

Modification of the tumor microenvironment to enhance immunity

Yu-Pei Liao, Dorthe Schaeue, William H. McBride

Department of Radiation Oncology, UCLA David Geffen School of Medicine, Los Angeles, CA 90095-1714

TABLE OF CONTENTS

1. Abstract
2. Overview
3. The tumor microenvironment
 - 3.1. Physiological influences of the tumor microenvironment
 - 3.2. Tumor-associated immune cells
 - 3.2.1. Tumor-infiltrating lymphocytes (TILs)
 - 3.2.2. Inhibitory T cell populations
 - 3.2.3. Tumor infiltrating dendritic cells (TIDCs) and tumor antigen presentation
 - 3.2.4. Tumor-associated macrophages (TAMs)
 - 3.2.5. The yin-yang of immune profiles in cancer
4. Strategies targeting the tumor-microenvironment to enhance tumor immunity
 - 4.1. Conventional therapies and their effects on the tumor microenvironment
 - 4.1.1. Radiation therapy
 - 4.1.2. Chemotherapy
 - 4.1.3. Surgery
 - 4.2. Specific targeting of the tumor microenvironment
 - 4.2.1. Hypoxia and tumor acidosis
 - 4.2.2. Vasculature and angiogenesis
 - 4.2.3. Extracellular matrix and fibroblasts
 - 4.3. Counteracting immune suppression in the tumor microenvironment
 - 4.3.1. Inflammation, cytokines and danger
 - 4.3.2. Dendritic cell and macrophage function and subsets
 - 4.3.3. T Regulatory cells and T cell imbalance
5. Conclusions
6. Acknowledgement
7. References

1. ABSTRACT

The growth and spread of cancer depends as much on the host response to tumor as on the biological characteristics of the tumor itself. This interaction is, at its most, intimate and dynamic within the tumor microenvironment. It is here that the battle is fought that leads to mutual evolution of tumor and host cell phenotypes. Contributing to this evolutionary process are physiological changes distinctive for the tumor microenvironment, such as hypoxia, low nutrient levels, low extracellular pH, and high interstitial fluid pressure. These largely result from the chaotic intratumoral vasculature but are impacted by the nature of the tumor and the inflammatory and wound healing responses that are generated. Numerous infiltrating immune cells, including macrophages, lymphocytes, natural killer cells and dendritic cells infiltrate the tumor, contributing to high levels of growth factors, hormones, and cytokines. We suggest that the integrated interplay between host and tumor factors results in distinct phenotypes that determine the response to therapy as well as tumor behavior.

Targeting the tumor microenvironment to awaken or reawaken immune cells, or to redirect it from a pro-tumor to an anti-tumor state, will require understanding of this phenotype. Current conventional therapies target tumors not tumor cells and clearly affect the host infiltrate and the physiological characteristics of the tumor microenvironment. This may be an advantage that has yet to be effectively exploited due to lack of knowledge of existing phenotypes resulting from the tumor-host interactions. The same lack of knowledge impacts outcomes of clinical immunotherapy (IT) trials that have so far not broken through the ceiling of 10% success rate that seems to exist even in melanoma. It seems obvious that more could be achieved by combining therapies that tackle malignancies from multiple angles, with the tumor microenvironment conditioned to support a powerful effector arm generated by IT. The challenge is how to design combination therapies that modify the tumor microenvironment so as to promote immunity and better combat both local and systemic disease.

The tumor microenvironment and immunity

Table 1. Immune escape mechanisms

Cell	Mechanism
Tumor-infiltrating lymphocytes	<ul style="list-style-type: none"> • Attenuated/defective antigen-specific lytic activity • Ineffective granule exocytosis • Low perforin levels • Immunosuppressive cytokine profile • Absent/low TCR zeta chain expression • Low TCR gamma chain expression • Susceptibility to spontaneous apoptosis
T suppressor (regulatory) cells	<ul style="list-style-type: none"> • Suppress immune responses by cell to cell contact and soluble factors • Attracted to tumor sites by tumor cells and TAMs through CCL22 • Expression of PD-1, ligand for co-inhibitory molecule B7-H1
Dendritic cells	<ul style="list-style-type: none"> • Defective mDC recruitment, maturation and differentiation within the suppressive cytokine and prostaglandin milieu • Dysfunctional DCs show compromised CD8 T cell priming • Immature, myeloid DCs release TGF-beta and stimulate T_{reg} proliferation leading to tolerance • Expression of co-inhibitory molecule B7-H1 with negative regulatory effects on T cells • Expression of IDO causing tryptophan depletion and T cell death • Increased levels of pDCs with angiogenesis stimulating potential and promotion of regulatory CD8 T cells and a Th2 response
Tumor-associated macrophages	<ul style="list-style-type: none"> • Increased levels of TAMs with a type 2 phenotype that sustain a suppressive cytokine milieu and promote angiogenesis, tumor growth and suppress T cell function • Expression of co-inhibitory B7-H4 (B7X-B7S1) • Production of NO that is immune suppressive at low levels • Considerable source of arginase limiting NO-mediated tumor cell lysis by TAMs and promoting growth factor availability for tumor cells
Tumor cells	<ul style="list-style-type: none"> • Suboptimal MHC and co-stimulatory molecule expression and antigen presentation • Rapid proliferation • Resistance to killing mechanisms • Antigen shedding • Suppressive cytokine profile

2. OVERVIEW

The immune system faces a tumor on a battlefield that is heavily influenced by multiple aspects of the host response. Disorganized angiogenesis and poor vascular structure seems to be a characteristic of many tumors. This has metabolic consequences and creates an ever-changing microenvironmental landscape with variable areas of (I) high interstitial fluid pressure, (II) low oxygen tension (hypoxia), (III) low extracellular pH and (IV) low nutrient levels. Host leukocyte infiltration into cancers was first noted by Rudolf Virchow in 1863 (1), but its significance is still in question. The abnormal tumor microenvironment generates an inflammatory response with infiltration by macrophages, neutrophils, lymphocytes and fibroblasts that contribute greatly to the tumor milieu. The tumor site becomes to resemble a chronic wound. The result is a mosaic of pro-inflammatory and anti-inflammatory forces with high levels of cytokines, growth factors, and hormones

that become a force to be reckoned with systemically as well as locally. These forces sculpt the tumor by selecting cells able to survive and avoid normal regulatory homeostatic forces, including those that are exerted by predatory immune cells. Tumors therefore continually reinvent themselves phenotypically in the face of the host assault and the extreme physiological environment that is created. The host co-evolves with these changes in tumor phenotype and is also continually redefined. The final outcome is often a mutually supportive tumor-host relationship. In this way, the tumor microenvironment provides a platform that is as critical for malignant development as the malignant transformation process itself.

Ehrlich in 1909 (2) was the first to forcibly express the concept that the immune system could recognize tumors and eradicate them. He proposed that “if these (immune) mechanisms did not exist we would expect that carcinomas would appear with enormous frequency”. Burnet in 1957 (3) and Thomas in 1959 (4) later formalized this belief as the theory of immune surveillance. Since then, the theory has been extensively and repeatedly modified to account for new findings. It is now acknowledged that, more often than not, cancer patients make detectable anti-tumor immune responses, both humoral and cell-mediated. In most cases, immune monitoring relies heavily on sampling of patient blood, i.e. measures of the systemic response, because temporal and functional immune profiling within the tumor microenvironment is difficult to perform. However, sufficient studies have been performed to establish that tumor-specific immune responses can occur intratumorally, even without vaccination, which may not be accurately reflected in what is measured in the circulation (5). This, more than anything else, points to the importance of studying immune function within the tumor microenvironment as clearly the keys to devising future immune-based strategies for cancer therapy lie here.

The fact that immune effector cells can find their way into tumors is amply demonstrated by the fact that tumor-infiltrating T cells (TILs) are frequently and increasingly used to identify tumor-associated antigens (TAAs). In many cases TIL lines have been established and used to identify immunodominant epitopes and TAAs for sequencing (6-8). These advances have allowed immunization schedules to be devised that can boost tumor-specific immunity. Most effort has been focused on active immunization for initiating and expanding tumor-specific cytolytic CD8⁺ T cell (CTL) responses that are often the most potent in causing tumor regression. Disappointingly, although tumor-specific immune responses have been shown to exist in patients that can be boosted systemically and locally, they only rarely translate into clinical responses, although, when they do, dramatic tumor regression can occur.

The infrequency with which clinical responses are made during cancer IT has been ascribed to the existence of multiple tumor escape mechanisms. The list is a long one and will not be dealt with fully here. Some are listed in Table 1. One important immunological concept, that post-dates Burnet, is that “danger” signals that mature

The tumor microenvironment and immunity

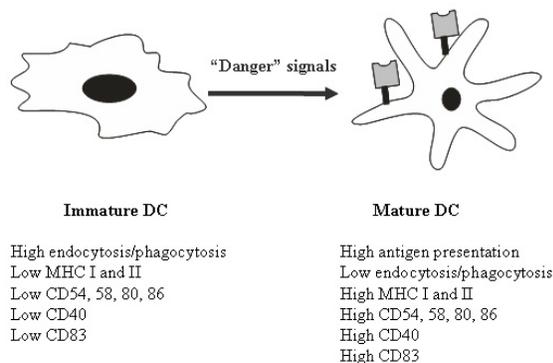


Figure 1. Functional maturation of dendritic cells. DCs capture antigen in the periphery and then migrate to the lymphoid organs to present antigen to T cells. Optimal antigen processing and presentation and the induction of immunity requires functional maturity of DCs that have transformed from an immature phenotype strong in antigen uptake to cells that focus on presenting the antigen with sufficient co-stimulation. Antigen presentation by immature DCs can lead to tolerance. The process of DC maturation is triggered by danger signals in the microenvironment such as Th1 cytokines.

dendritic cells (DC) into powerful antigen presenting cells are required for an immune response to occur (9, 10) (Figure 1). One cause of poor tumor immunity might be the inability of most tumors to generate such “danger” signals. Tumor vaccination strategies typically focus on enhancing this initial priming phase of an immune response. On the other hand, the ability of highly immunogenic, chemically-induced murine tumors to grow in the face of strong concomitant immunity has been known for decades and recently human tumor progression in the presence of a massive influx of activated CD8⁺ T cells has been documented (11). These point to the importance of tumor escape mechanisms that operate at the effector level of immunity (11).

The evidence seems suggest that divergent tumor immune phenotypes could result from the tumor-host interaction. Carcinogenesis-associated mutations and histological type may be influential to the outcome. Some tumor microenvironments may support the generation of immunity, with tumor escape mechanisms that depend heavily on activated proliferation and survival pathways. It is worth remembering that a 1 cm tumor contains 10^9 cells that are capable of dividing every few days and that tumor-specific T cells will be far fewer in number. Given the magnitude of their task, it is not unreasonable to suggest that tumors might grow in the face of robust concomitant immunity. At the other end of the spectrum, some tumor phenotypes might be generated with a highly immunosuppressive microenvironment that present a different therapeutic challenge.

The recent finding that the presence of immune cells in colorectal cancer with a Th1, but not an immunosuppressive or inflammatory, phenotype, as assessed by gene and tissue microarray analysis, together

with density and location, was a better predictor than current staging methods (13), speaks to the potential prognostic value of defining the immune cancer phenotype. Whether the immune phenotype has a positive or negative relationship with others, such as those representing a predominance of wound healing, inflammation, hypoxia, and acidosis profiles is of obvious interest as one would expect host profiles to be as predictive of outcome as tumor cell profiles and to be co-regulated. The fact that there are well documented examples of patients undergoing dramatic immune-mediated regression of large tumor burdens gives rise to the belief that if we knew the rules of engagement, immunotherapy would be integrated with other a front-line cancer therapies rather than being considered a last hope, independent therapy, which it tends to be currently.

3. THE TUMOR MICROENVIRONMENT

3.1. Physiological influences of the tumor microenvironment

Vascular abnormalities in tumors generate spatial and temporal heterogeneity of blood flow and regions of hypoxia, acidity and nutrient depletion. This host response to tumor growth serves as a force of nature to sculpt both. The microenvironment becomes acidic and chronically hypoxic as the oxygen diffusion limit is reached around 100 μ m away from a blood vessel. Additionally, the chaotic vasculature and high interstitial pressure generate areas that intermittently alternate between periods of hypoxia and reperfusion, changes that are particularly toxic to cells. These extremes in the microenvironment select for cells with mutations in death and survival pathways, with a glycolytic phenotype, and with increased ability to produce angiogenic factors. These activated pathways drive malignant progression and resistance to therapy, as does loss of the sensitizing effect of oxygen that local radiation therapy (RT) and certain forms of chemotherapy (CT) rely on for efficient cell kill (12).

Intratumoral microenvironmental heterogeneity is both considerable and inevitable. Intertumoral heterogeneity compounds this. Together, they place major demands on optimization of cancer treatment. It also makes hazardous attempts to generalize as to these physiological effects within the tumor microenvironment on immune mechanisms. Exceptions are likely to exist to any conclusion that is drawn. However, at the same time the uniqueness of the tumor microenvironment is an “Achilles Heel” that can be exploited for therapeutic benefit and its modifying it with a view to enhancing tumor immunity is a concept that is worth exploring.

3.2. Tumor-associated immune cells

3.2.1. Tumor-infiltrating lymphocytes (TILs)

The presence of a large number of TIL in a tumor microenvironment is often considered an indication of immune host-tumor interactions, although only a small percent may be tumor-specific. Much is known about the coordinated participation of selectins, chemokines and integrins in the multistep process that recruits lymphocytes from the circulation into normal tissues. Far less is known about the mechanisms of lymphocyte entry into tumor

The tumor microenvironment and immunity

tissue, but this may dictate TIL phenotype and specificity. The fact that tumors often express chemokines (13, 14) and receptors (13, 15) that are the same as those often used in T cell migration (16) may obfuscate the issue. Such tumor-derived chemokines may also assist tumor escape by blocking chemokine receptors on T cells (17) and decreasing tumor immunogenicity (18).

That being said, lymphocytes do find their way into tumors with T cells generally being the predominant population. However, TILs freshly isolated from tumor are generally less lytic than their systemic counterparts (19), although they can recover function after a brief *in vitro* culture period (20). Defective antigen-specific cytotoxic killing ability by TILs has been ascribed to many mechanisms (21, 22), including ineffective granule exocytosis (23) and low perforin levels (24, 25). Immunosuppressive cytokine profiles (26) and anergy have also been suggested (19, 26, 27). Importantly, while many TILs are CD3+ and express alpha/beta (28, 29) or gamma and zeta T cell receptors, Mizoguchi *et al.* (30) showed that splenic T cells from tumor-bearing mice had lost expression of TCR zeta chain and had low gamma chain expression. Since then, low TCR-associated zeta chain expression has since been consistently observed in patients with solid tumors with circulating T cells and NK cells, but most markedly TILs, being affected (31-34), and this has been associated with poor prognosis (31). Fresh TILs can form immunological synapses with tumor cells but TCR zeta signaling seems to be blocked at a proximal stage (35). Some studies have shown that these T cells also have increased susceptibility to spontaneous apoptosis (31, 36-39) and the presence of apoptotic TILs in the tumor has been correlated with Fas ligand (FasL) expression on the tumor cells (40). Multiple mechanisms have been proposed to down-regulate zeta chain expression (41). It can result from depletion of L-arginine (42) but many of the physiological conditions associated with the tumor microenvironment might also have a major impact.

