P. et al. CD4+CD25+ T cells in skin lesions of patients with cutaneous leishmaniasis exhibit phenotypic and functional characteristics of natural regulatory T cells. **J. of Infec. Dis.**, v. 193, p. 1313–1322, 2006.

CAMPBELL, M. J. et al. Proliferating macrophages associated with high grade, hormone receptor negative breast cancer and poor clinical outcome. **Breast Cancer Res Treat.,** v. 128, p. 703-711, 2011.

CASO, R. et al. Blood monocytes from mammary tumor-bearing micep. early targets of tumor-induced immune suppression? **Int. J. Oncol.,** v. 37, p. 891-900, 2010.

CAUX, C. et al. CD34+ hematopoietic progenitors from human cord blood differentiate along two independent dendritic cell pathways in response to GM-CSF+TNF alpha. **J. Exp. Med.,** v. 184, p. 695-706, 1996.

CAUX, C. et al. Activation of human dendritic cells through CD40 cross-linking. **J. Exp. Med.**, v. 180, p. 1263-1272, 1994a.

CAUX, C. et al. B70/B7-2 is identical to CD86 and is the major functional ligand for CD28 expressed on human dendritic cells. **J. Exp. Med**., v. 180, p. 1841-1877, 1994b.

CHANG, H. L. et al. Increased transforming growth factor β expression inhibits cell proliferation in vitro, yet increases tumorigenicity and tumor growth of Meth A sarcoma cells. **Cancer Res.,** v. 53, p. 4391–4398, 1993.

CHEN, W.; JIN, W.; HARDEGEN, N.; LEI, K. J.; LI, L.; MARINOS, N.; MCGRADY, G.; WAHL, S. M. Conversion of peripheral CD4+CD25- naive T cells to CD4+CD25+ regulatory T cells by TGF-beta induction of transcription factor Foxp3. **J. Exp. Med.** v.198, p. 1875–1886, 2003.

CHITTEZHATH, M. et al. Molecular profiling reveals a tumor-promoting phenotype of monocytes and macrophages in human cancer progression. **Immunity,** v. 41, n. 5, p. 815-829, 2014.

CLAUS, M. et al. Vascular permeability factorp. a tumor-derivated polypeptide that induces endothelial cell and monocyte procoagulant activity, and promotes monocyte migration. **J. Exp. Med.,** v. 172, p. 1535–1545, 1990.

COMMEREN, D. L.; VAN SOEST, P. L.; KARIMI, K.; LÖWENBERG, B.; CORNELISSEN, J. J.; BRAAKMAN, E. Paradoxical effects of interleukin-10 on the maturation of murine myeloid dendritic cells. **Immunology,** v. 110, n. 2, p. 188-196, 2003.

CURIEL, T. J. et al. Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. **Nat. Med.,** v. 10, p. 942-949, 2004.

CURIEL, T. J. Tregs and rethinking cancer immunotherapy. **J. Clin. Invest.,** v. 117, p. 1167-1174, 2007.

CURIEL, T. J. et al. Blockade of B7-H1 improves myeloid dendritic cell-mediated antitumor immunity. **Nat. Med.,** v. 9, p. 562-567, 2003.

DANNENMANN, S. R. et al. Tumor-associated macrophages subvert T-cell function and correlate with reduced survival in clear cell renal cell carcinoma. **Oncoimmunology**, v. 2, p. e23562, 2013.

DE CALISTO, J.; VILLABLANCA, E. J.; MORA, J. R. FcγRI (CD64)p. an identity card for intestinal macrophages. **Eur. J. Immunol.,** v. 42, n. 12, p. 3136-3140, 2012.

DE WAAL MALEFYT, R. et al. Interleukin 10 (IL-10) and viral IL-10 strongly reduce antigen-specific human T cell proliferation by diminishing the antigen-presenting capacity of monocytes via downregulation of class II major histocompatibility complex expression. **J. Exp. Med.,** v. 174, n. 4, p. 915-924, 1991.

DEHQANZADA, Z. A. et al. Assessing serum cytokine profiles in breast cancer patients receiving a HER2/neu vaccine using Luminex technology. **Oncol Rep.,** v. 17, n. 3, p. 687-694, 2007.

DENARDO, D. G. et al. Leukocyte complexity predicts breast cancer survival and functionally regulates response to chemotherapy. **Cancer Discov.,** v. 1, n. 1, p. 54-67, 2011.

DIEU-NOSJEAN, M. C. et al. Long-term survival for patients with non-small-cell lung cancer with intratumoral lymphoid structures. **J. Clin. Oncol.,** v. 26, p. 4410-4417, 2008.

DIVELLA, R. et al. Circulating levels of transforming growth factor-βeta (TGF-β) and chemokine (C-X-C motif) ligand-1 (CXCL1) as predictors of distant seeding of circulating tumor cells in patients with metastatic breast cancer. **Anticancer Res.,** v. 33, n. 4, p. 1491-1497, 2013.

DONG, H. et al. B7-H1, a third member of the B7 family, co-stimulates T-cell proliferation and interleukin-10 secretion. **Nat. Med.,** n. 5, p. 1365–1369, 1999.

DUNN, G. P. et al. Cancer immunoeditingp. from immunosurveillance to tumor escape. **Nat. Immunol.,** v. 3, n. 11, p. 991-998, 2002.

DUNN, G. P.; OLD, L. J.; SCHREIBER, R. D. The immunobiology of cancer immunosurveillance and immunoediting. **Immunity**, n. 21, p. 137–148, 2004.

EHRLICH, P. Über den jetzigen stand der karzinomforschung. **Ned. Tijdschr. Geneeskd.** v. 5, p. 273–290, 1909.

EPELMAN, S.; LAVINE, K.J.; RANDOLPH, G. J. Origin and functions of tissue macrophages. **Immunity,** v. 41, n. 1, p. 21-35, 2014.

FABRIEK, B. O. et al. The macrophage scavenger receptor CD163 functions as an innate immune sensor for bacteria. **Blood,** v. 113, p. 887-892, 2009.

FABRIEK, B. O.; DIJKSTRA, C. D.; VAN DEN BERG, T. K. The macrophage scavenger receptor CD163. **Immunobiology**, v. 210, p. 153-160, 2005.

FAGET, J. et al. Early detection of tumor cells by innate immune cells leads to T(reg) recruitment through CCL22 production by tumor cells. **Cancer Res.,** v. 71, p. 6143-6152, 2011.