Several studies have shown that acidic pH_e can affect proliferation of T cells (43) and cytotoxicity of CTLs (44), human lymphokine-activated killer (LAK) (45), and natural killer (NK) cells (46). For example, NK cell killing is most rapid at a slightly alkaline pH_e of 7.3-7.6 (47) and Loeffler *et al.* reported decreased NK cell cytolytic activity under anoxia (0% oxygen) and acidic pH (6.4 or 6.7) (46). While immune effector mechanisms may be compromised by hypoxia (48), others have shown that CTL can perform Fas ligand- and perforin-dependent killing as efficiently under low (0.5-4.5%) as under normal oxygen tensions, although their development may be delayed (49).

3.2.2. Inhibitory T cell populations

Berendt and North (50) first applied the T suppressor cell findings of Gershon (54) to cancer when they found that infusion of splenic T cells from tumor-bearing donors inhibited concomitant immunity in a mouse model. Twenty years later, the concept has been resurrected in the form of a small subset of CD4+ cells regulatory T cells (T_{reg}) that express high levels of glucocorticoid-inducible TNF receptor (GITR) and Foxp3 molecules and

can inhibit immune responses. Naturally occurring CD4+CD25+ and inducible CD4+CD25- T_{reg} cells subsets have been identified (51, 52). Using a mouse B16 melanoma model, Suttmiller *et al.* showed that removal of CD4+CD25+ T_{reg} enhanced anti-tumor responses (53, 54). In patients, CD4+CD25+T_{reg} cells have been reported to be increased in the blood and in various tumors (55-57), including of breast (55), non-small lung, and ovary (56, 58), and in human metastatic melanoma lymph nodes (59).

The mechanism of T_{reg} recruitment to the tumor site is poorly understood. Recently, Ghiringhelli *et al.* showed a subset of immature myeloid DC differentiates into TGF-beta producing cells that stimulate CD4+CD25+ T_{reg} proliferation in draining lymph node of colon cancer bearing mice (60). Curiel *et al.* (58) reported that the chemokine CCL22 produced by ovarian tumor cells and tumor associated macrophages (TAMs) mediate trafficking of T_{reg} to the tumor mass. Specific recruitment of T_{reg} into tumors may therefore be a powerful mechanism of immune escape for tumors.

Other mechanisms could down-regulate T cell responses in the tumor microenvironment. For example, while B7.1 and B7.2 (CD80 and CD86) molecules are important co-stimulatory molecules for T cell activation (61), a third member of the B7 family, B7-H1, seems to have a negative regulatory effect through IL-10 secretion (62). A subset of myeloid DCs in the tumor microenvironment expresses B7-H1 (63) and T_{reg} expresses PD-1, the ligand for B7-H1 (64). B7-H4 (B7X or B7S1) has recently been identified as another negative regulator of T cell responses that is expressed largely by TAMs (65). Overexpression of such molecules in the tumor microenvironment might serve as selective targets for intervention.

3.2.3. Tumor infiltrating dendritic cells (TIDCs) and TAA presentation

DCs are the most potent antigen presenting cells known, but belong to several lineages. TIDCs of the Langerhans, plasmacytoid (pDCs) and myeloid (mDCs) subtypes have been reported in human tumors (66-70). The extent of infiltration is variable and dependent on the tumor type and stage (67, 68), as is their precise location and their stage of maturation. One study reported that DCs were located at the invasion edge of the papillary thyroid carcinoma while TAMs were evenly distributed throughout the tumor (71). The mechanisms by which DCs traffick to the tumor site are poorly understood. Chemokine ligands 2, 3, 5, 7 and 8 and their receptors have been implicated (71-73). However, to trigger immunity, DCs need to reach the secondary lymphoid organs and present antigen to lymphocytes (Figure 1). This migration minimally involves interaction between chemokine receptor CCR7 expressed by DCs and the ligand CCL21 by the lymphatic endothelium (68).

The functional status of TIDCs is more important than their mere presence (65). Maturation of DCs is required for antigen presentation while immature DCs generally are thought to induce tolerance (74) (Figure 1).

The tumor microenvironment and immunity

Mature mDC (CD11a+,CD11c+,CD14-,HLA-DR+) are reportedly rare in human ovarian (69), breast (75, 76), prostate (77) and renal cell carcinoma (78). High levels of suppressive cytokines and growth factors in the tumor microenvironment may be a major cause of defective mDC maturation and differentiation. COX-2 and VEGF can suppress mDC maturation and function (79-81) while IL-6 and M-CSF can direct monocytes away from TIDCs towards TAM differentiation (82). In addition, mDCs in breast tumor and lymph nodes in melanoma, breast, colon, lung and pancreatic cancer patients express indoleamine-2,3-deoxygenase (IDO) that catalyzes oxidation of tryptophan (83) and can suppress T cell proliferation and cause their death by tryptophan depletion. There are few studies that assess intratumoral DCs at the functional level but antigen presenting capacity of cells from within an immunogenic murine fibrosarcoma was intact and the immunopotentiating potential of TAMs was considerable (84, 85).

In contrast to mDCs, pDCs (86) and vascular DCs (87), which can stimulate angiogenesis, are abundant, at least in ovarian tumors. pDCs can induce angiogenesis through production of TNF-alpha and IL-8 (86) and, in the absence of appropriate stimulation in tumor microenvironment, can promote development of regulatory CD8 T cells (88) and a Th2 response (89).

3.2.4. Tumor-associated macrophages (TAMs)

TAMs are present in substantial, though variable, numbers (10%-65%) in different tumors (90, 91). Obviously, they have a major impact on gene expression profiles, functional imaging features, and many characteristics often ascribed to "tumors". TAMs are most often found in the tumor margin but in some cancers, such as breast (92) and endometrial cancers (93) they accumulate around necrotic foci, and in others they may be distributed throughout the tumor. In breast cancers, Leek *et al.* (94) showed that the degree of necrosis was correlated with both focal TAM infiltration and high vascular density.

Any one transplanted tumor cell line maintains a remarkably constant proportion of TAMs after the first few *in vivo* passes (95), indicating that tumor-derived products, most likely cytokines such as the colony stimulating factors GM-CSF and M-CSF, determine the number and state of functional activation of these cells. TAMs demonstrate considerable heterogeneity in size, phenotype, and function within any tumor (96) and are major sources of a wide range of intratumoral cytotoxic mediators, cytokines, growth factors, angiogenic factors and proteases (97). Many of the vascular effects within the tumor microenvironment are mediated or influenced by TAMs. They respond to hypoxia with production of hypoxia-inducible factor (HIF) (98).

There has been considerable controversy as to the role TAMs play in tumor progression. One way this might be rationalized is by considering whether they have predominantly a type 1 (M1) or a type 2 (M2) phenotype (see reviews by (99, 100)) (Figure 2). The former are better able to kill tumor cells, present antigen, and produce

immune-stimulatory cytokines, while the latter promote angiogenesis, tumor growth and metastasis, and suppress T cell function. It will be important to understand this yin-yang balance if immune intervention strategies are to be successful. It seems reasonable to suggest that over the long, prolonged battle for supremacy between the tumor and host there is likely to be selection for tumors that enlist the help of M2 TAMs to grow, however the ability of tumors to grow in the face of robust immunity suggests that M1 TAM phenotypes will also be present.

The M2 phenotype may be promoted by tumor-derived factors such as the interleukins IL-4, IL-6, IL-10, transforming growth factor beta (TGF-beta), PGE2, and M-CSF (101). M2 TAMs in turn assist the tumor by producing high levels of similar cytokines and prostaglandins, and factors such as vascular endothelial growth factor (VEGF) that promote angiogenesis (102). M2 TAMs suppress T (103) and NK (104) cell activity (105, 106). At the other end of the spectrum, M1 TAMs may be generated by certain tumor phenotypes that are more immunosupportive, and by TAA-directed Th1 and CTL responses that release IFN-gamma and IL-3 to activate TAMs. Under these circumstances, tumors presumably rely on a different battery of escape mechanisms such as increased resistance to cytotoxicity or increased proliferation.

The cytostatic and cytolytic actions of M1 TAMs can be mediated either by direct contact with target cells or release of soluble mediators. Two major mediators are TNF-alpha and nitric oxide (NO). Since the action of TNF-alpha is dependent on the generation of hydroxyl radicals, the cytotoxic ability of this population would be diminished in hypoxic areas (107-109). In contrast, hypoxia can enhance NO production (110), which at high levels can inhibit tumor cell proliferation and enhance cell death (111). On the other hand, at lower concentrations, NO will protect tumor cells from apoptosis (112, 113), suppress immune responses (114), stimulate angiogenesis (115), and increase tumor blood flow (116, 117). The balance of inducible nitric oxide synthase (iNOS) to negative regulators of this pathway is therefore likely to be critical to outcome. A major negative regulator of NO is arginase, which can be produced in large amounts by M2 TAMs. Depletion of L-arginine by arginase can suppress NO-mediated macrophage cytotoxicity and T cell function (121), while polyamines and other products of the arginase pathway are growth factors for tumor cells, and depletion of L-arginine by arginase can stimulate tumor cell proliferation (118). Production of iNOS or arginase is therefore one expression of their M1 or M2 phenotype of TAMs and their ability to hinder or promote tumor growth (Figure 3). A major issue that is critical to planning cancer treatments with the best therapeutic outcome is the extent to which TAMs can switch their phenotype from M2 to M1 in response to stimuli, such as immune activation (127) and whether this requires a fresh host cell infiltrate.

It seems reasonable to suggest that over the long, prolonged battle for supremacy between the tumor and host, some tumors will be selected that enlist help from M2

The tumor microenvironment and immunity

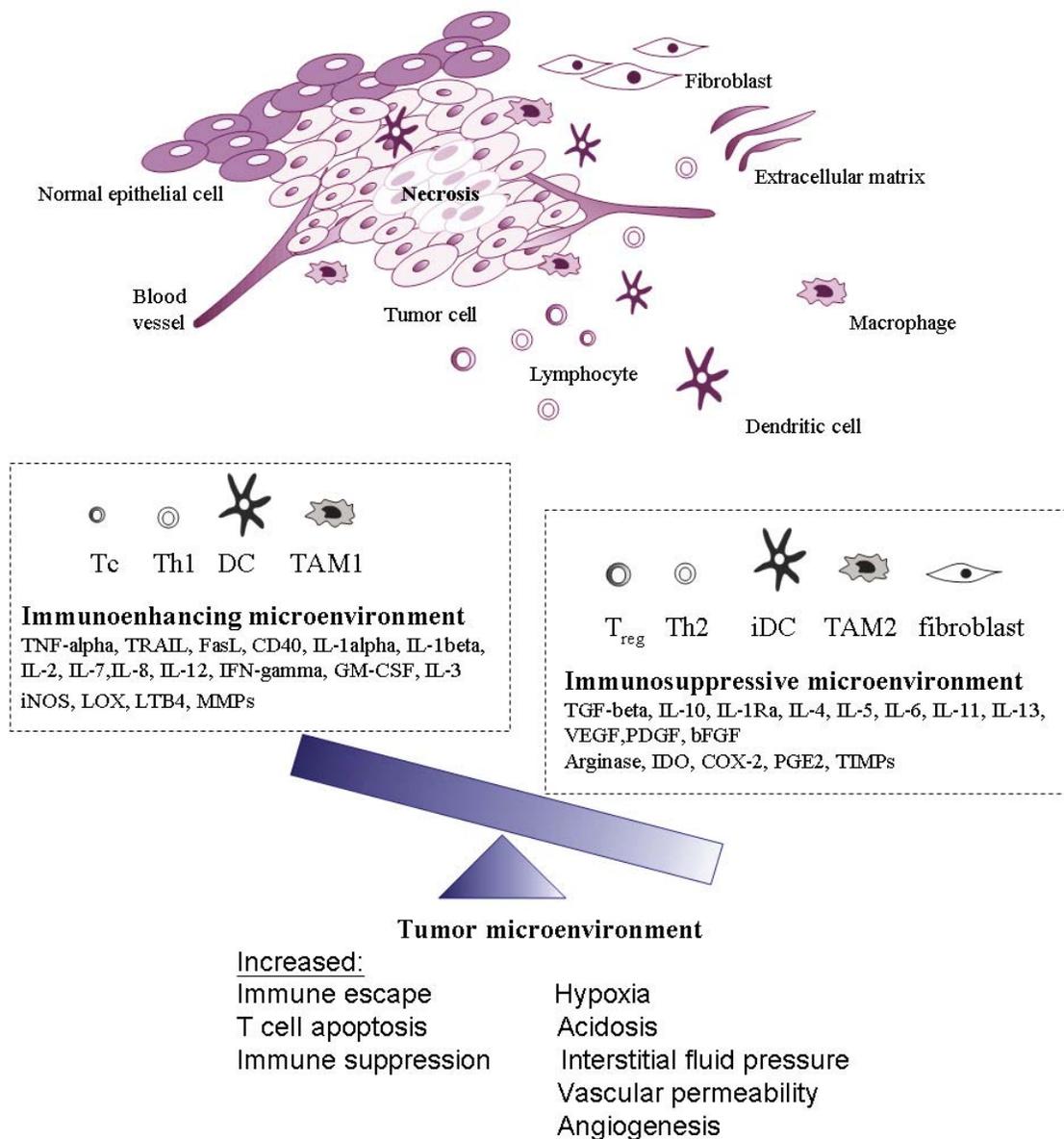


Figure 2. Cartoon depicting the tumor microenvironment. Extreme physiological conditions in the tumor microenvironment such as vascular abnormalities lead to acidity and chronic hypoxia. This ultimately selects for a resistant cellular phenotype that drives malignant progression and immune escape, which is exemplified by chronic inflammation, cytokine imbalance favoring T helper type 2 (Th2), dendritic cell (DC) dysfunction, dendritic cell subset imbalance, suboptimal tumor-associated antigen presentation, insufficient effector T cell priming, T cell receptor dysfunction, suppressive T regulatory (T_{reg}) cells and tumor-associated macrophages (TAMs) and T cell subset imbalance.

TAMs to escape immunity and grow. This phenotype may be promoted by tumor-derived factors such as the interleukins IL-4, IL-6, IL-10, transforming growth factor beta (TGF-beta, PGE2, and M-CSF (101). M2 TAMs in turn assist the tumor by producing high levels of similar cytokines and prostaglandins, and factors such as vascular endothelial growth factor (VEGF) that promote angiogenesis (102). M2 TAMs suppress T (103) and NK (104) cell activity (105, 106). At the other end of the spectrum, M1 TAMs may be generated by certain tumor phenotypes that are more immunosupportive, with the

tumors relying on other escape mechanisms such as increased resistance to cytotoxicity or increased proliferation. A major issue that is critical to planning cancer treatments aimed at achieving the best therapeutic outcome is whether or not TAMs switch their phenotype in response to certain specific stimuli, such as immune activation (119).