FAGET, J. et al. Early detection of tumor cells by innate immune cells leads to T(reg) recruitment through CCL22 production by tumor cells. **Can. Res.**, v. 17, p. 6143–6152, 2011.

FAILLI, A.; LEGITIMO, A.; ORSINI, G.; ROMANINI, A.; CONSOLINI, R. Numerical defect of circulating dendritic cell subsets and defective dendritic cell generation from monocytes of patients with advanced melanoma. **Cancer Lett.,** v. 337, p. 184-192, 2013.

FAINARU, O. et al. Dendritic cells support angiogenesis and promote lesion growth in a murine model of endometriosis. **FASEB J.,** n. 22, p. 522-529, 2008.

FAKHRAI, H. et al. Eradication of established intracranial rat gliomas by transforming growth factor β antisense gene therapy. **Proc. Natl. Acad. Sci. USA,** v. 93, p. 2909–2914, 1996.

FARIA, A. M.; WEINER, H. L. Oral tolerance. **Immunol. Rev.,** v. 206, p. 232–259, 2005.

FARREN, M. R. et al. Tumor-induced STAT3 signaling in myeloid cells impairs dendritic cell generation by decreasing PKCβII abundance. **Sci. Signal.,** v. 7, n. 313, p. ra16, 2014.

FINBLOOM, D. S.; WINESTOCK, K. D. IL-10 induces the tyrosine phosphorylation of tyk2 and Jak1 and the differential assembly of STAT1 alpha and STAT3 complexes in human T cells and monocytes. **J. Immunol.,** v. 155, n. 3, p. 1079-1090, 1995.

FINGERLE-ROWSON, G. Expansion of CD14+CD16+ monocytes in critically ill cardiac surgery patients. **Inflammation,** v. 22, p. 367–379, 1998.

FOLKMAN, J. et al. Induction of angiogenesis during the transition from hyperplasia to neoplasia. **Nature,** v. 339, n. 6219, p. 58-61, 1989.

FREEMAN, G. J. et al. Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. **J. Exp. Med.,** v. 192, p. 1027–1034, 2000.

FRIDMAN, W. H. et al. The immune microenvironment of human tumors: general significance and clinical impact. **Cancer Microenviron**., v. 6, p. 117-122, 2013.

FRIDMAN, W. H.; PAGÈS, F.; SAUTÈS-FRIDMAN, C.; GALON, J. The immune contexture in human tumoursp. impact on clinical outcome. **Nat. Rev. Cancer**, v. 12, p. 298-306, 2012.

GABRILOVICH, D. I. et al. Production of vascular endothelial growth factor by human tumors inhibits the functional maturation of dendritic cells. **Nat. Med.,** v. 2, n. 10, p. 1096-1103, 1996.

GABRILOVICH, D. I. et al. Decreased antigen presentation by dendritic cells in patients with breast cancer. **Clin. Cancer Res.,** v. 3, n. 3, p. 483-490, 1997.

GABRILOVICH, D. Mechanisms and functional significance of tumour-induced dendritic-cell defects. **Nat. Rev. Imm.**, v. 4, p. 941–952, 2004.

GABRILOVICH, D. I. ; OSTRAND-ROSENBERG, S.; BRONTE, V. Coordinated regulation of myeloid cells by tumours. **Nat. Rev. Immunol.,** v. 12, p. 253–268, 2012.

GALON, J. et al. Cancer classification using the Immunoscorep. a worldwide task force. **J. Transl. Med.,** v. 10, n. 205, p. 1-9, 2012.

GASTL, G. A. et al. Interleukin-10 production by human carcinoma cell lines and its relationship to interleukin-6 expression. **Int. J. Cancer**., v. 55, n. 1, p. 96-101, 1993.

GATTI, R. A.; GOOD, R. A. Occurrence of malignancy in immunodeficiency diseases. A literature review. **Cancer,** v. 28, n. 1, p. 89-98, 1971.

GERLACH, K. et al. TH9 cells that express the transcription factor PU.1 drive T cell-mediated colitis via IL-9 receptor signaling in intestinal epithelial cells. **Nat. Immunol.,** v. 15, n. 7, p. 676-686, 2015.

GHIRINGHELLI, F.; PUIG, P. E.; ROUX, S.; PARCELLIER, A.; SCHMITT, E.; SOLARY, E.; KROEMER, G.; MARTIN, F.; CHAUFFERT, B.; ZITVOGEL, L. Tumor cells convert immature myeloid dendritic cells into TGF-β-secreting cells inducing CD4+CD25+ regulatory T cell proliferation. **J. Exp. Med.**, v. 202, p. 919–929, 2005.

GOBERT, M. et al. Regulatory T cells recruited through CCL22/CCR4 are selectively activated in lymphoid infiltrates surrounding primary breast tumors and lead to an adverse clinical outcome. **Cancer Res**., v. 69, p. 2000-2009, 2009.

GOC, J. et al. Dendritic cells in tumor-associated tertiary lymphoid structures signal a Th1 cytotoxic immune contexture and license the positive prognostic value of infiltrating CD8+ T cells. **Cancer Res**., v. 74, p. 705-715, 2014.

GORDON, N.; KLEINERMAN, E. S. The role of Fas/FasL in the metastatic potential of osteosarcoma and targeting this pathway for the treatment of osteosarcoma lung metastases. **Cancer Treat. Res.**, v. 152, p. 497-508, 2009.

GORDON, S. Alternative activation of macrophages. **Nat. Rev. Immunol.** v. 3, p. 23–35, 2003.

GORDON, S.; MARTINEZ, F. O. Alternative activation of macrophage: mechanism and functions. **Immunity,** v. 32, p. 593–604, 2010.

GORELIK, L.; FLAVELL, R. A. Abrogation of TGF-β signaling in T cells leads to spontaneous T cell differentiation and autoimmune disease. **Immunity,** v. 12, p. 171–181, 2000.

GORELIK, L.; CONSTANT, S.; FLAVELL, R. A. Mechanism of transforming growth factor beta-induced inhibition of T helper type 1 differentiation. **J. Exp. Med.,** v. 195, p. 1499–1505, 2002.

GRAGE-GRIEBENOW, E. et al. Identification of a novel dendritic cell-like subset of CD64+/CD16+ blood monocytes. **Eur. J. Immunol**., v. 31, p. 48–56, 2001.

GROUX, H.; BIGLER, M.; DE VRIES, J. E.; RONCAROLO, M. G. Interleukin-10 induces a long-term antigen-specific anergic state in human CD4+ T cells. **J. Exp. Med.,** v. 184, p. 19-29, 1996.

GUERMONPREZ, P. et al. Antigen presentation and T cell stimulation by dendritic cells. **Annu. Rev. Immunol**., v. 20, p. 621-667, 2002.

GUERY, L. et al. Ag-presenting CpG-activated pDCs prime Th17 cells that induce tumor regression. **Cancer Res.,** v. 74, n. 22, p. 6430-6440, 2014.

HANAHAN, D.; WEINBERG, R. A. The hallmarks of cancer. **Cell,** v. 100, n. 1, p. 57-70, 2000.

HANAHAN, D.; WEINBERG, R. A. Hallmarks of cancer: the next generation. **Cell,** v. 144, p. 646-674, 2011.

HARTKOPF, A. D. et al. Prognostic relevance of disseminated tumour cells from the bone marrow of early stage breast cancer patients - results from a large single-centre analysis. **Eur. J. Cancer,** v. 50, n. 15, p. 2550-2559, 2014.

HÉMONT, C.; NEEL, A.; HESLAN, M.; BRAUDEAU, C.; JOSIEN, R. Human blood mDC subsets exhibit distinct TLR repertoire and responsiveness. **J. Leukoc. Biol.,** v. 93, p. 599-609, 2013.

HERBST, R. S. et al. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. **Nature,** v. 515, n. 7528, p. 563-567, 2014.

HEUSINKVELD, M.; VAN DER BURG, S.H. Identification and manipulation of tumor associated macrophages in human cancers. **J. Transl. Med.**, v. 9, n. 216, p. 1-13, 2011.

HO, I. C.; TAI, T. S.; PAI, S. Y. GATA3 and the T-cell lineagep. essential functions before and after T-helper-2-cell differentiation. **Nat. Rev. Immunol.,** v. 9, p. 125-135, 2009.

HODI, F. S.; O'DAY, S. J.; MCDERMOTT, D. F. et al. Improved survival with ipilimumab in patients with metastatic melanoma. **N. England J. Med.**, v. 363, p. 711– 723, 2010.

HORELT, A.; BELGE, K. U.; STEPPICH, B.; PRINZ, J.; ZIEGLER-HEITBROCK, L. The CD14+CD16+ monocytes in erysipelas are expanded and show reduced cytokine production. **Eur. J. Immunol**., v. 32, p. 1319–1327, 2002.

HOTAMISLIGIL, G. S.; ERBAY, E. Nutrient sensing and inflammation in metabolic diseases. **Nat. Rev. Immunol.,** v. 8, p. 923–934, 2008.

IKUSHIMA, H.; MIYAZONO, K. TGFB 2 signallingp. a complex web in cancer progression. **Nat. Rev. Can.**, v. 10, p. 415–424, 2010.

INSTITUTO NACIONAL DO CÂNCER. (Brazil). Available from: <httpp.//www2.inca.gov.br/wps/wcm/connect/tiposdecancer/site/home/mama>. Acessed in: 10 Sep. 2015.

INSTITUT NACIONAL DU CANCER. (France). Available from: <httpp.//www.e-cancer.fr/soins/les-chiffres-du-cancer-en-france/epidemiologie-des-cancers>. Acessed in: 1 Sep. 2015.

INO, Y. et al. Immune cell infiltration as an indicator of the immune microenvironment of pancreatic cancer. **Br. J. Cancer**, v. 108, p. 914-923, 2013.

ISAKSSON, M. et al. Plasmacytoid DC promote priming of autoimmune Th17 cells and EAE. **Eur. J. Immunol.**, v. 39, p. 2925-2935, 2009.

IVANOVIĆ, V. et al. Elevated plasma levels of transforming growth factor-beta 1 (TGF-beta1) in patients with advanced breast cancerp. association with disease progression. **Eur. J. Cancer,** v. 39, n. 4, p. 454-461, 2003.

IWAI, Y. et al. Involvement of PD-L1 on tumor cells in the escape from host immune system and tumor immunotherapy by PD-L1 blockade. **Proc. Natl Acad. Sci. USA,** v. 99, p. 12293–12297, 2002.

JAGUIN, M.; HOULBERT, N.; FARDEL, O.; LECUREUR V. Polarization profiles of human M-CSF-generated macrophages and comparison of M1-markers in classically activated macrophages from GM-CSF and M-CSF origin. **Cell Immunol.,** v. 281, n. 1, p. 51-61, 2013.

JANNI, W. et al. Persistence of disseminated tumor cells in the bone marrow of breast cancer patients predicts increased risk for relapse - a European pooled analysis. **Clin. Cancer Res.,** v. 17, n. 9, p. 2967-2976, 2011.

JEMAL, A. et al. Cancer statistics. **CA. Cancer J. Clin.**, v. 54, p. 8-29, 2004

JONGBLOED, S. L. et al. Human CD141+ (BDCA-3) dendritic cells (DCs) represent a unique myeloid DC subset that cross-presents necrotic cell antigens. **J. Exp. Med.**, v. 207, p. 1247-1260, 2010.

KAMINSKA, J. et al. Pretreatment serum levels of cytokines and cytokine receptors in patients with non-small cell lung cancer, and correlations with clinicopathological features and prognosis. M-CSF - an independent prognostic factor. **Oncology,** v. 70, p. 115-125, 2006.

KAPLAN, D. H. et al. Demonstration of an interferon gamma-dependent tumor surveillance system in immunocompetent mice. **Proc. Natl. Acad. Sci. U S A.,** v. 95, n. 13, p. 7556-7561, 1998.

KAWAMURA, K. et al. Detection of M2 macrophages and colony stimulating factor 1 expression in serous and mucinous ovarian epithelial tumors. **Pathol. Int.,** v. 59, p. 300–305, 2009.

KEIR, M. E. et al. PD-1 and its ligands in T-cell immunity. **Curr. Opin. Immunol,** v. 19, p. 309–314, 2007.

KESSENBROCK, K. et al. Matrix metalloproteinasesp. regulators of the tumor microenvironment. **Cell**, v. 141, p. 52–67, 2010.

KEHRL, J. H. et al. Production of transforming growth factor β by human T lymphocytes and its potential role in the regulation of T cell growth. **J. Exp. Med.,** v. 163, p. 1037–1050, 1986.