3.2.5. The yin-yang of immune profiles in cancer

The yin-yang balance observed in TAM phenotypes yields cytokine profiles that have both pro- and

The tumor microenvironment and immunity

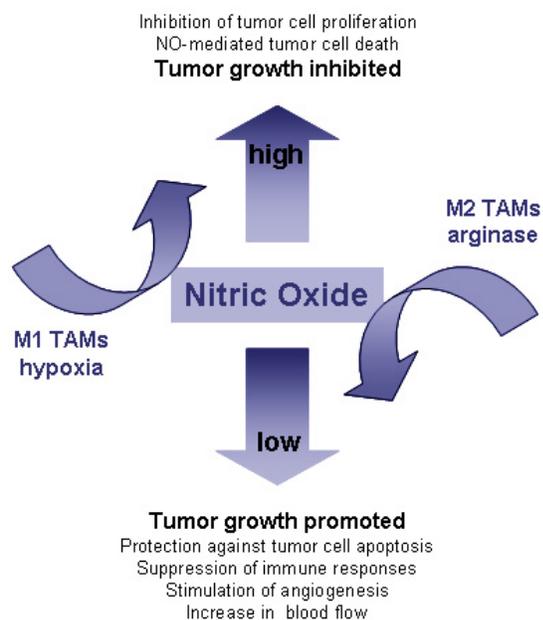


Figure 3. The phenotype of tumor-associated macrophages (TAMs) with respect to Nitric oxide (NO) production affects tumor growth. M1 TAMs are effective tumor cell killers partly because of their ability to produce high levels of NO. In contrast, TAMs of the M2 phenotype are a considerable source of the negative NO-regulator, arginase. As a result M2 TAMs skew the balance in favor of low levels of NO and hence promote tumor growth.

anti-inflammatory components, although one tends to predominate. For example, in breast carcinoma, TNF-alpha is highly expressed (120-122) that could be cytotoxic to tumor cells but more often may select for TNF-resistant cells and even promote tumor cell proliferation (123), angiogenesis, and metastasis (121, 122). A similar argument can be made for IL-1 and other pro-inflammatory cytokines that are also frequently overexpressed in breast (120), squamous cell (124) and head and neck cancer (125).

In contrast to expression of pro-inflammatory cytokines, immunosuppressive cytokines, in particular IL-10 and TGF-beta (126), are also often elevated in advanced tumors and correlate with a poor clinical prognosis (65, 127-129). Both are generally anti-proliferative for many cell types, including T cells, but many tumor cells have lost the ability to respond to these cytokines by mutation (130, 131). In contrast, T cell expansion and activation (142), LAK cell activity (143) and NK cell function (144) are inhibited while T_{reg} cell expansion is promoted (132). IL-10 also blocks DC-mediated priming of CD8⁺ T cells (133) and DC recruitment (134). Production of these cytokines may be stimulated by COX2 and prostaglandins, which many human tumors express at high levels (82, 135-137). Angiogenic cytokines, such as VEGF and erythropoietin, seem to be favored by such an environment, in addition to being induced by hypoxia (138).

Overall, the data suggest that the tumor microenvironment can present many profiles that might be

viewed as ranging from being highly immunosuppressive to immunosupportive (Figure 2). These might be linked to characteristic physiologic profiles, such as mean vessel density, acidosis, hypoxia, extent of nutrient deprivation, *etc.* Defining these profiles is still in its infancy but the concept is an important one since such profiles may reflect the extent of immune recognition and point to the likelihood of immunity being involved in response to conventional therapies. They also might offer targets for novel intervention with the aim of changing the phenotype of the tumor microenvironment to be more immunosupportive, which may be an absolute requirement for effective IT either deliberately generated or passively acquired during other therapies.

4. MODIFYING THE TUMOR MICROENVIRONMENT TO ENHANCE TUMOR IMMUNITY

4.1. Conventional therapies affect the tumor microenvironment

It is axiomatic that therapy aimed at tumors targets the tumor microenvironment and the host cell infiltrate as well as the tumor cells. Given that the tumor microenvironment may range from immunosuppressive to immunosupportive leads to questions as to how conventional treatments impact these profiles and vice-versa, how conventional treatments might be optimized to bring out the best in the immune system, how IT might best be integrated into a conventional schedule, and how the tumor microenvironment might be modified to support the development of tumor immunity.

It should be noted that the use of preclinical transplanted tumor models can easily mislead investigators into thinking that conventional treatments enhance immunity. In mice, immunogenic tumors grow in the face of concomitant immunity, they are easier to cure by conventional RT or CT than non-immunogenic tumors, and if complete regression occurs, or if tumor is surgically removed, a state of systemic immunity can easily be demonstrated. In these situations, therapy most likely unmasks the potential of the immune system to combat tumor growth, as opposed to acting as a positive stimulus to generate immunity. Most human tumors that arise spontaneously are only weakly immunogenic and have a wide range of phenotypes that may not be well represented by a single transplantable tumor model. The important questions are whether RT or CT can create an immunosupportive from an immunosuppressive tumor microenvironment, under what conditions, and whether additional manipulations are needed to better translate therapy-induced cell death into the generation of immunity.

4.1.1. Radiation therapy

Traditionally, ionizing radiation has been thought of as a “silent” killer, in that radiation-induced cell death does not readily translate into tumor immunity. This is somewhat surprising since radiation often induces immunologically ‘loud’ necrotic cell death, in addition to apoptosis (139, 140). The presumed liberation of TAA and

The tumor microenvironment and immunity

the release of nucleic acids that follow RT might be expected to have powerful immune activating properties (141-143), which may favor antigen capture and maturation of DCs (144). Perhaps the most exciting hypothetical possibility is that RT might broaden the spectrum of TAA epitopes that are recognized by the immune system, either by altering the molecules themselves so as to provide more helper recognition or the amount of rare TAAs released at any one time. There has also been much recent discussion as to the importance of RT in facilitating antigen presentation by induced phenotypic changes on irradiated tumor cells and DCs. For example, there is ample evidence for RT-induced up-regulation of molecules, such as MHC class I and II, TAA, the co-stimulatory molecule CD80 and CD86, and the expression of members of the death TNF receptor family and their ligands (145-150) (McBride unpublished). Importantly, *in vivo* RT has been shown to up-regulate MHC class I expression on the leading edge of invasive glioma cells in mice (151). It is easy to see how anti-tumor immunity and tumor control might be enhanced by these mechanisms (152), but it seems that up-regulation in expression of all molecules is not a universal phenomenon and further work is needed to elucidate the mechanisms involved and their relevance. Also, there is no consensus as to what the best radiation dose or scheduling might be or even the circumstances, if any, under which this would be effective.

Another potentially immunologically positive feature of irradiation is that it causes recurring waves of cytokines, chemokines and growth factors, most prominently TNF-alpha, IL-1, TGF-beta, and EGF (153-159), that might be expected to act as 'danger' signals to activate DCs to present TAA (160). The synergy seen between RT and various IT approaches in achieving better tumor control in mice encourages enthusiasm for this view (148, 161-164). As mentioned above, the critical issue is whether RT acts as an immune adjuvant in these situations or simply unmasks existing immunity. Irradiated tumor cell vaccines made from most spontaneous mouse tumors are unable to generate a state of protective immunity (165, 166) (the classical definition of "non-immunogenic"), suggesting that irradiation does not readily confer immunogenicity on tumors and there is little evidence that release of debris from non-immunogenic tumors *in vivo* caused by local RT translates into immune activation (167, Liao unpublished). By analogy, one might expect little immune activation following RT of human cancer. On the other hand, basic parameters such as the optimal radiation dose, dose rate, and fractionation schema have not been extensively investigated and could have an impact.

Unmasking of an existing immune state by RT is, of course, a potentially positive contribution providing such a state exists or can be generated. In addition, the ability of RT to shrink tumor bulk dramatically and slow the rate of tumor cell proliferation could be enough to give the immune system an advantage (168). Irradiation may also increase trafficking of cells into tumor, as it does for normal tissues as a result of increased expression of adhesion molecules on the surface of endothelial cells involved in extravasation, such as ICAM-1, E-selectin,

VCAM-1 and CD31 (169, 170) (171-173). In tumors, the promotion of host cell trafficking may be offset by radiation damage to endothelial cells and the tortuous tumor vessels and defective angiogenesis, but the overall outcome may still be in favor of tumor control (174-176), as has been shown in some mouse tumor models (152). This may be a result of RT-induced decreases in interstitial fluid pressure, remodeling of the extracellular matrix, tumor re-oxygenation, and/or changes in vascular flow more than radiation-induced changes in adhesion molecules (177-181).

A particularly important question is whether RT can make an immunosuppressive intratumoral environment into an immunosupportive one. There is little data on this but at least one study suggests otherwise. Tsai *et al.* (182) showed that single and fractionated radiation doses in a murine prostate cancer model did not generate much TNF-alpha, which may polarize TAMs towards a M1 inflammatory phenotype with predominantly tumoricidal activities (183), but simply accentuated the production of arginase and COX-2 and the generally immunosuppressive and pro-tumor microenvironment. There was little increase in anti-tumor immunity following RT. This is clearly not the final word on this important subject.

Having said that, RT, because it is a powerful cytotoxic modality, will directly and indirectly affect immune cell survival and function within the tumor microenvironment, which will have immunomodulatory effects. Different intrinsic radiosensitivities will shape the balance of intratumoral TIL subsets. In general, memory T and NK cells are relatively radioresistant when compared to B cells and naïve T cells (184, 185). Importantly, North and colleagues (186) demonstrated, in murine models, that sublethal whole body gamma-irradiation led to partial or complete tumor regression in immuno-competent but not in immuno-incompetent animals. They argued that radiation-induced elimination of suppressor T cells or their precursors shifted the immune-balance in favor of effector T cells (187-189). Again, tumor immunogenicity may contribute an immunosupportive milieu to these findings and concomitant immunity may be simply unmasked. However, Yu has shown that localized depletion of suppressive T cells can translate into tumor rejection (190). Whether this is as effective as whole body irradiation has yet to be evaluated but it argues that RT may be a useful therapeutic immune adjuvant even at low doses. Whole body irradiation may have an additional advantage that it would enrich for systemic T memory cells and allow space for their selective lymphocyte expansion, as it does in cell transfer studies.

Macrophages and DCs tend to be radioresistant but may be functionally affected by RT. Irradiation of DCs *in vitro* can inhibit their ability to endogenously process and present MART-1 TAA to T cells and to generate protective tumor immunity (191), which has very evident clinical implications. Interestingly, cross-presentation of TAA was enhanced after RT and this may be a positive aspect of RT that could be further exploited.

The tumor microenvironment and immunity

One rationale for combining IT with RT is that IT may be able to increase the likelihood of achieving local tumor control with RT while eliminating disease outside the field by immune mechanisms. In spite of all the caveats and the lack of rigorous mechanistic evidence on certain critical issues, numerous *in vivo* studies have demonstrated that local RT can enhance the systemic effect of IT. These studies have utilized mainly DC-based and/or viral vaccines and/or local or systemic GM-CSF, IL-2 and IL-3 therapy (150, 162-164, 192, 193) (167, 194, 195). For example, Lumniczky *et al.* (161) showed that administration of vaccines producing GM-CSF, IL-4, or IL-12 in combination with local RT resulted in >80% cure of murine brain tumors. Others (167, 196-198) have shown the same for vaccines expressing IL-3, which induces multiple alterations in tumor cell phenotype, including MHC class I expression and TNF- α sensitivity, while enhancing intratumoral DC and T cell infiltrates. Remarkably, IL-3 expression renders a classically non-immunogenic irradiated tumor cell vaccine immunogenic and is able to generate immunity against the original parental tumor. In other words, cytokines can reshape the cellular profile of tumors changing an immunosuppressive into an immunosupportive phenotype while unmasking TAA expression (196). It is not surprising that approaches integrating IT with local RT of prostate cancer, pancreatic cancer, melanoma, glioblastoma multiforme, non-small cell lung cancer have entered phase I and II of clinical testing under NCI approval (199, www.clinicaltrials.gov).

The impact of RT on immune defenses is obviously complex and multifactorial but there are many facets of relevance to the tumor microenvironment that are worthy of further investigation. In this age of combination therapies, it is however also necessary to consider the impact CT might have on such responses and how its effects on immune modulation in the tumor microenvironment might differ from those of RT.

4.1.2. Chemotherapy

Similar to RT, CT appears to augment IT in ways that are difficult to explain by a simple de-bulking hypothesis alone (reviewed in (200-202)). General and selective lymphopenias are often manifest that may allow for the selective recovery of tumor-specific immune machinery, especially when used in conjunction with vaccination or adoptive cell transfer (201, 203). As for RT, this concept is based, in part, on the old observations that the administration of cyclophosphamide in rodents and indeed in humans depletes circulating T regulatory cells and famously counteracts peripheral tolerance (187, 204-208). Conversely, cyclophosphamide also may favor memory T cell proliferation, possibly through controlling T cell growth factors and iNOS expression (209-211). The nucleoside analogue Gemcitabine is also immune modulatory. It selectively reduces B cells and myeloid suppressor cells in tumor-bearing mice while increasing the anti-tumor activity of CD8 T cells and NK cells (212, 213). Importantly, while resculpting the T cell balance, these cytotoxic drugs do not appear to greatly reduce the frequency of tumor-specific T cells (200). In any event, the relative resistance of memory T cells to CT seems a

consistent finding that could be exploited, as it could for RT.

Many cytotoxic drugs can prime the local tumor microenvironment to facilitate anti-tumor immune attack. Gemcitabine, increases T cell infiltration into solid tumors in rodents, while cyclophosphamide does not (214, 215). Docetaxel on the other hand greatly compromises the motility of DCs, especially at very low doses (216). Whether or not this depends on drug-induced changes in the cytokine milieu or is due to vascular changes is not clear. In fact, endothelial proliferation and tumor angiogenesis are inhibited by many cytotoxic drugs, especially when given in metronomic doses (217-219), although agents such as 5,6-dimethylxanthenone-4-acetic acid (DMXAA) that directly target the vasculature can also have an immune-modulatory component as they can increase cell trafficking to the tumor site (220). In general, cancer chemotherapeutic drugs that are currently in the clinic vary considerably regarding their modes of anti-tumor action and there is no reason to believe that this isn't also true for effects they might have on the immune response.

Some chemotherapeutic drugs also modify events at the immunological synapse. Tumor cell death will liberate potential antigenic peptides for cross presentation (221). Gemcitabine, for example, has this immune priming effect (222). Taxol is particularly intriguing in that it appears to activate macrophages and DCs through toll-like receptors to produce IL-12 and hence could alter the Th1/2 balance in the tumor microenvironment, while also being able to suppress lymphocyte proliferation (223-225). Improved tumor-antigen presentation and T cell activation due to drug-induced expression of MHC and co-stimulatory molecules on tumor and DCs are not uncommon. They have been reported in preclinical studies for 5-aza-2'-deoxycytidine, 5-fluorouracil, melphalan, chlorambucil, cytosine arabinoside and bryostatins (144, 226-231). There is substantial evidence that CT can also enhance the effector arm of the immune response by 'weakening' the target. Drugs such as 5-fluorouracil, dacarbazine, cisplatin and cytosine arabinoside sensitize tumor cells to antigen-specific CTL-mediated lysis by enhancing fas-dependent and -independent pathways (230, 232-235).