KNUTSON, K. L. et al. Regulatory T cells, inherited variation, and clinical outcome in epithelial ovarian cancer. **Cancer Immunol. Immunother.,** 2015 Aug 23. [Epub ahead of print]

KOPPELMAN, B.; NEEFJES, J. J.; DE VRIES, J. E.; DE WAAL MALEFYT, R. Interleukin-10 down-regulates MHC class II alphabeta peptide complexes at the plasma membrane of monocytes by affecting arrival and recycling. **Immunity,** v. 7, n. 6, p. 861-871, 1997.

KORTYLEWSKI, M. et al. Inhibiting Stat3 signaling in the hematopoietic system elicits multicomponent antitumor immunity. **Nat. Med.,** v. 11, p. 1314–1321, 2005.

KRÜGER, J. M. et al. Combat or surveillance? Evaluation of the heterogeneous inflammatory breast cancer microenvironment. **J. Pathol.,** v. 229, p. 569-578, 2013.

KRYCZEK, I. et al. B7-H4 expression identifies a novel suppressive macrophage population in human ovarian carcinoma. **J. Exp. Med.**, v. 203, p. 871-881, 2006.

KUANG, D. M. et al. Activated monocytes in peritumoral stroma of hepatocellular carcinoma foster immune privilege and disease progression through PD-L1. **J. Exp. Med.,** v. 206, p. 1327-1337, 2009.

LABIDI-GALY, S. I. et al. Quantitative and functional alterations of plasmacytoid dendritic cells contribute to immune tolerance in ovarian cancer. **Cancer Res.,** v. 71, n. 16, p. 5423-5434, 2011.

LACEY, D. C. et al. Defining GM-CSF- and macrophage-CSF-dependent macrophage responses by in vitro models. **J. Immunol.,** v. 188, p. 5752-5765, 2012.

LARKIN, J. et al. Combined Nivolumab and Ipilimumab or Monotherapy in Untreated Melanoma. **N. Engl. J. Med.,** v. 373, n. 1, p. 23-34, 2015.

LEE, J. et al. Restricted dendritic cell and monocyte progenitors in human cord blood and bone marrow. **J. Exp. Med.,** v. 212, n. 3, p. 385-399, 2015.

LE MERCIER, I.; POUJOL, D.; SANLAVILLE, A.; SISIRAK, V. et al. Tumor promotion by intratumoral plasmacytoid dendritic cells is reversed by TLR7 ligand treatment. **Cancer Res.,** v. 73, n. 15, p. 4629-4640, 2013.

LEPIQUE, A. P.; DAGHASTANLI, K. R.; CUCCOVIA, I. M.; VILLA. L. L. HPV16 tumor associated macrophages suppress antitumor T cell responses. **Clin. Cancer Res.,** v.15, p. 4391-4400, 2009.

LEWIS, C. E.; HUGHES, R. Inflammation and breast cancer. Microenvironmental factors regulating macrophage function in breast tumour: hypoxia and angiopoietin-2. **Breast Cancer Res.,** v. 9, n. 3, p. 209-212, 2007.

LEWIS, C. E.; POLLARD, J. W. Distinct role of macrophages in different tumor microenvironments. **Cancer Res**., v. 66, p. 605–612, 2006.

LI, M. O. et al. Transforming growth factor-beta regulation of immune responses. **Annu. Rev. Immunol.,** v. 24, p. 99-146, 2006.

LINDENBERG, J. J. et al., IL-10 conditioning of human skin affects the distribution of migratory dendritic cell subsets and functional T cell differentiation. **PLoS One,** v. 8, p. e70237, 2013.

LIYANAGE, U. K. et al. Prevalence of regulatory T cells is increased in peripheral blood and tumor microenvironment of patients with pancreas or breast adenocarcinoma. **J. Immunol.,** v. 169, p. 2756-2761, 2002.

LIPSON, E. J. et al. Durable cancer regression off-treatment and effective reinduction therapy with an anti-PD-1 antibody, **Clin. Can. Res.**, v. 19, p. 462–468, 2013.

LU, H. TLR Agonists for Cancer Immunotherapyp. Tipping the Balance between the Immune Stimulatory and Inhibitory Effects. **Front. Immunol.,** v. 5, p. 83, eCollection, 2014.

ŁUKASZEWICZ-ZAJĄC, M. et al. Clinical significance of serum macrophage-colony stimulating factor (M-CSF) in esophageal cancer patients and its comparison with classical tumor markers. **Clin. Che. and Lab. Med.**, v. 48, p. 1467–1473, 2010.

MA, G. F. et al. Transforming growth factor-ß1 and -ß2 in gastric precancer and cancer and roles in tumor-cell interactions with peripheral blood mononuclear cells in vitro. **PLoS ONE**, v.8, p. 542–549, 2013.

MA, J. et al. Targeting of erbB3 receptor to overcome resistance in cancer treatment. **Mol. Cancer,** v. 13, n. 1, p. 105-113, 2014.

MACATONIA, S. E.; DOHERTY, T. M.; KNIGHT, S. C.; O'GARRA, A. Differential effect of IL-10 on dendritic cell-induced T cell proliferation and IFN-gamma production. **J. Immunol.,** v. 150, n. 9, p. 3755-3765, 1993.

MANTOVANI, A. et al. Macrophage-derived chemokine (MDC). **J. Leukoc. Biol.** v. 68, n. 3, p. 400-404, 2000.

MANTOVANI, A.; SOZZANI, S.; LOCATI, M.; ALLAVENA, P.; SICA, A. Macrophage olarization. Tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. **Trends Immunol.,** v. 23, p. 549-555, 2002.

MASSAGUE, J. How cells read TGF-β signals. **Nat. Rev. Mol. Cell. Biol**. v.1, p. 169–178, 2000.

MATHAN, T. S.; FIGDOR, C. G.; BUSCHOW, S. I. Human plasmacytoid dendritic cellsp. from molecules to intercellular communication network. **Front Immunol.,** v. 4, n. 372, p. 1-15, 2013.

MCALLISTER, S.S.; WEINBERG, R.A. The tumour-induced systemic environment as a critical regulator of cancer progression and metastasis. **Nat. Cell Biol.,** v. 16, n. 8, p. 717-727, 2014.

MCNUTT, M. Cancer immunotherapy. **Science**, v. 342, p. 1417, 2013.

MEDREK, C.; PONTEN, F.; JIRSTROM, K.; LEANDERSSON, K. The presence of tumor associated macrophages in tumor stroma as a prognostic marker for breast cancer patients. **BMC. Cancer,** v. 12, p. 306-315, 2012.