Clearly, these cytotoxic drugs could shape an immune-mediated tumor rejection mechanism either acting through modification of the tumor microenvironment or by systemic action, making a strong case for combining CT with IT. Ultimately, the combination chosen, and how timing and dosing are applied will have to be carefully assessed in order not to compromise the efficacy of each individual modality alone. For example, in mice, the combination of cyclophosphamide, doxorubicin and taxol can enhance the anti-tumor potency of a GM-CSF vaccine, but only when given in a specific sequence, while cisplatin remains ineffective (236). IL-12 can delay the growth of murine tumors only when given after taxol treatment, while simultaneous administration of drug and cytokine can not (237). An increasingly utilized way of combining CT and IT in clinical cancer trials is

The tumor microenvironment and immunity

myeloablative/lymphodepleting CT followed by autologous stem cell transplant and low-dose CT, in combination with various biological therapies (IL-2 or GM-CSF-based, cellular adoptive IT, with or without vaccine therapy with autologous DCs), or alternatively lower CT doses combined with similar vaccines. These strategies are currently being tested for patients with lymphoma and leukemia, as well as cancer of the breast, prostate, lung, testis, kidney, rectum, ovaries, multiple myeloma, and, of course, melanoma (www.clinicaltrials.gov).

4.1.3. Surgery

The influence of surgery on immune function has been largely examined from the immunosuppressive aspect consequent to trauma. Chemotaxis, serum cytokine levels, and cellular activities have been reported to be severely suppressed, and this could increase the chance for metastases formation, although there are few reports on the impact of surgically-induced inflammation on presentation of TAA for tumor-specific immunity. The bigger the surgical incision is, the more pronounced the immune suppression will be. The ongoing development of minimally invasive techniques, such as laparoscopy, may provide the surgeon with an alternative that helps preserve immune activity (238). This may be especially important for combating postoperative or residual disease. It is not clear if innate and adaptive responses are equally affected (239-243), but in either event, the effects of surgery on the immune system may need to be taken into account when used in conjunction with other modalities. The jury is still out on whether or not the immunological benefits of keyhole surgery outweigh potentially serious adverse effects, such as port wound tumors (244).

4.2. Specific targeting of the tumor microenvironment

It is almost axiomatic that cytotoxic agents, including RT and CT, will affect hypoxic status and acidosis in the tumor microenvironment, simply by causing cell death. They will also affect immune function, directly through affecting the balance and activation of host cells, as well as indirectly through altering the tumor microenvironment. These effects could be critical to the outcome of conventional treatment but their influence is difficult to assess and may depend upon the extent of cell depletion. Conventional cytotoxic therapies roil the tumor microenvironment non-specifically, but new biological targeting agents are coming online that promise more selective control, although their lack of cytotoxicity generally means that these will generally be used in conjunction with conventional treatments

4.2.1. Hypoxia and tumor acidosis

Various strategies have been used to target hypoxia itself or the biological responses to it, while others have been found to do it passively. Approaches to alter hypoxia include hyperbaric gas inhalation, transfusions, NSAIDs, mild hyperthermia, and VEGF blockade (245, 246). In addition, HIF-1 may also be destabilized and/or inhibited either directly or through targeting Hsp90, topoisomerase I, or COX-2 (247-253). There have also been considerable efforts to screen for novel HIF-1 pharmacological inhibitors (254-257). Some of these

compounds exhibited beneficial effects in murine tumor models and have entered clinical trials (151, 258-260). These might be expected to affect angiogenesis through VEGF inhibition, but also to have unexpected effects on the immune system.

Tight regulation of HIF-1 is essential for the normal development of many cells and tissues, including lymphocytes and monocytes, as well as for the expression of inflammation (261-268). Targeting of HIF-1 in attempts to eliminate hypoxic cells or inhibit their role in angiogenesis may therefore have the incidental effect of enhancing general and tumor-specific immune function. This may explain why antisense to HIF-1 α caused a murine tumor to regress in a NK cell dependent fashion (269). The number of TAMs with an immune-suppressive phenotype that typically develop under low oxygen tension may also decrease (270) and their energy balance alter, which can translate into impairment in motility, invasiveness, adhesion and antibacterial function (267, 271). Targeting hypoxia may also restore CD80 expression and possibly MCP-1 and MMP-9 production by macrophages, which should aid co-stimulation and leukocyte recruitment (272-274). Based on studies with ischemia-reperfusion and systemic hypoxia, restoring oxygenation may be expected to enhance adhesion and migration of DC and NK cells and the activation and survival of TILs (263, 275-277).

Ultimately, targeting hypoxia may also change tumor acidosis, although the link between two phenomena is not entirely clear (278-280). Acidosis may be counteracted indirectly through targeting hypoxia by temporal O₂ conditioning of tumors or by inhibition of the hypoxia-inducible carbonic anhydrase (43, 281). Strategies that modify metabolism or ion pumps to transiently increase or decrease acidosis have been tested in animal tumor models and clearly enhance the toxicity of certain cytotoxic drugs (282). However, by and large, the issue of how targeting acidosis will shape the tumor microenvironment and tumor immunity has not been addressed. Based on what we know about immune function and the importance of extracellular pH, acidosis intervention may enhance lymphocyte proliferation and cytotoxicity, chemotaxis of leukocytes, and macrophage function, although the antigen-presentation skills of DCs might be compromised by such approaches (283-287). Perhaps the introduction of vascular targeting agents into the clinic will put more urgency into these studies.

4.2.2. Vasculature and angiogenesis

Targeting either angiogenesis or the established vasculature in tumors has been the focus of much recent attention and both have profound effects on the tumor microenvironment. They are conceptually different approaches as the former aims to prevent new vasculature growth while the latter aims to disrupt existing tumor vasculature to cause necrosis. Several agents are currently undergoing clinical evaluation, especially as part of multimodality treatments (reviewed in (288)). The most clinically advanced is the FDA-approved humanized

The tumor microenvironment and immunity

monoclonal antibody against VEGF-A, Avastin (Bevacizumab) (289, 290).

Avastin appears paradoxically to cause transient vascular normalization, reduced vascular permeability and reduction in interstitial fluid pressure (291). This may be because VEGF is also vascular permeability factor. Targeting VEGF enhances drug delivery and there is no reason to believe that it shouldn't also improve immune cell access, provided normalization persists. Sun *et al.* (292) showed that the potent anti-angiogenic factor angiostatin in combination with IT involving T cell costimulation caused murine tumor rejection, whereas either therapy alone did not. The vascular disrupting agent DMXAA increased the traffic of macrophages and lymphocytes to the tumor site ultimately leading to the generation of an anti-tumor immune response (220). On the other hand, vascular targeting by anti-VEGF may inhibit TAMs, since they are its major source and respond to it with migration and activation (293-295). This need not necessarily be a bad thing for cancer therapy if the TAMs exhibit a M2 phenotype (296, 297). There is yet another dimension to vascular targeting, in that cells of haematopoietic origin appear to contribute if not initiate tumor spreading through expression of VEGFR1. An elegant study by Kaplan and colleagues showed that bone marrow progenitor cells expressing this receptor create a pre-metastatic niche long before the tumor cells arrive (298).

One particularly novel strategy to target the endothelium is by vaccination. For example, at least in preclinical models (299), immunization with VEGFR2-pulsed DCs, or VEGFR2-derived peptides, or VEGFR2-based DNA vaccines elicited humoral and/or cell-mediated immune toxicities towards tumor endothelial cells that prevented tumor angiogenesis and tumor growth and in the last case slowed the growth of established murine tumors (300-303). Humanized A2/K^b transgenic mice responded to VEGFR2 peptide vaccination and had better tumor control (304). The same group demonstrated that responses could be generated in cancer patients, at least *ex vivo*. Tumor control may be further increased by targeting both vasculature-associated antigens and TAAs (305).

4.2.3. Extracellular matrix and fibroblasts

According to traditional belief, tumor angiogenesis, tumor invasion and even early tumorigenesis are not possible until the extracellular matrix (ECM) and the basement membrane are degraded. Consequently, inhibiting the enzymes that are involved in this remodeling process such as matrix metalloproteases (MMPs), cysteine and serine proteases, and heparanase may improve tumor control (reviewed in (288)). The unfortunate failure of some of the 1st and 2nd generation broad-spectrum MMP inhibitors in the clinic made apparent the spatial and temporal complexity of the ECM balance associated with tumorigenesis (reviewed in (306, 307)). However, since proteases are so intimately involved with molecules such as TNF- α , IL-1 β and TGF- β , chemokines, and adhesion molecules, chemotaxis and immunity are likely to be affected by their inhibition (307-309). Tissue inhibitors

of MMPs (TIMP-1) inhibited the migration of immature dendritic cells and TIMPs are therefore possible targets for intervention (310). Although the relationship may be circumstantial, skin cancer prevention in mice with a polyphenolic botanical supplement both inhibited MMP2 and MMP9 and increased cytotoxic T cell numbers (311). TIMP-1 also restored IL-2-dependent proliferation of TILs, at least *ex vivo* (312). Any structural modification of the ECM can potentially lead to the activation of cryptic ECM activities, so-called matricryptic sites within ECM molecules (313, 314) that encourage adaptive immunity.

Antibody-mediated or pharmacological targeting of integrins that maintain the communication between cells and the ECM proteins have also shown promise in preclinical studies and are undergoing evaluation in the clinic (315). Strategies that selectively target activated fibroblasts or their products within the ECM framework are under development and preliminary data are encouraging. These approaches are based on the fact that fibroblasts can act as tumor suppressors under normal condition, but alter their phenotype to an activated one that enhances epithelial transformation and migration in established tumors (288). All in all, although our knowledge of how ECM targeting will affect tumor immunity is currently very limited, there is evidence that ECM modulation is likely to have multiple effects on tumor immunity.

4.3. Counteracting immune suppression in the tumor microenvironment

4.3.1. Inflammation, cytokines and “danger”

A relationship between many cancers and chronic inflammation has long been suggested, and is perhaps best exemplified in the tumor preventative potential of non-steroidal anti-inflammatory drugs (316-321). Such treatments might be expected to enhance tumor immunity but it is still not clear what is the optimal inflammatory profile to generate such responses. This is even more important if the cytokine balance is to be therapeutically skewed from being immunosuppressive to being immunosupportive, as may already be being achieved with some strategies. For example, preclinical studies with IL-2 gene therapy along with RT has shown efficacy that may be due to decreased intratumoral hypoxia (322), and might be expected to be immunosupportive. IL-3 gene therapy also decreases hypoxia in experimental tumors (McBride, unpublished) and is immune enhancing. Perhaps this is why cytokine gene therapy can be particularly effective in combination with RT. On the other hand, essentially any therapy will alter tumor hypoxia. Whether this is sufficient to change the intratumoral milieu into one that is immunosupportive is not clear. Perhaps local delivery of cytokine mediators may be required to change the intratumoral profile and for highly toxic agents such as TNF- α this may be the only route possible, and it has shown efficacy in patients with sarcoma (323, 324). It would be of interest to know how tumor immunity is affected.

Anti-inflammatory drugs, especially the new generation of selective, yet controversial COX-2 inhibitors also show promise in systemic targeting of the chronic

The tumor microenvironment and immunity

inflammatory profile. Pharmacological targeting of COX-2 appears to counteract some tumor escape mechanism *in vivo* such as a suppressive cytokine milieu, suppressed DC function, preferential Th2 response, limited T cell proliferation, MHC-mediated NK cytotoxicity and IDO expression (325-328). Not surprisingly, COX-2 inhibitors such as celecoxib or rofecoxib can boost the efficacy of cancer vaccines by enhancing tumor-specific CTLs in mice (329, 330). When thinking further upstream of inflammatory signaling cascades, NF-kappaB – the master regulator is certainly an attractive target (331, 332). Mouse colon cancer cells expressing a super repressor of NF-kappaB, for example, are unable to form inflammation-induced lung metastasis (333). Taking this further, reducing inflammation and its mediators may also dampen HIF-1 activity, which in itself would be a desirable ‘side’ effect (see above, (266)).

4.3.2. Dendritic cell and macrophage function and subsets

Numerous IT trials apply dendritic cell vaccines with TAAs in the form of DNA, peptides, proteins or in viral vectors, in attempts to boost immune responses to cancer. Others use gene therapy approaches to modify immunity within the tumor environment. The cytokines GM-CSF and IL-3 are thought to, in part, bring about some of their immune enhancing effects through generating DCs (173-175). Other approaches target using co-stimulator molecules such as B7.1 and B7.2 (334). Melanoma patients subjected to intralesional vaccinia virus vector expressing B7.1 developed systemic T cell responses against tumor antigens that in some cases translated into partial responses or stabilization and prolonged survival (335). That there is room for improvement was recently shown in mice whereby a vaccination with 4-1BBL in addition to B7.1 led to even higher levels of tumor-specific T cells (336). Whether this approach is better than systemic administration is not clear and may depend on the strategy. For example, blocking chemokines or receptors involved in the recruitment of immune-suppressive DC subsets such as plasmacytoid DCs and myeloid suppressor DCs would suggest an intratumoral approach, as would use of AMD3100, which is a CXCR4 antagonist that can counteract the chemorepulsion of T cells associated with CXCL12 expression by tumor (337).

4.3.3. T regulatory cells and T cell imbalance

Another approach to counter T cell anergy is aimed at the effector arm of immunity (338). Of particular interest is the recent use of adoptive transfer of T cells genetically modified to express monoclonal TCR with specificity for a TAA epitope in lymphodepleted patients (339). Such studies are easily adapted to imaging of the adoptively transferred cells and examination of their ability to localize to the tumor and assessing the effects of therapies on such infiltrates are likely to give valuable information on how to modify the tumor microenvironment to optimize IT. In some of these adoptive transfer experiments, depletion of T_{regs} with antibodies, which have been shown to enhance anti-tumor responses (340-343), may be an essential element. How necessary this will prove to be will have to be determined. Metabolic intervention

with production of IDO, the enzyme responsible for tryptophan deprivation and hence T cell anergy or death, improved tumor control in mice in a T cell dependent fashion (344). Even more promising is IDO inhibition when used in combination with cytotoxic treatments. Simultaneous targeting of IDO by 1-methyl-tryptophan (1MT) with paxlitaxel, cisplatin, cyclophosphamide or doxorubicin translated into tumor regression in a mouse model for breast cancer (345). Even though IT approaches based on combination therapies that enhance certain elements of tumor immunity and simultaneously inhibit others, most notably CTLA-4 blockade, give more encouraging results than single approaches, finding the optimal clinical strategy for IT has in many cases proved elusive (346). Perhaps the key is that it is necessary to think and examine the local intratumoral events rather than what happens systemically. In many cases, the impact of therapies on the tumor microenvironment was simply not investigated.

5. CONCLUSIONS

This review has summarized some of the most recent developments in cancer therapies. We have tried to focus on effects of conventional and novel therapies on the tumor microenvironment and how this might influence tumor immunity. What is obvious is that therapies must affect the tumor microenvironment if they are to be effective and these changes may be used as a barometer of efficacy. It is also evident that the fact that most IT strategies will remain disappointing if used on their own and the key to improved cure rates will most likely lie in combination therapies that treat tumors for what they are, namely the product of a development process that has used many mechanisms to escape from normal growth control and immune recognition. Every therapy should be chosen with great care because they are based on a variety of concepts, some of which might be mutually antagonistic when used in combination. Finally, we suggest that the tumor microenvironment presents several distinct integrated profiles of gene expression and cell content that incorporate physiological changes and host cell content as well as tumor cell characteristics. Recognition of whether the milieu is immunosupportive or immunosuppressive will be critical to our understanding of how to best use therapies that aim to promote tumor immunity. It is also true that, irrespective of the proposed target of a therapy, alterations that are wrought in the tumor microenvironment could lead to unexpected involvement of immune mechanisms that could contribute to tumor cure. This is particularly true if animal models are used that are limited in their applicability to the human situation.

6. ACKNOWLEDGEMENT

All authors contributed equally to the preparation of this manuscript. This work was supported by National Institutes of Health/National Cancer Institute Grant RO1 CA-101752, (WHM), United States Army Medical Research and Materiel Command Grants W81XWH-04-1-0126 (WHM) and W81XWH-07-1-0114 (DS) and a Jonsson Comprehensive Cancer Center research grant (DS).

7. REFERENCES

1. Balkwill, F. & A. Mantovani: Inflammation and cancer: back to Virchow? *Lancet*, 357, 539-545 (2001)
2. Ehrlich, P.: Ueber den jetzigen Stand der Karzinomforschung. *Ned. Tijdschr. Geneesk.*, 5, 273-290 (1909)
3. Burnet, M.: Cancer; a biological approach. I. The processes of control. *Br Med J* 779-86 (1957)
4. Thomas, L.: In: Cellular and Humoral Aspects of the Hypersensitive States. Ed: H. S. Lawrence. P.B Hoeber, New York (1959)
5. thor Straten, P., P. Guldberg, K. Gronbak, M. R. Hansen, A. F. Kirkin, T. Seremet, J. Zeuthen & J. C. Becker: *In situ* T Cell Responses Against Melanoma Comprise High Numbers of Locally Expanded T Cell Clonotypes. *J Immunol*, 163, 443-447 (1999)
6. Rosenberg, S. A.: Progress in the development of immunotherapy for the treatment of patients with cancer. *Journal of Internal Medicine*, 250, 462-475 (2001)
7. Bremers, A. J. A. & G. Parmiani: Immunology and immunotherapy of human cancer: present concepts and clinical developments. *Critical Reviews in Oncology/Hematology*, 34, 1-25 (2000)
8. Boon, T., P. G. Coulie & B. Van den Eynde: Tumor antigens recognized by T cells. *Immunol Today*, 18, 267-8 (1997)
9. Matzinger, P.: The danger model: a renewed sense of self. *Science*, 296, 301-305 (2002)
10. Fuchs, E. J. & P. Matzinger: Is cancer dangerous to the immune system? *Semin. Immunol.*, 8, 271-280 (1996)
11. Gajewski, T. F., Y. Meng & H. Harlin: Immune suppression in the tumor microenvironment. *J Immunother*, 29, 233-40 (2006)
12. Gray, L. H., A. D. Conger, M. Ebert, S. Hornsey & O. C. Scott: The concentration of oxygen dissolved in tissues at the time of irradiation as a factor in radiotherapy. *Br J Radiol*, 26, 638-48 (1953)
13. Hahnegahdar, H., J. Du, D. Wang, R. M. Strieter, M. D. Burdick, L. B. Nannay, N. Cardwell, J. Luan, R. Shattuck-Brandt & A. Richmond: The tumorigenic and angiogenic effects of MGSA/GRO proteins in melanoma. *J Leukoc Biol*, 67, 53-62 (2000)
14. Mrowietz, U., U. Schwenk, S. Maune, J. Bartels, M. Kupper, I. Fichtner, J. M. Schroder & D. Schadendorf: The chemokine RANTES is secreted by human melanoma cells and is associated with enhanced tumour formation in nude mice. *Br J Cancer*, 79, 1025-31 (1999)
15. Loukinova, E., G. Dong, I. Enamorado-Ayalya, G. R. Thomas, Z. Chen, H. Schreiber & C. Van Waes: Growth regulated oncogene-alpha expression by murine squamous cell carcinoma promotes tumor growth, metastasis, leukocyte infiltration and angiogenesis by a host CXC receptor-2 dependent mechanism. *Oncogene*, 19, 3477-86 (2000)
16. Soler, D., T. L. Humphreys, S. M. Spinola & J. J. Campbell: CCR4 versus CCR10 in human cutaneous TH lymphocyte trafficking. *Blood*, 101, 1677-82 (2003)
17. Kurt, R. A., A. Baher, K. P. Wisner, S. Tackitt & W. J. Urba: Chemokine receptor desensitization in tumor-bearing mice. *Cell Immunol*, 207, 81-8 (2001)
18. Peng, L., S. Shu & J. C. Krauss: Monocyte chemoattractant protein inhibits the generation of tumor-reactive T cells. *Cancer Res*, 57, 4849-54 (1997)
19. Van den Hove, L. E., S. W. Van Gool, H. Van Poppel, L. Baert, L. Coorevits, B. Van Damme & J. L. Ceuppens: Phenotype, cytokine production and cytolytic capacity of fresh (uncultured) tumour-infiltrating T lymphocytes in human renal cell carcinoma. *Clin Exp Immunol*, 109, 501-9 (1997)
20. Hellstrom, I., J. A. Ledbetter, N. Scholler, Y. Yang, Z. Ye, G. Goodman, J. Pullman, M. Hayden-Ledbetter & K. E. Hellstrom: CD3-mediated activation of tumor-reactive lymphocytes from patients with advanced cancer. *Proc Natl Acad Sci U S A*, 98, 6783-8 (2001)
21. Guilloux, Y., C. Viret, N. Gervois, E. Le Drean, M. C. Pandolfino, E. Diez & F. Jotereau: Defective lymphokine production by most CD8+ and CD4+ tumor-specific T cell clones derived from human melanoma-infiltrating lymphocytes in response to autologous tumor cells *in vitro*. *Eur J Immunol*, 24, 1966-73 (1994)
22. Roussel, E., M. C. Gingras, E. A. Grimm & J. A. Roth: High expression of adhesion molecules/activation markers with little interleukin-2, interferon gamma, and tumor necrosis factor beta gene activation in fresh tumor-infiltrating lymphocytes from lung adenocarcinoma. *Cancer Immunol Immunother*, 41, 1-9 (1995)
23. Radoja, S., M. Saio, D. Schaer, M. Koneru, S. Vukmanovic & A. B. Frey: CD8 (+) tumor-infiltrating T cells are deficient in perforin-mediated cytolytic activity due to defective microtubule-organizing center mobilization and lytic granule exocytosis. *J Immunol*, 167, 5042-51 (2001)
24. Mortarini, R., A. Piris, A. Maurichi, A. Molla, I. Bersani, A. Bono, C. Bartoli, M. Santinami, C. Lombardo, F. Ravagnani, N. Cascinelli, G. Parmiani & A. Anichini: Lack of terminally differentiated tumor-specific CD8+ T cells at tumor site in spite of antitumor immunity to self-antigens in human metastatic melanoma. *Cancer Res*, 63, 2535-45 (2003)
25. Zippelius, A., P. Batard, V. Rubio-Godoy, G. Bioley, D. Lienard, F. Lejeune, D. Rimoldi, P. Guillaume, N. Meidenbauer, A. Mackensen, N. Rufer, N. Lubenow, D. Speiser, J. C. Cerottini, P. Romero & M. J. Pittet: Effector function of human tumor-specific CD8 T cells in melanoma lesions: a state of local functional tolerance. *Cancer Res*, 64, 2865-73 (2004)
26. Nakagomi, H., P. Pisa, E. K. Pisa, Y. Yamamoto, E. Halapi, K. Backlin, C. Juhlin & R. Kiessling: Lack of interleukin-2 (IL-2) expression and selective expression of IL-10 mRNA in human renal cell carcinoma. *Int J Cancer*, 63, 366-71 (1995)
27. Roussel, E., M. C. Gingras, E. A. Grimm, J. M. Bruner & R. P. Moser: Predominance of a type 2 intratumoral immune response in fresh tumour-infiltrating lymphocytes from human gliomas. *Clin Exp Immunol*, 105, 344-52 (1996)
28. Whiteside, T. L. & G. Parmiani: Tumor-infiltrating lymphocytes: their phenotype, functions and clinical use. *Cancer Immunol Immunother*, 39, 15-21 (1994)
29. Vose, B. M. & M. Moore: Human tumor-infiltrating lymphocytes: a marker of host response. *Semin Hematol*, 22, 27-40 (1985)

30. Mizoguchi, H., J. O'Shea, D. Longo, C. Loeffler, D. McVicar & A. Ochoa: Alterations in signal transduction molecules in T lymphocytes from tumor-bearing mice 10.1126/science.1465616. *Science*, 258, 1795-1798 (1992)
31. Kuss, I., T. Saito, J. T. Johnson & T. L. Whiteside: Clinical Significance of Decreased zeta Chain Expression in Peripheral Blood Lymphocytes of Patients with Head and Neck Cancer. *Clin Cancer Res*, 5, 329-334 (1999)
32. Lai, P., H. Rabinowich, P. A. Crowley-Nowick, M. C. Bell, G. Mantovani & T. L. Whiteside: Alterations in expression and function of signal-transducing proteins in tumor-associated T and natural killer cells in patients with ovarian carcinoma. *Clin Cancer Res*, 2, 161-73 (1996)
33. Healy, C. G., J. W. Simons, M. A. Carducci, T. L. DeWeese, M. Bartkowski, K. P. Tong & W. E. Bolton: Impaired expression and function of signal-transducing zeta chains in peripheral T cells and natural killer cells in patients with prostate cancer. *Cytometry*, 32, 109-19 (1998)
34. Riccobon, A., R. Gunelli, R. Ridolfi, F. De Paola, E. Flamini, M. Fiori, C. Saltutti, M. Petrini, L. Fiammenghi, M. Stefanelli, A. M. Granato, D. E. Cuzzocrea & D. Amadori: Immunosuppression in renal cancer: differential expression of signal transduction molecules in tumor-infiltrating, near-tumor tissue, and peripheral blood lymphocytes. *Cancer Invest*, 22, 871-7 (2004)
35. Koneru, M., D. Schaer, N. Monu, A. Ayala & A. B. Frey: Defective proximal TCR signaling inhibits CD8+ tumor-infiltrating lymphocyte lytic function. *J Immunol*, 174, 1830-40 (2005)
36. Chen, X., A. Woiciechowsky, S. Raffegerst, D. Schendel, H.-J. Kolb & M. Roskrow: Impaired expression of the CD3-zeta chain in peripheral blood T cells of patients with chronic myeloid leukaemia results in an increased susceptibility to apoptosis doi:10.1046/j.1365-2141.2000.02415.x. *British Journal of Haematology*, 111, 817-825 (2000)
37. Dworacki, G., N. Meidenbauer, I. Kuss, T. K. Hoffmann, W. Gooding, M. Lotze & T. L. Whiteside: Decreased zeta chain expression and apoptosis in CD3+ peripheral blood T lymphocytes of patients with melanoma. *Clin Cancer Res*, 7, 947s-957s (2001)
38. Reichert, T. E., H. Rabinowich, J. T. Johnson & T. L. Whiteside: Mechanisms responsible for signaling and functional defects. *J Immunother*, 21, 295-306 (1998)
39. Bauernhofer, T., I. Kuss, B. Henderson, A. S. Baum & T. L. Whiteside: Preferential apoptosis of CD56dim natural killer cell subset in patients with cancer. *Eur J Immunol*, 33, 119-24 (2003)
40. Whiteside, T. L.: Tumor-induced death of immune cells: its mechanisms and consequences. *Semin Cancer Biol*, 12, 43-50 (2002)
41. Whiteside, T. L.: Down-regulation of zeta-chain expression in T cells: a biomarker of prognosis in cancer? *Cancer Immunol Immunother*, 53, 865-78 (2004)
42. Zea, A. H., P. C. Rodriguez, K. S. Culotta, C. P. Hernandez, J. DeSalvo, J. B. Ochoa, H. J. Park, J. Zabaleta & A. C. Ochoa: L-Arginine modulates CD3zeta expression and T cell function in activated human T lymphocytes. *Cell Immunol*, 232, 21-31 (2004)
43. Loeffler, D. A., P. L. Juneau & S. Masserant: Influence of tumour physico-chemical conditions on interleukin-2-stimulated lymphocyte proliferation. *Br J Cancer*, 66, 619-22 (1992)
44. Masson, D., P. J. Peters, H. J. Geuze, J. Borst & J. Tschopp: Interaction of chondroitin sulfate with perforin and granzymes of cytolytic T-cells is dependent on pH. *Biochemistry*, 29, 11229-35 (1990)
45. Severin, T., B. Muller, G. Giese, B. Uhl, B. Wolf, S. Hauschildt & W. Kreutz: pH-dependent LAK cell cytotoxicity. *Tumour Biol*, 15, 304-10 (1994)
46. Loeffler, D. A., P. L. Juneau & G. H. Heppner: Natural killer-cell activity under conditions reflective of tumor micro-environment. *Int J Cancer*, 48, 895-9 (1991)
47. Radosevic, K., B. G. de Grooth & J. Greve: Changes in intracellular calcium concentration and pH of target cells during the cytotoxic process: a quantitative study at the single cell level. *Cytometry*, 20, 281-9 (1995)
48. Le, Q. T., G. Shi, H. Cao, D. W. Nelson, Y. Wang, E. Y. Chen, S. Zhao, C. Kong, D. Richardson, K. J. O'Byrne, A. J. Giaccia & A. C. Koong: Galectin-1: a link between tumor hypoxia and tumor immune privilege. *J Clin Oncol*, 23, 8932-41 (2005)
49. Caldwell, C. C., H. Kojima, D. Lukashev, J. Armstrong, M. Farber, S. G. Apasov & M. V. Sitkovsky: Differential effects of physiologically relevant hypoxic conditions on T lymphocyte development and effector functions. *J Immunol*, 167, 6140-9 (2001)
50. Berendt, M. J. & R. J. North: T-cell-mediated suppression of anti-tumor immunity. An explanation for progressive growth of an immunogenic tumor. *J Exp Med*, 151, 69-80 (1980)
51. Roncarolo, M. G. & M. K. Levings: The role of different subsets of T regulatory cells in controlling autoimmunity. *Curr Opin Immunol*, 12, 676-83 (2000)
52. Bach, J. F.: Regulatory T cells under scrutiny. *Nat Rev Immunol*, 3, 189-98 (2003)
53. Suttmuller, R. P., L. M. van Duivenvoorde, A. van Elsas, T. N. Schumacher, M. E. Wildenberg, J. P. Allison, R. E. Toes, R. Offringa & C. J. Melief: Synergism of cytotoxic T lymphocyte-associated antigen 4 blockade and depletion of CD25 (+) regulatory T cells in antitumor therapy reveals alternative pathways for suppression of autoreactive cytotoxic T lymphocyte responses. *J Exp Med*, 194, 823-32 (2001)
54. Tanaka, H., J. Tanaka, J. Kjaergaard & S. Shu: Depletion of CD4+ CD25+ regulatory cells augments the generation of specific immune T cells in tumor-draining lymph nodes. *J Immunother*, 25, 207-17 (2002)
55. Liyanage, U. K., T. T. Moore, H. G. Joo, Y. Tanaka, V. Herrmann, G. Doherty, J. A. Drebin, S. M. Strasberg, T. J. Eberlein, P. S. Goedegebuure & D. C. Linehan: Prevalence of regulatory T cells is increased in peripheral blood and tumor microenvironment of patients with pancreas or breast adenocarcinoma. *J Immunol*, 169, 2756-61 (2002)
56. Woo, E. Y., C. S. Chu, T. J. Goletz, K. Schlienger, H. Yeh, G. Coukos, S. C. Rubin, L. R. Kaiser & C. H. June: Regulatory CD4 (+)CD25 (+) T cells in tumors from patients with early-stage non-small cell lung cancer and late-stage ovarian cancer. *Cancer Res*, 61, 4766-72 (2001)
57. Woo, E. Y., H. Yeh, C. S. Chu, K. Schlienger, R. G. Carroll, J. L. Riley, L. R. Kaiser & C. H. June: Cutting Edge: Regulatory T Cells from Lung Cancer Patients