MEDZHITOV, R. Origin and physiological roles of inflammation. **Nature**, v. 454, p. 428–435, 2008.

MELLMAN, I.; STEINMAN, R. M. Dendritic cellsp. specialized and regulated antigen processing machines. **Cell**, v. 106, p. 255–258, 2001.

MÉNÉTRIER-CAUX, C.; BAIN, C.; FAVROT, M. C.; DUC, A.; BLAY, J. Y. Renal cell carcinoma induces interleukin 10 and prostaglandin E2 production by monocytes. **Br. J. Cancer,** v. 79, p. 119-130, 1999.

MENETRIER-CAUX, C.; MONTMAIN, G.; DIEU, M. C.; BAIN, C.; FAVROT, M. C.; CAUX, C.; BLAY, J. Y. Inhibition of the differentiation of dendritic cells from CD34(+) progenitors by tumor cellsp. role of interleukin-6 and macrophage colony-stimulating factor. **Blood**, v. 92, p. 4778-4791, 1998.

MÉNÉTRIER-CAUX, C.; THOMACHOT, M. C.; ALBERTI, L.; MONTMAIN, G.; BLAY, J. Y. IL-4 prevents the blockade of dendritic cell differentiation induced by tumor cells. **Cancer Res.,** v. 61, p. 3096-3104, 2001.

MIA, S. et al. An optimized protocol for human M2 macrophages using M-CSF and IL-4/IL-10/TGF-β yields a dominant immunosuppressive phenotype. **Scand. J. Immunol.,** v. 79, n. 5, p. 305-314, 2014.

MITCHEM, J. B. et al. Targeting tumor-infiltrating macrophages decreases tumor-initiating cells, relieves immunosuppression, and improves chemotherapeutic responses. **Cancer Res.,** v. 73, n. 3, p. 1128-1141, 2013.

MIZUNO, K. et al. Selective expansion of CD16highCCR2− subpopulation of circulating monocytes with preferential production of heme oxygenase (HO)-1 in response to acute inflammation. **Clin. Exp. Immunol**., v. 142, p. 461–470, 2005.

MOSSER, D. M.; EDWARDS, J. P. Exploring the full spectrum of macrophage activation. **Nat. Rev. Immunol.,** v. 8, n. 12, p. 958-969, 2008.

NAIK, S. H. Demystifying the development of dendritic cell subtypes, a little. **Immunol. Cell Biol.,** v. 86, n. 5, p. 439-452, 2008.

NAROD, S. A.; IQBAL, J.; GIANNAKEAS, V.; SOPIK, V.; SUN, P. Breast Cancer mortality after a diagnosis of Ductal Carcinoma *In Situ*. **JAMA. Oncol.,** 2015 [Epub ahead of print].

NEVES, A. R.; ENSINA, L. F.; ANSELMO, L. B.; LEITE, K. R.; BUZAID, A. C.; CÂMARA-LOPES, L. H.; BARBUTO, J. A. Dendritic cells derived from metastatic cancer patients vaccinated with allogeneic dendritic cell-autologous tumor cell hybrids express more CD86 and induce higher levels of interferon-gamma in mixed lymphocyte reactions. **Cancer Immunol. Immunother**., v. 54, p. 61-66, 2005.

NIZZOLI, G. et al. Human CD1c+ dendritic cells secrete high levels of IL-12 and potently prime cytotoxic T-cell responses. **Blood**, v. 122, p. 932-942, 2013.

OCHANDO, J. C. et al. Alloantigen-presenting plasmacytoid dendritic cells mediate tolerance to vascularized grafts. **Nat. Immunol.**, v. 7, p. 652-662, 2006.

OESTREICH, K. J.; WEINMANN, A. S. Transcriptional mechanisms that regulate T helper 1 cell differentiation. **Curr Opin Immunol.,** v. 24, p. 191-195, 2012.

OGINO, T. et al. Increased Th17-inducing activity of CD14+ CD163 low myeloid cells in intestinal lamina propria of patients with Crohn's disease **Gastroenterology,** v. 145, p. 1380-1391.e1, 2013.

ORSINI, E. et al. The circulating dendritic cell compartment in patients with chronic lymphocytic leukemia is severely defective and unable to stimulate an effective T-cell response. **Cancer Res.,** v. 63, n. 15, p. 4497-4506, 2003.

ORSINI, G.; LEGITIMO, A.; FAILLI, A.; FERRARI, P.; NICOLINI, A.; SPISNI, R.; MICCOLI, P.; CONSOLINI, R. Defective generation and maturation of dendritic cells from monocytes in colorectal cancer patients during the course of disease. **Int. J. Mol. Sci.**, v. 14, p. 22022-22041, 2013.

OSTUNI, R.; KRATOCHVILL, F.; MURRAY, P.J.; NATOLI, G. Macrophages and cancerp. from mechanisms to therapeutic implications. **Trends Immunol.,** v. 36, n. 4, p. 229-239, 2015.

OUABED, A.; HUBERT, F. X.; CHABANNES, D.; GAUTREAU, L.; HESLAN, M.; JOSIEN, R. Differential control of T regulatory cell proliferation and suppressive activity by mature plasmacytoid versus conventional spleen dendritic cells. **J. Immunol.**, v. 180, p. 5862-5870, 2008.

PALUCKA, K.; BANCHEREAU, J. Cancer immunotherapy via dendritic cells. **Nat. Rev. Can.**, v. 12, p. 265–277, 2012.

PASSLICK, B.; FLIEGER, D.; ZIEGLER-HEITBROCK, H. W. Identification and characterization of a novel monocyte subpopulation in human peripheral blood. **Blood**, v. 74, p. 2527–2534, 1989.

PEREIRA, E. R. et al. Endoplasmic reticulum (ER) stress and hypoxia response pathways interact to potentiate hypoxia-inducible factor 1 (HIF-1) transcriptional activity on targets like vascular endothelial growth factor (VEGF). **J. Biol. Chem.,** v. 289, n. 6, p. 3352-3364, 2014.

PEROU, C. M. et al. Molecular portraits of human breast tumours. **Nature,** v. 406, n. 6797, p. 747-752, 2000.

PETZELBAUER, P.; FÖDINGER, D.; RAPPERSBERGER, K.; VOLC-PLATZER, B.; WOLFF K. CD68 positive epidermal dendritic cells. **J. Invest. Dermatol.,** v. 101, n. 3, p. 256-261,1993.