The tumor microenvironment and immunity

- Directly Inhibit Autologous T Cell Proliferation. *J Immunol*, 168, 4272-4276 (2002)
58. Curiel, T. J., G. Coukos, L. Zou, X. Alvarez, P. Cheng, P. Mottram, M. Evdemon-Hogan, J. R. Conejo-Garcia, L. Zhang, M. Burow, Y. Zhu, S. Wei, I. Kryczek, B. Daniel, A. Gordon, L. Myers, A. Lackner, M. L. Disis, K. L. Knutson, L. Chen & W. Zou: Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. *Nat Med*, 10, 942-9 (2004)
59. Viguier, M., F. Lemaître, O. Verola, M. S. Cho, G. Gorochoy, L. Dubertret, H. Bachelez, P. Kourilsky & L. Ferradini: Foxp3 expressing CD4+CD25 (high) regulatory T cells are overrepresented in human metastatic melanoma lymph nodes and inhibit the function of infiltrating T cells. *J Immunol*, 173, 1444-53 (2004)
60. Ghiringhelli, F., P. E. Puig, S. Roux, A. Parcellier, E. Schmitt, E. Solary, G. Kroemer, F. Martin, B. Chauffert & L. Zitvogel: Tumor cells convert immature myeloid dendritic cells into TGF-beta-secreting cells inducing CD4+CD25+ regulatory T cell proliferation. *J Exp Med*, 202, 919-29 (2005)
61. June, C. H., J. A. Bluestone, L. M. Nadler & C. B. Thompson: The B7 and CD28 receptor families. *Immunol Today*, 15, 321-31 (1994)
62. Dong, H., G. Zhu, K. Tamada & L. Chen: B7-H1, a third member of the B7 family, co-stimulates T-cell proliferation and interleukin-10 secretion. *Nature Med.*, 5, 1365-1369 (1999)
63. Curiel, T. J.: Blockade of B7-H1 improves myeloid dendritic cell-mediated antitumor immunity. *Nature Med.*, 9, 562-567 (2003)
64. Chen, L.: Co-inhibitory molecules of the B7-CD28 family in the control of T-cell immunity. *Nat Rev Immunol*, 4, 336-47 (2004)
65. Zou, W.: Immunosuppressive networks in the tumour environment and their therapeutic relevance. *Nat Rev Cancer*, 5, 263-74 (2005)
66. Vermi, W., R. Bonecchi, F. Facchetti, D. Bianchi, S. Sozzani, S. Festa, A. Berenzi, M. Cella & M. Colonna: Recruitment of immature plasmacytoid dendritic cells (plasmacytoid monocytes) and myeloid dendritic cells in primary cutaneous melanomas. *J Pathol*, 200, 255-68 (2003)
67. Preynat-Seauve, O., P. Schuler, E. Contassot, F. Beermann, B. Huard & L. E. French: Tumor-infiltrating dendritic cells are potent antigen-presenting cells able to activate T cells and mediate tumor rejection. *J Immunol*, 176, 61-7 (2006)
68. Vicari, A. P., I. Treilleux & S. Lebecque: Regulation of the trafficking of tumour-infiltrating dendritic cells by chemokines. *Semin Cancer Biol*, 14, 161-9 (2004)
69. Zou, W., V. Machelon, A. Coulomb-L'Hermin, J. Borvak, F. Nome, T. Isaeva, S. Wei, R. Krzysiek, I. Durand-Gasselín, A. Gordon, T. Pustilnik, D. T. Curiel, P. Galanaud, F. Capron, D. Emilie & T. J. Curiel: Stromal-derived factor-1 in human tumors recruits and alters the function of plasmacytoid precursor dendritic cells. *Nat Med*, 7, 1339-46 (2001)
70. Salio, M.: Plasmacytoid dendritic cells prime IFN-[gamma]-secreting melanoma-specific CD8 lymphocytes and are found in primary melanoma lesions. *Eur. J. Immunol.*, 33, 1052-1062 (2003)
71. Scarpino, S., A. Stoppacciaro, F. Ballerini, M. Marchesi, M. Prat, M. C. Stella, S. Sozzani, P. Allavena, A. Mantovani & L. P. Ruco: Papillary carcinoma of the thyroid: hepatocyte growth factor (HGF) stimulates tumor cells to release chemokines active in recruiting dendritic cells. *Am J Pathol*, 156, 831-7 (2000)
72. Negus, R. P., G. W. Stamp, J. Hadley & F. R. Balkwill: Quantitative assessment of the leukocyte infiltrate in ovarian cancer and its relationship to the expression of C-C chemokines. *Am J Pathol*, 150, 1723-34 (1997)
73. Tang, K. F., S. Y. Tan, S. H. Chan, S. M. Chong, K. S. Loh, L. K. Tan & H. Hu: A distinct expression of CC chemokines by macrophages in nasopharyngeal carcinoma: implication for the intense tumor infiltration by T lymphocytes and macrophages. *Hum Pathol*, 32, 42-9 (2001)
74. Tan, J. K. & H. C. O'Neill: Maturation requirements for dendritic cells in T cell stimulation leading to tolerance versus immunity. *J Leukoc Biol*, 78, 319-24 (2005)
75. Bell, D., P. Chomarat, D. Broyles, G. Netto, G. M. Harb, S. Lebecque, J. Valladeau, J. Davoust, K. A. Palucka & J. Banchereau: In breast carcinoma tissue, immature dendritic cells reside within the tumor, whereas mature dendritic cells are located in peritumoral areas. *J Exp Med*, 190, 1417-26 (1999)
76. Iwamoto, M., H. Shinohara, A. Miyamoto, M. Okuzawa, H. Mabuchi, T. Nohara, G. Gon, M. Toyoda & N. Tanigawa: Prognostic value of tumor-infiltrating dendritic cells expressing CD83 in human breast carcinomas. *Int J Cancer*, 104, 92-7 (2003)
77. Troy, A., P. Davidson, C. Atkinson & D. Hart: Phenotypic characterisation of the dendritic cell infiltrate in prostate cancer. *J Urol*, 160, 214-9 (1998)
78. Troy, A. J., K. L. Summers, P. J. Davidson, C. H. Atkinson & D. N. Hart: Minimal recruitment and activation of dendritic cells within renal cell carcinoma. *Clin Cancer Res*, 4, 585-93 (1998)
79. Kalinski, P., J. H. Schuitemaker, C. M. Hilkens & M. L. Kapsenberg: Prostaglandin E2 induces the final maturation of IL-12-deficient CD1a+CD83+ dendritic cells: the levels of IL-12 are determined during the final dendritic cell maturation and are resistant to further modulation. *J Immunol*, 161, 2804-2809 (1998)
80. Sombroek, C. C.: Prostanoids play a major role in the primary tumor-induced inhibition of dendritic cell differentiation. *J Immunol.*, 168, 4333-4343 (2002)
81. Akasaki, Y.: Induction of a CD4+ T regulatory type 1 response by cyclooxygenase-2-overexpressing glioma. *J Immunol.*, 173, 4352-4359 (2004)
82. Menetrier-Caux, C.: Inhibition of the differentiation of dendritic cells from CD34+ progenitors by tumor cells: role of interleukin-6 and macrophage colony-stimulating factor. *Blood*, 92, 4778-4791 (1998)
83. Munn, D. H., M. D. Sharma, J. R. Lee, K. G. Jhaver, T. S. Johnson, D. B. Keskin, B. Marshall, P. Chandler, S. J. Antonia, R. Burgess, C. L. Slingluff, Jr. & A. L. Mellor: Potential regulatory function of human dendritic cells expressing indoleamine 2,3-dioxygenase. *Science*, 297, 1867-70 (2002)

84. Dougherty, G. J. & W. H. McBride: Immunoregulating activity of tumor-associated macrophages. *Cancer Immunol Immunother*, 23, 67-72 (1986)
85. Dougherty, G. J. & W. H. McBride: Accessory cell activity of murine tumor-associated macrophages. *J Natl Cancer Inst*, 76, 541-8 (1986)
86. Curiel, T. J., P. Cheng, P. Mottram, X. Alvarez, L. Moons, M. Evdemon-Hogan, S. Wei, L. Zou, I. Kryczek, G. Hoyle, A. Lackner, P. Carmeliet & W. Zou: Dendritic cell subsets differentially regulate angiogenesis in human ovarian cancer. *Cancer Res*, 64, 5535-8 (2004)
87. Conejo-Garcia, J. R., F. Benencia, M. C. Courreges, E. Kang, A. Mohamed-Hadley, R. J. Buckanovich, D. O. Holtz, A. Jenkins, H. Na, L. Zhang, D. S. Wagner, D. Katsaros, R. Carroll & G. Coukos: Tumor-infiltrating dendritic cell precursors recruited by a beta-defensin contribute to vasculogenesis under the influence of Vegf-A. *Nat Med*, 10, 950-8 (2004)
88. Gilliet, M. & Y. J. Liu: Generation of human CD8 T regulatory cells by CD40 ligand-activated plasmacytoid dendritic cells. *J. Exp. Med.*, 195, 695-704 (2002)
89. Rissoan, M. C., V. Soumelis, N. Kadowaki, G. Grouard, F. Briere, R. de Waal Malefyt & Y. J. Liu: Reciprocal control of T helper cell and dendritic cell differentiation. *Science*, 283, 1183-6 (1999)
90. van Ravenswaay Claasen, H. H., P. M. Kluin & G. J. Fleuren: Tumor infiltrating cells in human cancer. On the possible role of CD16+ macrophages in antitumor cytotoxicity. *Lab Invest*, 67, 166-74 (1992)
91. Toomey, D., J. Harmey, C. Condron, E. Kay & D. Bouchier-Hayes: Phenotyping of immune cell infiltrates in breast and colorectal tumours. *Immunol Invest*, 28, 29-41 (1999)
92. Leek, R. D.: The prognostic role of angiogenesis in breast cancer. *Anticancer Res*, 21, 4325-31 (2001)
93. Hashimoto, I., J. Kodama, N. Seki, A. Hongo, Y. Miyagi, M. Yoshinouchi & T. Kudo: Macrophage infiltration and angiogenesis in endometrial cancer. *Anticancer Res*, 20, 4853-6 (2000)
94. Leek, R. D., R. J. Landers, A. L. Harris & C. E. Lewis: Necrosis correlates with high vascular density and focal macrophage infiltration in invasive carcinoma of the breast. *Br J Cancer*, 79, 991-5 (1999)
95. McBride, W. H.: Phenotype and functions of intratumoral macrophages. *Biochim Biophys Acta*, 865, 27-41 (1986)
96. Moore, K. & W. H. McBride: Enhanced Fc receptor expression by a sub-population of murine intra-tumour macrophages following intravenous Corynebacterium parvum therapy. *Br J Cancer*, 47, 797-802 (1983)
97. Mantovani, A.: Tumor-associated macrophages in neoplastic progression: a paradigm for the *in vivo* function of chemokines. *Lab Invest*, 71, 5-16 (1994)
98. Burke, B., N. Tang, K. P. Corke, D. Tazzyman, K. Ameri, M. Wells & C. E. Lewis: Expression of HIF-1alpha by human macrophages: implications for the use of macrophages in hypoxia-regulated cancer gene therapy. *J Pathol*, 196, 204-12 (2002)
99. Lewis, C. E. & J. W. Pollard: Distinct role of macrophages in different tumor microenvironments. *Cancer Res*, 66, 605-12 (2006)
100. Condeelis, J. & J. W. Pollard: Macrophages: obligate partners for tumor cell migration, invasion, and metastasis. *Cell*, 124, 263-6 (2006)
101. Elgert, K. D., D. G. Alleva & D. W. Mullins: Tumor-induced immune dysfunction: the macrophage connection. *J. Leukoc. Biol.*, 64, 275-290 (1998)
102. Harmey, J. H., E. Dimitriadis, E. Kay, H. P. Redmond & D. Bouchier-Hayes: Regulation of macrophage production of vascular endothelial growth factor (VEGF) by hypoxia and transforming growth factor beta-1. *Ann Surg Oncol*, 5, 271-8 (1998)
103. Alleva, D. G., C. J. Burger & K. D. Elgert: Tumor-induced regulation of suppressor macrophage nitric oxide and TNF-alpha production. Role of tumor-derived IL-10, TGF-beta, and prostaglandin E2. *J Immunol*, 153, 1674-86 (1994)
104. Aso, H., K. Tamura, O. Yoshie, T. Nakamura, S. Kikuchi & N. Ishida: Impaired NK response of cancer patients to IFN-alpha but not to IL-2: correlation with serum immunosuppressive acidic protein (IAP) and role of suppressor macrophage. *Microbiol Immunol*, 36, 1087-97 (1992)
105. Naama, H. A., V. E. Mack, G. P. Smyth, P. P. Stapleton & J. M. Daly: Macrophage effector mechanisms in melanoma in an experimental study. *Arch Surg*, 136, 804-9 (2001)
106. al-Sarireh, B. & O. Eremin: Tumour-associated macrophages (TAMS): disordered function, immune suppression and progressive tumour growth. *J R Coll Surg Edinb*, 45, 1-16 (2000)
107. Park, Y. M., R. L. Anderson, D. R. Spitz & G. M. Hahn: Hypoxia and resistance to hydrogen peroxide confer resistance to tumor necrosis factor in murine L929 cells. *Radiat Res*, 131, 162-8 (1992)
108. Naldini, A., S. Cesari & V. Bocci: Effects of hypoxia on the cytotoxicity mediated by tumor necrosis factor-alpha. *Lymphokine Cytokine Res*, 13, 233-7 (1994)
109. Lynch, E. M., L. E. Sampson, A. A. Khalil, M. R. Horsman & D. J. Chaplin: Cytotoxic effect of tumour necrosis factor -alpha on sarcoma F cells at tumour relevant oxygen tensions. *Acta Oncol*, 34, 423-7 (1995)
110. Park, S. Y., H. Lee, J. Hur, S. Y. Kim, H. Kim, J. H. Park, S. Cha, S. S. Kang, G. J. Cho, W. S. Choi & K. Suk: Hypoxia induces nitric oxide production in mouse microglia via p38 mitogen-activated protein kinase pathway. *Brain Res Mol Brain Res*, 107, 9-16 (2002)
111. Lee, V. Y., D. S. McClintock, M. T. Santore, G. R. Budinger & N. S. Chandel: Hypoxia sensitizes cells to nitric oxide-induced apoptosis. *J Biol Chem*, 277, 16067-74 (2002)
112. Zhao, H., N. Dugas, C. Mathiot, A. Delmer, B. Dugas, F. Sigaux & J. P. Kolb: B-cell chronic lymphocytic leukemia cells express a functional inducible nitric oxide synthase displaying anti-apoptotic activity. *Blood*, 92, 1031-43 (1998)
113. Mannick, J. B., X. Q. Miao & J. S. Stamler: Nitric oxide inhibits Fas-induced apoptosis. *J Biol Chem*, 272, 24125-8 (1997)
114. Hegardt, P., B. Widegren & H. O. Sjogren: Nitric-oxide-dependent systemic immunosuppression in animals with progressively growing malignant gliomas. *Cell Immunol*, 200, 116-27 (2000)