PIOLI, P. A. et al. TGF-beta regulation of human macrophage scavenger receptor CD163 is Smad3-dependent. **J. Leukoc. Biol.,** v. 76, n. 2, p. 500-508, 2004.

PLANTE, M. et al. Interleukin-6 level in serum and ascites as a prognostic factor in patients with epithelial ovarian cancer. **Cancer**, v. 73, p. 1882–1888, 1994.

POLLARD, J. W. Trophic macrophages in development and disease. **Nat. Rev. Immunol.** v. 9, p. 259–270, 2009.

POLLARD, J. W. Tumour-educated macrophages promote tumour progression and metastasis. **Nat. Rev. Cancer,** v. 4, p. 71-78, 2004.

PYONTECK, S. M. et al. CSF-1R inhibition alters macrophage polarization and blocks glioma progression. **Nat. Med.,** v. 19, n. 10, p. 1264-1272, 2013.

QIAN, B. Z.; POLLARD, J. W. Macrophage diversity enhances tumor progression and metastasis. **Cell,** v. 141, p. 39–51, 2010.

RABINOVICH, G. A.; GABRILOVICH, D.; SOTOMAYOR, E. M. Immunosuppressive strategies that are mediated by tumor cells. **Annu. Rev. Immunol.,** v. 25, p. 267-296, 2007.

RADICE, D.; REDAELLI, A. Breast cancer management: quality-of-life and cost considerations. **Pharmacoeconomics**, v. 21, p. 383-396, 2003.

RAMOS, R. N. **Investigação de um possivel vies imunossupressor em celulas dendriticas derivadas de individuos portadores de cancer**. 2011. 101 f. Dissertação (Mestrado em Imunologia) – Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, 2011.

RAMOS, R. N.; CHIN, L. S.; DOS SANTOS, A. P., BERGAMI-SANTOS, P. C.; LAGINHA, F.; BARBUTO, J. A. Monocyte-derived dendritic cells from breast cancer patients are biased to induce CD4+CD25+Foxp3+ regulatory T cells. **J. Leukoc. Biol.,** v. 92, p. 673-682, 2012a.

RAMOS, R. N.; OLIVEIRA, C. E.; GASPAROTO, T. H. et al. CD25+ T cell depletion impairs murine squamous cell carcinoma development via modulation of antitumor immune responses. **Carcinogenesis**, v. 33, p. 902–909, 2012b.

RAMOS, R. N.; MORAES, C. J. DE.; ZELANTE, B.; BARBUTO, J. A. What Are the Molecules Involved in Regulatory T-Cells Induction by Dendritic Cells in Cancer? **Clin. & Dev. Immun**., p.806025, 2013.

REINARTZ, S. et al., Mixed-polarization phenotype of ascites-associated macrophages in human ovarian carcinomap. Correlation of CD163 expression, cytokine levels and early relapse **Int. J. Cancer.,** v. 134, p. 32–42, 2014.

RICHARDSEN, E. et al. Macrophage-colony stimulating factor (CSF1) predicts breast cancer progression and mortality. **Anticancer Res.,** v. 35, n. 2, p. 865-874, 2015.

RIES, C. H. et al. Targeting tumor-associated macrophages with anti-CSF-1R antibody reveals a strategy for cancer therapy. **Cancer Cell,** v. 25, n. 6, p. 846-859, 2014.

ROBERT, C. et al. Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. **N. England J. Med.**, v. 364, p. 2517–2526, 2011.

ROBINSON, B. D. et al. Tumor microenvironment of metastasis in human breast carcinomap. a potential prognostic marker linked to hematogenous dissemination. **Clin. Cancer Res.,** v. 15, p. 2433–2441, 2009.

RODRÍGUEZ-GARCÍA, M. et al. Expression of PD-L1 and PD-L2 on human macrophages is up-regulated by HIV-1 and differentially modulated by IL-10. **J. Leukoc. Biol.,** v. 89, p. 507-515, 2011.

ROLLA, S. et al. Th22 cells are expanded in multiple sclerosis and are resistant to IFN-β. **J. Leukoc. Biol.,** v. 96, n. 6, p. 1155-1164, 2014.

RONCAROLO, M. G. et al. Interleukin-10-secreting type 1 regulatory T cells in rodents and humans. **Immunol. Rev.,** v. 212, p. 28–50, 2006.

ROSSI, R. E. et al. The role of dietary factors in prevention and progression of breast cancer. **Anticancer Res.,** v. 34, n. 12, p. 6861-6875, 2014.

ROY, S. et al. Defective dendritic cell generation from monocytes is a potential reason for poor therapeutic efficacy of interferon α2b (IFNα2b) in cervical cancer. **Transl. Res.,** v. 158, n. 4, p. 200-213, 2011.

RUFFELL, B.; AU, A.; RUGO, H. S.; ESSERMAN, L. J.; HWANG, E. S.; COUSSENS, L. M. Leukocyte composition of human breast cancer. **Proc. Natl. Acad. Sci. U.S.A.**, v. 109, p. 2796-2801, 2012.

RUFFELL, B. et al. Macrophage IL-10 blocks CD8+ T cell-dependent responses to chemotherapy by suppressing IL-12 expression in intratumoral dendritic cells. **Cancer Cell,** v. 26, n. 5, p. 623-637, 2014.

RUFFELL, B.; COUSSENS, L. M. Macrophages and therapeutic resistance in cancer. **Cancer Cell,** v. 27, n. 4, p. 462-472, 2015.

RUSSELL, D. G. et al. Foamy macrophages and the progression of the human tuberculosis granuloma. **Nat. Immunol.,** v. 10, p. 943–948, 2009.

RYGIEL, T. P.; MEYAARD, L. CD200R signaling in tumor tolerance and inflammationp. A tricky balance. **Curr. Opin. Immunol.,** v. 24, p. 233-238, 2012.

SAKAGUCHI, S. Naturally arising Foxp3-expressing CD25+CD4+ regulatory T cells in immunological tolerance to self and non-self. **Nature Immunol.**, v. 6, p. 345–352, 2005.

SAKAGUCHI, S.; MIYARA, M.; COSTANTINO, C. M.; HAFLER, D. A. FOXP3+ regulatory T cells in the human immune system. **Nat. Rev. Immun.**, v. 10, p. 490–500, 2010.

SAKUISHI, K. et al. Targeting Tim-3 and PD-1 pathways to reverse T cell exhaustion and restore anti-tumor immunity. **J. Exp. Med.,** v. 207, p. 2187–2194, 2010.

SALLUSTO, F.; LANZAVECCHIA, A. Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor alpha. **J. Exp. Med.**, v. 179, p. 1109-1118, 1994.

SANFORD, D. E. et al. Inflammatory monocyte mobilization decreases patient survival in pancreatic cancerp. a role for targeting the CCL2/CCR2 axis. **Clin. Cancer Res.**, v. 19, p. 3404-3015, 2013.

SATTHAPORN, S. et al. Dendritic cells are dysfunctional in patients with operable breast cancer. **Cancer Immunol. Immunother.,** v. 53, n. 6, p. 510-518, 2004.

SCAMBIA, G. et al. Prognostic significance of interleukin 6 serum levels in patients with ovarian cancer. **Br. J. Cancer**, v. 71, p. 354–356, 1995.

SEGURA, E.; DURAND, M.; AMIGORENA S. Similar antigen cross-presentation capacity and phagocytic functions in all freshly isolated human lymphoid organ-resident dendritic cells. **J. Exp. Med.**, v. 210, p. 1035-1047, 2013a.

SEGURA, E., et al. Human inflammatory dendritic cells induce Th17 cell differentiation. **Immunity,** v. 38, p. 336-348, 2013b.

SELIGER, B. et al. Analysis of the major histocompatibility complex class I antigen presentation machinery in normal and malignant renal cellsp. evidence for deficiencies associated with transformation and progression. **Cancer Res.,** v. 56, n. 8, p. 1756-1760, 1996.

SERHAN, C. N.; SAVILL, J. Resolution of inflammation: the beginning programs the end. **Nat. Immunol.,** v. 6, p. 1191–1197, 2005.

SHABO, I.; STAL, O.; OLSSON, H.; DORÉ, S.; SVANVIK, J. Breast cancer expression of CD163, a macrophage scavenger receptor, is related to early distant recurrence and reduced patient survival. **Int. J. Cancer,** v. 123, n. 4, p. 780-786, 2008.

SHANKARAN, V. et al. IFNgamma and lymphocytes prevent primary tumour development and shape tumour immunogenicity. **Nature,** v. 410, n. 6832, p. 1107-1111, 2001.

SHARMA, M. D.; BABAN, B.; CHANDLER, P.; HOU, D. Y.; SINGH, N.; YAGITA, H. et al. Plasmacytoid dendritic cells from mouse tumor-draining lymph nodes directly activate mature Tregs via indoleamine 2,3-dioxygenase. **J. Clin. Invest.**, v. 117, p. 2570-2582, 2007.

SHARMA, M. et al. Analysis of stromal signatures in the tumor microenvironment of ductal carcinoma in situ. **Breast Cancer Res. Treat.,** v. 123, p. 397-404, 2010.

SHARMA, P. et al. Early detection of breast cancer based on gene-expression patterns in peripheral blood cells. **Breast Cancer Res.,** v. 7, n. 5, p. R634–644, 2005.

SHEIL, A. G. Cancer after transplantation. **World J. Surg.,** v. 10, n. 3, p. 389-396, 1986.

SHEVACH, E. CD4+CD25+ suppressor T cells: more questions than answers. **Nat. Rev. Immunol.,** v. 2, p. 389-400, 2002.

SICA, A.; MANTOVANI, A. Macrophage plasticity and polarizationp. in vivo veritas. **J. Clin. Invest.**, v. 122, p. 787-795, 2012.

SILVA, F. S. et al. Mesenchymal stem cells derived from human exfoliated deciduous teeth (SHEDs) induce immune modulatory profile in monocyte-derived dendritic cells. **Plos One**, v. 9, p. e98050, 2014.

SISIRAK, V. et al. Impaired IFN-alpha production by plasmacytoid dendritic cells favors regulatory T-cell expansion that may contribute to breast cancer progression. **Cancer Res.**, v. 72, p. 5188-5197, 2012.

SISIRAK, V.; VEY, N.; GOUTAGNY, N.; RENAUDINEAU, S.; MALFROY, M.; THYS, S. et al. Breast cancer-derived transforming growth factor-β and tumor necrosis factor-α compromise

interferon-α production by tumor-associated plasmacytoid dendritic cells. **Int. J. Cancer.,** v. 133, n. 3, p. 771-778, 2013.

SKRZECZYŃSKA-MONCZNIK, J. et al. Peripheral blood CD14high CD16+ monocytes are main producers of IL-10. **Scand. J. Immunol.,** v. 67, n. 2, p. 152-159, 2008.

SONG, H. et al. Intratumoral heterogeneity impacts the response to anti-neu antibody therapy. **BMC Cancer,** v. 14, n. 647, p. 1-12, 2014.

SOUSA, S.; BRION, R.; LINTUNEN, M.; KRONQVIST, P.; SANDHOLM, J.; MÖNKKÖNEN, J. et al. Human breast cancer cells educate macrophages toward the M2 activation status. **Breast Cancer Res.,** v. 17, n. 1, p. 101, 2015.

STEIDL, C. et al. Tumor associated macrophages and survival in classic Hodgkin's lymphoma. **N. England J. Med.,** v. 362, p. 875–885, 2010.

STONE, S. C.; ROSSETTI, R. A.; BOLPETTI, A.; BOCCARDO, E.; SOUZA, P. S.; LEPIQUE, A. P. HPV16-associated tumors control myeloid cell homeostasis in lymphoid organs, generating a suppressor environment for T cells. **J. Leukoc. Biol.,** v. 96, n. 4, p. 619-631, 2014.

STRACHAN, D. C. et al. CSF1R inhibition delays cervical and mammary tumor growth in murine models by attenuating the turnover of tumor-associated macrophages and enhancing infiltration by CD8+ T cells. **Oncoimmunology,** v. 2, n. 12, p. e26968, 2013.

STREET, S. E.; CRETNEY, E.; SMYTH, M. J. Perforin and interferon-gamma activities independently control tumor initiation, growth, and metastasis. **Blood,** v. 97, n. 1, p. 192-197, 2001.

STUTMAN, O. Tumor development after 3-methylcholanthrene in immunologically deficient athymic-nude mice. **Science,** v. 183, n. 4124, p. 534-536, 1974.

STUTMAN O. Chemical carcinogenesis in nude micep. comparison between nude mice from homozygous matings and heterozygous matings and effect of age and carcinogen dose. **J. Natl. Cancer Inst.,** v. 62, n. 2, p. 353-358, 1979.