115. Polverini, P. J. & S. J. Leibovich: Induction of neovascularization *in vivo* and endothelial proliferation *in vitro* by tumor-associated macrophages. *Lab Invest*, 51, 635-42 (1984)
116. Fukumura, D. & R. K. Jain: Role of nitric oxide in angiogenesis and microcirculation in tumors. *Cancer Metastasis Rev*, 17, 77-89 (1998)
117. Fukumura, D., S. Kashiwagi & R. K. Jain: The role of nitric oxide in tumour progression. *Nat Rev Cancer*, 6, 521-34 (2006)
118. Chang, C. I., J. C. Liao & L. Kuo: Macrophage arginase promotes tumor cell growth and suppresses nitric oxide-mediated tumor cytotoxicity. *Cancer Res*, 61, 1100-6 (2001)
119. Stout, R. D., C. Jiang, B. Matta, I. Tietzel, S. K. Watkins & J. Suttles: Macrophages sequentially change their functional phenotype in response to changes in microenvironmental influences. *J Immunol*, 175, 342-9 (2005)
120. Ben-Baruch, A.: Host microenvironment in breast cancer development: inflammatory cells, cytokines and chemokines in breast cancer progression: reciprocal tumor-microenvironment interactions. *Breast Cancer Res*, 5, 31-6 (2003)
121. Miles, D. W., L. C. Happerfield, M. S. Naylor, L. G. Bobrow, R. D. Rubens & F. R. Balkwill: Expression of tumour necrosis factor (TNF alpha) and its receptors in benign and malignant breast tissue. *Int J Cancer*, 56, 777-82 (1994)
122. Leek, R. D., R. Landers, S. B. Fox, F. Ng, A. L. Harris & C. E. Lewis: Association of tumour necrosis factor alpha and its receptors with thymidine phosphorylase expression in invasive breast carcinoma. *Br J Cancer*, 77, 2246-51 (1998)
123. Balkwill, F.: Tumor necrosis factor or tumor promoting factor? *Cytokine Growth Factor Rev*, 13, 135-41 (2002)
124. Partridge, M., D. Chantry, M. Turner & M. Feldmann: Production of interleukin-1 and interleukin-6 by human keratinocytes and squamous cell carcinoma cell lines. *J Invest Dermatol*, 96, 771-6 (1991)
125. Mann, E. A., J. D. Spiro, L. L. Chen & D. L. Kreutzer: Cytokine expression by head and neck squamous cell carcinomas. *Am J Surg*, 164, 567-73 (1992)
126. Mosmann, T. R., H. Cherwinski, M. W. Bond, M. A. Giedlin & R. L. Coffman: Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *J Immunol*, 136, 2348-57 (1986)
127. Wittke, F., R. Hoffmann, J. Buer, I. Dallmann, K. Oevermann, S. Sel, T. Wandert, A. Ganser & J. Atzpodien: Interleukin 10 (IL-10): an immunosuppressive factor and independent predictor in patients with metastatic renal cell carcinoma. *Br J Cancer*, 79, 1182-4 (1999)
128. Shim, K. S., K. H. Kim, W. S. Han & E. B. Park: Elevated serum levels of transforming growth factor-beta1 in patients with colorectal carcinoma: its association with tumor progression and its significant decrease after curative surgical resection. *Cancer*, 85, 554-61 (1999)
129. Bohlen, H., M. Kessler, M. Sextro, V. Diehl & H. Tesch: Poor clinical outcome of patients with Hodgkin's disease and elevated interleukin-10 serum levels. Clinical significance of interleukin-10 serum levels for Hodgkin's disease. *Ann Hematol*, 79, 110-3 (2000)
130. Villanueva, A., C. Garcia, A. B. Paules, M. Vicente, M. Megias, G. Reyes, P. de Villalonga, N. Agell, F. Lluís, O. Bachs & G. Capella: Disruption of the antiproliferative TGF-beta signaling pathways in human pancreatic cancer cells. *Oncogene*, 17, 1969-78 (1998)
131. Jong, H. S., H. S. Lee, T. Y. Kim, Y. H. Im, J. W. Park, N. K. Kim & Y. J. Bang: Attenuation of transforming growth factor beta-induced growth inhibition in human hepatocellular carcinoma cell lines by cyclin D1 overexpression. *Biochem Biophys Res Commun*, 292, 383-9 (2002)
132. Peng, Y., Y. Laouar, M. O. Li, E. A. Green & R. A. Flavell: TGF-beta regulates *in vivo* expansion of Foxp3-expressing CD4+CD25+ regulatory T cells responsible for protection against diabetes. *Proc Natl Acad Sci U S A*, 101, 4572-7 (2004)
133. Steinbrink, K., H. Jonuleit, G. Muller, G. Schuler, J. Knop & A. H. Enk: Interleukin-10-treated human dendritic cells induce a melanoma-antigen-specific anergy in CD8 (+) T cells resulting in a failure to lyse tumor cells. *Blood*, 93, 1634-42 (1999)
134. Qin, Z., G. Noffz, M. Mohaupt & T. Blankenstein: Interleukin-10 prevents dendritic cell accumulation and vaccination with granulocyte-macrophage colony-stimulating factor gene-modified tumor cells. *J Immunol*, 159, 770-6 (1997)
135. Wolff, H.: Expression of cyclooxygenase-2 in human lung carcinoma. *Cancer Res*, 58, 4997-5001 (1998)
136. Shono, T., P. J. Tofilon, J. M. Bruner, O. Owolabi & F. F. Lang: Cyclooxygenase-2 expression in human gliomas: prognostic significance and molecular correlations. *Cancer Res*, 61, 4375-4381 (2001)
137. Joki, T.: Expression of cyclooxygenase 2 (COX-2) in human glioma and *in vitro* inhibition by a specific COX-2 inhibitor, NS-398. *Cancer Res*, 60, 4926-4931 (2000)
138. Sanchez-Elsner, T., J. R. Ramirez, F. Rodriguez-Sanz, E. Varela, C. Bernabeu & L. M. Botella: A Cross-Talk Between Hypoxia and TGF-[beta] Orchestrates Erythropoietin Gene Regulation Through SP1 and Smads. *Journal of Molecular Biology*, 336, 9-24 (2004)
139. Dynlacht, J. R., M. Earles, J. Henthorn, Z. V. Roberts, E. W. Howard, J. D. Seno, D. Sparling & M. D. Story: Degradation of the nuclear matrix is a common element during radiation-induced apoptosis and necrosis. *Radiat Res*, 152, 590-603 (1999)
140. Rainaldi, G., A. Ferrante, P. L. Indovina & M. T. Santini: Induction of apoptosis or necrosis by ionizing radiation is dose-dependent in MG-63 osteosarcoma multicellular spheroids. *Anticancer Res*, 23, 2505-18 (2003)
141. Larmonier, N., D. Merino, A. Nicolas, D. Cathelin, A. Besson, A. Bateman, E. Solary, F. Martin, E. Katsanis & B. Bonnotte: Apoptotic, necrotic, or fused tumor cells: An equivalent source of antigen for dendritic cell loading. *Apoptosis* (2006)
142. Dillman, R., N. Barth, L. Vandermolen, K. Mahdavi, L. Beutel, C. de Leon, C. DePriest & S. Nayak: Autologous tumor cell line-derived vaccine for patient-specific treatment of advanced renal cell carcinoma. *Cancer Biother Radiopharm*, 19, 570-80 (2004)

143. Galili, U.: Autologous tumor vaccines processed to express alpha-gal epitopes: a practical approach to immunotherapy in cancer. *Cancer Immunol Immunother*, 53, 935-45 (2004)
144. Galetto, A., S. Buttiglieri, S. Forno, F. Moro, A. Mussa & L. Matera: Drug- and cell-mediated antitumor cytotoxicities modulate cross-presentation of tumor antigens by myeloid dendritic cells. *Anticancer Drugs*, 14, 833-43 (2003)
145. Santin, A. D., J. C. Hiserodt, J. Fruehauf, P. J. DiSaia, S. Pecorelli & G. A. Granger: Effects of irradiation on the expression of surface antigens in human ovarian cancer. *Gynecol Oncol*, 60, 468-74 (1996)
146. Morel, A., N. Fernandez, A. de La Coste, H. Haddada, M. Viguier, B. S. Polla, B. Antoine & A. Kahn: Gamma-ray irradiation induces B7.1 co-stimulatory molecule neoexpression in various murine tumor cells. *Cancer Immunol Immunother*, 46, 277-82 (1998)
147. Seo, A., F. Ishikawa, H. Nakano, H. Nakazaki, K. Kobayashi & T. Kakiuchi: Enhancement of B7-1 (CD80) expression on B-lymphoma cells by irradiation. *Immunology*, 96, 642-8 (1999)
148. Chakraborty, M., S. I. Abrams, K. Camphausen, K. Liu, T. Scott, C. N. Coleman & J. W. Hodge: Irradiation of tumor cells up-regulates Fas and enhances CTL lytic activity and CTL adoptive immunotherapy. *J Immunol*, 170, 6338-47 (2003)
149. Garnett, C. T., C. Palena, M. Chakraborty, K. Y. Tsang, J. Schlom & J. W. Hodge: Sublethal irradiation of human tumor cells modulates phenotype resulting in enhanced killing by cytotoxic T lymphocytes. *Cancer Res*, 64, 7985-94 (2004)
150. Reits, E. A., J. W. Hodge, C. A. Herberts, T. A. Groothuis, M. Chakraborty, E. K. Wansley, K. Camphausen, R. M. Luiten, A. H. de Ru, J. Neijssen, A. Griekspoor, E. Mesman, F. A. Verreck, H. Spits, J. Schlom, P. van Veelen & J. J. Neefjes: Radiation modulates the peptide repertoire, enhances MHC class I expression, and induces successful antitumor immunotherapy. *J Exp Med*, 203, 1259-71 (2006)
151. Newcomb, E. W., S. Demaria, Y. Lukyanov, Y. Shao, T. Schnee, N. Kawashima, L. Lan, J. K. Dewyngaert, D. Zagzag, W. H. McBride & S. C. Formenti: The combination of ionizing radiation and peripheral vaccination produces long-term survival of mice bearing established invasive GL261 gliomas. *Clin Cancer Res*, 12, 4730-7 (2006)
152. Lugade, A. A., J. P. Moran, S. A. Gerber, R. C. Rose, J. G. Frelinger & E. M. Lord: Local radiation therapy of B16 melanoma tumors increases the generation of tumor antigen-specific effector cells that traffic to the tumor. *J Immunol*, 174, 7516-23 (2005)
153. McBride, W. H.: Cytokine cascades in late normal tissue radiation responses. *Int J Radiat Oncol Biol Phys*, 33, 233-4 (1995)
154. Hong, J. H., C. S. Chiang, I. L. Campbell, J. R. Sun, H. R. Withers & W. H. McBride: Induction of acute phase gene expression by brain irradiation. *Int J Radiat Oncol Biol Phys*, 33, 619-26 (1995)
155. Hong, J. H., C. S. Chiang, C. Y. Tsao, P. Y. Lin, W. H. McBride & C. J. Wu: Rapid induction of cytokine gene expression in the lung after single and fractionated doses of radiation. *Int J Radiat Biol*, 75, 1421-7 (1999)
156. Rube, C. E., H. P. Rodemann & C. Rube: [The relevance of cytokines in the radiation-induced lung reaction. Experimental basis and clinical significance]. *Strahlenther Onkol*, 180, 541-9 (2004)
157. Anscher, M. S., I. R. Crocker & R. L. Jirtle: Transforming growth factor-beta 1 expression in irradiated liver. *Radiat Res*, 122, 77-85 (1990)
158. Barcellos-Hoff, M. H., R. Derynck, M. L. Tsang & J. A. Weatherbee: Transforming growth factor-beta activation in irradiated murine mammary gland. *J Clin Invest*, 93, 892-9 (1994)
159. Ruifrok, A. C., K. A. Mason, G. Lozano & H. D. Thames: Spatial and temporal patterns of expression of epidermal growth factor, transforming growth factor alpha and transforming growth factor beta 1-3 and their receptors in mouse jejunum after radiation treatment. *Radiat Res*, 147, 1-12 (1997)
160. McBride, W. H., C. S. Chiang, J. L. Olson, C. C. Wang, J. H. Hong, F. Pajonk, G. J. Dougherty, K. S. Iwamoto, M. Pervan & Y. P. Liao: A sense of danger from radiation. *Radiat Res*, 162, 1-19 (2004)
161. Lumniczky, K., S. Desaknai, L. Mangel, B. Szende, H. Hamada, E. J. Hidvegi & G. Safrany: Local tumor irradiation augments the antitumor effect of cytokine-producing autologous cancer cell vaccines in a murine glioma model. *Cancer Gene Ther*, 9, 44-52 (2002)
162. Teitz-Tennenbaum, S., Q. Li, S. Rynkiewicz, F. Ito, M. A. Davis, C. J. McGinn & A. E. Chang: Radiotherapy potentiates the therapeutic efficacy of intratumoral dendritic cell administration. *Cancer Res*, 63, 8466-75 (2003)
163. Chakraborty, M., S. I. Abrams, C. N. Coleman, K. Camphausen, J. Schlom & J. W. Hodge: External beam radiation of tumors alters phenotype of tumor cells to render them susceptible to vaccine-mediated T-cell killing. *Cancer Res*, 64, 4328-37 (2004)
164. Kudo-Saito, C., J. Schlom, K. Camphausen, C. N. Coleman & J. W. Hodge: The requirement of multimodal therapy (vaccine, local tumor radiation, and reduction of suppressor cells) to eliminate established tumors. *Clin Cancer Res*, 11, 4533-44 (2005)
165. Hewitt, H. B., E. Blake & E. H. Proter: The effect of lethally irradiated cells on the transplantability of murine tumours. *Br J Cancer*, 28, 123-35 (1973)
166. Hewitt, H. B. & E. R. Blake: Failure of preoperative C. parvum vaccine to modify secondary disease following excision of two non-immunogenic murine carcinomas. *Br J Cancer*, 38, 219-23 (1978)
167. Tsai, C. H., J. H. Hong, K. F. Hsieh, H. W. Hsiao, W. L. Chuang, C. C. Lee, W. H. McBride & C. S. Chiang: Tetracycline-regulated intratumoral expression of interleukin-3 enhances the efficacy of radiation therapy for murine prostate cancer. *Cancer Gene Ther* (2006)
168. Wenger, F. A., C. A. Jacobi, J. Zieren, W. Docke, H. D. Volk & J. M. Muller: Tumor size and lymph-node status in pancreatic carcinoma - is there a correlation to the preoperative immune function? *Langenbecks Arch Surg*, 384, 473-8 (1999)
169. Hallahan, D., J. Kuchibhotla & C. Wyble: Cell adhesion molecules mediate radiation-induced leukocyte