TABAS, I. Macrophage death and defective inflammation resolution in atherosclerosis. **Nat. Rev. Immunol.,** v. 10, p. 36–46, 2010.

TADA, T.; OHZEKI, S.; UTSUMI, K.; TAKIUCHI, H.; MURAMATSU, M.; LI, X. F. et al. Transforming growth factor-beta-induced inhibition of T cell function. Susceptibility difference in T cells of various phenotypes and functions and its relevance to immunosuppression in the tumor-bearing state. **J. Immunol.**, v. 146, p. 1077-1082, 1991.

TAMIMI, R. M. et al. Circulating colony stimulating factor-1 and breast cancer risk. **Cancer Res.,** v. 68, n. 1, p. 18-21, 2008.

TAMOUTOUNOUR, S. et al. CD64 distinguishes macrophages from dendritic cells in the gut and reveals the Th1-inducing role of mesenteric lymph node macrophages during colitis. **Eur. J. Immunol.,** v. 42, p. 3150–3166, 2012.

TAKAGI, H.; FUKAYA, T.; EIZUMI K, et al. Plasmacytoid dendritic cells are crucial for the initiation of inflammation and T cell immunity in vivo. **Immunity**, v. 35, n. 6, p. 958-971, 2011.

THOMACHOT, M. C. et al. Breast carcinoma cells promote the differentiation of $CD34^+$ progenitors towards 2 different subpopulations of dendritic cells with CD1a(high)CD86(-)

Langerin- and CD1a(+)CD86(+)Langerin+ phenotypes. **Int. J. Cancer,** v. 110, p. 710-720, 2004.

THOMAS, L. **Cellular and Humoral aspects of the hypersinsitive States**. New York: Lawrence, 1959.

TIAINEN, S. et al. High numbers of macrophages, especially M2-like (CD163-positive), correlate with hyaluronan accumulation and poor outcome in breast cancer. **Histopathology,** v. 66, n. 6, p. 873-883, 2015.

TOPALIAN, S. L. et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer, **The New Engl. J. Med.,** v. 366, p. 2443–2454, 2012.

TORROELLA-KOURI, M. et al. Identification of a subpopulation of macrophages in mammary tumor-bearing mice that are neither M1 nor M2 and are less differentiated. **Cancer Res.,** v. 69, n. 11, p. 4800-4809, 2009.

TORROELLA-KOURI, M.; RODRÍGUEZ, D.; CASO, R. Alterations in macrophages and monocytes from tumor-bearing micep. evidence of local and systemic immune impairment. **Immunol Res.,** v. 57, p. 86-98, 2013.

TREILLEUX, I. et al. Dendritic cell infiltration and prognosis of early stage breast cancer. **Clin. Cancer Res.,** v. 10, p. 7466-7474, 2004.

TSAI, W. L.; CHUNG, R. T. Viral hepatocarcinogenesis. **Oncogene,** v. 29, n. 16, p. 2309-2324, 2010.

UZZAN, B. et al. Microvessel density as a prognostic factor in women with breast cancerp. a systematic review of the literature and meta-analysis. **Cancer Res**., v. 64, p. 2941–2955, 2004.

VARNEY, M. L. et al. Tumour-associated macrophage infiltration, neovascularization and aggressiveness in malignant melanomap. role of monocyte chemotactic protein-1 and vascular endothelial growth factor-A. **Melanoma Res.,** v. 15, p. 417–425, 2005.

VOGEL, D. Y. et al. Human macrophage polarization in vitrop. maturation and activation methods compared. **Immunobiology,** v. 219, n. 9, p. 695-703, 2014.

VORON, T. et al. VEGF-A modulates expression of inhibitory checkpoints on CD8+ T cells in tumors. **J. Exp. Med.,** v. 212, n. 2, p. 139-148, 2015.

WAHL, S. M. et al. Transforming growth factor type β induces monocyte chemotaxis and growth factor production. **Proc. Natl. Acad. Sci. USA,** v. 84, p. 5788–5792, 1987.

WANG, H. et al. The osteogenic niche promotes early-stage bone colonization of disseminated breast cancer cells. **Cancer Cell,** v. 27, n. 2, p. 193-210, 2015.

WANG, L. et al. Programmed death 1 ligand signaling regulates the generation of adaptive Foxp3+CD4+ regulatory T cells. **Proc. Natl. Acad. Sci. U.S.A.**, v. 105, p. 9331–9336, 2008.

WARREN, G. W.; SOBUS, S.; GRITZ, E. R. The biological and clinical effects of smoking by patients with cancer and strategies to implement evidence-based tobacco cessation support. **Lancet Oncol.,** v. 15, n. 12, p. e568-580, 2014.

WEBER, C. et al. Differential chemokine receptor expression and function in human monocyte subpopulations. **J. Leukoc. Biol**., v. 67, p. 699–704, 2000.

WORLD HEALTH ORGANIZATION. Available from: <httpp.//www.who.int/mediacentre/factsheets/fs297/en/>. Acessed in: 1 sep. 2015.

WOO, E. Y. et al. Regulatory T cells from lung cancer patients directly inhibit autologous T cell proliferation. **J. Immunol.,** v. 168, p. 4272–4726, 2002.

YAMAMURA, M.; MODLIN, R. L.; OHMEN, J. D.; MOY, R. L. Local expression of antiinflammatory cytokines in cancer. **J. Clin. Invest.**, v. 91, p. 1005-1010, 1993.

YANAGIHARA, S. et al. EBI1/CCR7 is a new member of dendritic cell chemokine receptor that is upregulated upon maturation. **J. Immunol.**, v. 161, p. 3096-3102, 1998.

ZIEGLER-HEITBROCK, H. W. et al. Differential expression of cytokines in human blood monocyte subpopulations. **Blood,** v. 79, p. 503–511, 1992.

ZIEGLER-HEITBROCK, L.; HOFER, T. P. Toward a refined definition of monocyte subsets **Front Immunol**., v. 4, p. 23, 2013.

ZIELINSKI, C. E. et al. Pathogen-induced human TH17 cells produce IFN-γ or IL-10 and are regulated by IL-1β. **Nature,** v. 484, n. 7395, p. 514-518, 2012.

ZOU, W. Immunosuppressive networks in the tumour environment and their therapeutic relevance. **Nat. Rev. Cancer**, v. 5, p. 263-274, 2005.

ZOU, W. Regulatory T cells, tumour immunity and immunotherapy. **Nat. Rev. Immunol.**, v. 6, p. 295-307, 2006.