The tumor microenvironment and immunity

- adhesion to the vascular endothelium. *Cancer Res*, 56, 5150-5 (1996)
170. Quarmby, S., R. D. Hunter & S. Kumar: Irradiation induced expression of CD31, ICAM-1 and VCAM-1 in human microvascular endothelial cells. *Anticancer Res*, 20, 3375-81 (2000)
171. Heckmann, M., K. Douwes, R. Peter & K. Degitz: Vascular activation of adhesion molecule mRNA and cell surface expression by ionizing radiation. *Exp Cell Res*, 238, 148-54 (1998)
172. Onoda, J. M., S. S. Kantak, M. P. Piechocki & C. A. Diglio: Low dose radiation induces endothelial cell eicosanoid metabolism and cytoskeletal rearrangement. *Adv Exp Med Biol*, 400B, 879-87 (1997)
173. Prabhakarandian, B., D. J. Goetz, R. A. Swerlick, X. Chen & M. F. Kiani: Expression and functional significance of adhesion molecules on cultured endothelial cells in response to ionizing radiation. *Microcirculation*, 8, 355-64 (2001)
174. Mao, X. W.: A quantitative study of the effects of ionizing radiation on endothelial cells and capillary-like network formation. *Technol Cancer Res Treat*, 5, 127-34 (2006)
175. Tsai, J. H., S. Makonnen, M. Feldman, C. M. Sehgal, A. Maity & W. M. Lee: Ionizing radiation inhibits tumor neovascularization by inducing ineffective angiogenesis. *Cancer Biol Ther*, 4, 1395-1400 (2005)
176. Garcia-Barros, M., F. Paris, C. Cordon-Cardo, D. Lyden, S. Rafii, A. Haimovitz-Friedman, Z. Fuks & R. Kolesnick: Tumor response to radiotherapy regulated by endothelial cell apoptosis. *Science*, 300, 1155-9 (2003)
177. Kalofonos, H., G. Rowlinson & A. A. Epenetos: Enhancement of monoclonal antibody uptake in human colon tumor xenografts following irradiation. *Cancer Res*, 50, 159-63 (1990)
178. Barcellos-Hoff, M. H.: Radiation-induced transforming growth factor beta and subsequent extracellular matrix reorganization in murine mammary gland. *Cancer Res*, 53, 3880-6 (1993)
179. Znati, C. A., M. Rosenstein, Y. Boucher, M. W. Epperly, W. D. Bloomer & R. K. Jain: Effect of radiation on interstitial fluid pressure and oxygenation in a human tumor xenograft. *Cancer Res*, 56, 964-68 (1996)
180. Znati, C. A., M. Rosenstein, T. D. McKee, E. Brown, D. Turner, W. D. Bloomer, S. Watkins, R. K. Jain & Y. Boucher: Irradiation reduces interstitial fluid transport and increases the collagen content in tumors. *Clin Cancer Res*, 9, 5508-13 (2003)
181. Crockart, N., B. F. Jordan, C. Baudelet, R. Ansiaux, P. Sonveaux, V. Gregoire, N. Beghein, J. DeWever, C. Bouzin, O. Feron & B. Gallez: Early reoxygenation in tumors after irradiation: determining factors and consequences for radiotherapy regimens using daily multiple fractions. *Int J Radiat Oncol Biol Phys*, 63, 901-10 (2005)
182. Tsai, C. H., F. H. Chen, C. C. Wang, H. L. Huang, S. M. Jung, C. J. Wu, C. C. Lee, W. H. McBride, C. S. Chiang & J. H. Hong: Macrophages from irradiated tumors express higher levels of iNOS, Arginase-I, and promote tumor growth. (submitted)
183. Mantovani, A., A. Sica, S. Sozzani, P. Allavena, A. Vecchi & M. Locati: The chemokine system in diverse forms of macrophage activation and polarization. *Trends Immunol*, 25, 677-86 (2004)
184. Philippe, J., H. Louagie, H. Thierens, A. Vral, M. Cornelissen & L. De Ridder: Quantification of apoptosis in lymphocyte subsets and effect of apoptosis on apparent expression of membrane antigens. *Cytometry*, 29, 242-9 (1997)
185. Belka, C., H. Ottinger, E. Kreuzfelder, M. Weinmann, M. Lindemann, A. Lepple-Wienhues, W. Budach, H. Grosse-Wilde & M. Bamberg: Impact of localized radiotherapy on blood immune cells counts and function in humans. *Radiother Oncol*, 50, 199-204 (1999)
186. North, R. J.: Radiation-induced, immunologically mediated regression of an established tumor as an example of successful therapeutic immunomanipulation. Preferential elimination of suppressor T cells allows sustained production of effector T cells. *J Exp Med*, 164, 1652-66 (1986)
187. Awwad, M. & R. J. North: Sublethal, whole-body ionizing irradiation can be tumor promotive or tumor destructive depending on the stage of development of underlying antitumor immunity. *Cancer Immunol Immunother*, 26, 55-60 (1988)
188. Hellstrom, K. E., I. Hellstrom, J. A. Kant & J. D. Tamerius: Regression and inhibition of sarcoma growth by interference with a radiosensitive T-cell population. *J Exp Med*, 148, 799-804 (1978)
189. Tilkin, A. F., N. Schaaf-Lafontaine, A. Van Acker, M. Boccadoro & J. Urbain: Reduced tumor growth after low-dose irradiation or immunization against blastic suppressor T cells. *Proc Natl Acad Sci U S A*, 78, 1809-12 (1981)
190. Yu, P., Y. Lee, W. Liu, T. Krausz, A. Chong, H. Schreiber & Y. X. Fu: Intratumor depletion of CD4+ cells unmasks tumor immunogenicity leading to the rejection of late-stage tumors. *J Exp Med*, 201, 779-91 (2005)
191. Liao, Y. P., C. C. Wang, L. H. Butterfield, J. S. Economou, A. Ribas, W. S. Meng, K. S. Iwamoto & W. H. McBride: Ionizing radiation affects human MART-1 melanoma antigen processing and presentation by dendritic cells. *J Immunol*, 173, 2462-9 (2004)
192. Chen, Z., D. Xia, X. Bi, A. Saxena, N. Sidhu, A. El-Gayed & J. Xiang: Combined radiation therapy and dendritic cell vaccine for treating solid tumors with liver micro-metastasis. *J Gene Med* (2004)
193. Younes, E., G. P. Haas, B. Dezso, E. Ali, R. L. Maughan, M. A. Kukuruga, E. Montecillo, J. E. Pontes & G. G. Hillman: Local tumor irradiation augments the response to IL-2 therapy in a murine renal adenocarcinoma. *Cell Immunol*, 165, 243-51 (1995)
194. Nishisaka, N., R. F. Jones, P. Morse, K. Kuratsukuri, R. Romanowski, C. Y. Wang & G. P. Haas: Inhibition of lung metastases of murine renal cell carcinoma by the combination of radiation and interferon-alpha-producing tumor cell vaccine. *Cytokines Cell Mol Ther*, 6, 199-206 (2000)
195. Lee, J., J. P. Moran, B. M. Fenton, C. J. Koch, J. G. Frelinger, P. C. Keng & E. M. Lord: Alteration of tumour response to radiation by interleukin-2 gene transfer. *Br J Cancer*, 82, 937-44 (2000)
196. McBride, W. H., G. D. Dougherty, A. E. Wallis, J. S. Economou & C. S. Chiang: Interleukin-3 in gene therapy of cancer. *Folia Biol (Praha)*, 40, 62-73 (1994)

197. Chiang, C. S., J. H. Hong, Y. C. Wu, W. H. McBride & G. J. Dougherty: Combining radiation therapy with interleukin-3 gene immunotherapy. *Cancer Gene Ther*, 7, 1172-8 (2000)
198. Chiang, C. S., R. G. Syljuasen, J. H. Hong, A. Wallis, G. J. Dougherty & W. H. McBride: Effects of IL-3 gene expression on tumor response to irradiation *in vitro* and *in vivo*. *Cancer Res*, 57, 3899-903 (1997)
199. Gulley, J. L., P. M. Arlen, A. Bastian, S. Morin, J. Marte, P. Beetham, K. Y. Tsang, J. Yokokawa, J. W. Hodge, C. Menard, K. Camphausen, C. N. Coleman, F. Sullivan, S. M. Steinberg, J. Schlom & W. Dahut: Combining a recombinant cancer vaccine with standard definitive radiotherapy in patients with localized prostate cancer. *Clin Cancer Res*, 11, 3353-62 (2005)
200. Lake, R. A. & B. W. Robinson: Immunotherapy and chemotherapy--a practical partnership. *Nat Rev Cancer*, 5, 397-405 (2005)
201. Emens, L. A. & E. M. Jaffee: Leveraging the activity of tumor vaccines with cytotoxic chemotherapy. *Cancer Res*, 65, 8059-64 (2005)
202. van der Most, R. G., A. Currie, B. W. Robinson & R. A. Lake: Cranking the immunologic engine with chemotherapy: using context to drive tumor antigen cross-presentation towards useful antitumor immunity. *Cancer Res*, 66, 601-4 (2006)
203. Dudley, M. E., J. R. Wunderlich, J. C. Yang, R. M. Sherry, S. L. Topalian, N. P. Restifo, R. E. Royal, U. Kammula, D. E. White, S. A. Mavroukakis, L. J. Rogers, G. J. Gracia, S. A. Jones, D. P. Mangiameli, M. M. Pelletier, J. Gea-Banacloche, M. R. Robinson, D. M. Berman, A. C. Filie, A. Abati & S. A. Rosenberg: Adoptive cell transfer therapy following non-myeloablative but lymphodepleting chemotherapy for the treatment of patients with refractory metastatic melanoma. *J Clin Oncol*, 23, 2346-57 (2005)
204. Polak, L. & J. L. Turk: Reversal of immunological tolerance by cyclophosphamide through inhibition of suppressor cell activity. *Nature*, 249, 654-6 (1974)
205. North, R. J.: Cyclophosphamide-facilitated adoptive immunotherapy of an established tumor depends on elimination of tumor-induced suppressor T cells. *J Exp Med*, 155, 1063-74 (1982)
206. Hoover, S. K., S. K. Barrett, T. M. Turk, T. C. Lee & H. D. Bear: Cyclophosphamide and abrogation of tumor-induced suppressor T cell activity. *Cancer Immunol Immunother*, 31, 121-7 (1990)
207. Ghiringhelli, F., N. Larmonier, E. Schmitt, A. Parcellier, D. Cathelin, C. Garrido, B. Chauffert, E. Solary, B. Bonnotte & F. Martin: CD4+CD25+ regulatory T cells suppress tumor immunity but are sensitive to cyclophosphamide which allows immunotherapy of established tumors to be curative. *Eur J Immunol*, 34, 336-44 (2004)
208. Berd, D. & M. J. Mastrangelo: Effect of low dose cyclophosphamide on the immune system of cancer patients: depletion of CD4+, 2H4+ suppressor-inducer T-cells. *Cancer Res*, 48, 1671-5 (1988)
209. Schiavoni, G., F. Mattei, T. Di Pucchio, S. M. Santini, L. Bracci, F. Belardelli & E. Proietti: Cyclophosphamide induces type I interferon and augments the number of CD44 (hi) T lymphocytes in mice: implications for strategies of chemoimmunotherapy of cancer. *Blood*, 95, 2024-30 (2000)
210. Loeffler, M., J. A. Kruger & R. A. Reisfeld: Immunostimulatory effects of low-dose cyclophosphamide are controlled by inducible nitric oxide synthase. *Cancer Res*, 65, 5027-30 (2005)
211. Proietti, E., G. Greco, B. Garrone, S. Baccharini, C. Mauri, M. Venditti, D. Carlei & F. Belardelli: Importance of cyclophosphamide-induced bystander effect on T cells for a successful tumor eradication in response to adoptive immunotherapy in mice. *J Clin Invest*, 101, 429-41 (1998)
212. Nowak, A. K., B. W. Robinson & R. A. Lake: Gemcitabine exerts a selective effect on the humoral immune response: implications for combination chemoimmunotherapy. *Cancer Res*, 62, 2353-8 (2002)
213. Suzuki, E., V. Kapoor, A. S. Jassar, L. R. Kaiser & S. M. Albelda: Gemcitabine selectively eliminates splenic Gr-1+/CD11b+ myeloid suppressor cells in tumor-bearing animals and enhances antitumor immune activity. *Clin Cancer Res*, 11, 6713-21 (2005)
214. Nowak, A. K., B. W. Robinson & R. A. Lake: Synergy between chemotherapy and immunotherapy in the treatment of established murine solid tumors. *Cancer Res*, 63, 4490-6 (2003)
215. Ben-Hur, H., G. Kossoy, J. Zandbank & I. Zusman: Response of the immune system of mammary tumor-bearing rats to cyclophosphamide and soluble low-molecular-mass tumor-associated antigens: rate of lymphoid infiltration and distribution of T lymphocytes in tumors. *Int J Mol Med*, 9, 425-30 (2002)
216. Nakashima, H., A. Tasaki, M. Kubo, H. Kuroki, K. Matsumoto, M. Tanaka, M. Nakamura, T. Morisaki & M. Katano: Effects of docetaxel on antigen presentation-related functions of human monocyte-derived dendritic cells. *Cancer Chemother Pharmacol*, 55, 479-87 (2005)
217. Browder, T., C. E. Butterfield, B. M. Kraling, B. Shi, B. Marshall, M. S. O'Reilly & J. Folkman: Antiangiogenic scheduling of chemotherapy improves efficacy against experimental drug-resistant cancer. *Cancer Res*, 60, 1878-86 (2000)
218. Bocci, G., K. C. Nicolaou & R. S. Kerbel: Protracted low-dose effects on human endothelial cell proliferation and survival *in vitro* reveal a selective antiangiogenic window for various chemotherapeutic drugs. *Cancer Res*, 62, 6938-43 (2002)
219. Drevs, J., J. Fakler, S. Eisele, M. Medinger, G. Bing, N. Esser, D. Marme & C. Unger: Antiangiogenic potency of various chemotherapeutic drugs for metronomic chemotherapy. *Anticancer Res*, 24, 1759-63 (2004)
220. Jassar, A. S., E. Suzuki, V. Kapoor, J. Sun, M. B. Silverberg, L. Cheung, M. D. Burdick, R. M. Strieter, L. M. Ching, L. R. Kaiser & S. M. Albelda: Activation of tumor-associated macrophages by the vascular disrupting agent 5,6-dimethylxanthenone-4-acetic acid induces an effective CD8+ T-cell-mediated antitumor immune response in murine models of lung cancer and mesothelioma. *Cancer Res*, 65, 11752-61 (2005)
221. Heath, W. R., G. T. Belz, G. M. Behrens, C. M. Smith, S. P. Forehan, I. A. Parish, G. M. Davey, N. S. Wilson, F. R. Carbone & J. A. Villadangos: Cross-presentation, dendritic cell subsets, and the generation of immunity to cellular antigens. *Immunol Rev*, 199, 9-26 (2004)

222. Nowak, A. K., R. A. Lake, A. L. Marzo, B. Scott, W. R. Heath, E. J. Collins, J. A. Frelinger & B. W. Robinson: Induction of tumor cell apoptosis *in vivo* increases tumor antigen cross-presentation, cross-priming rather than cross-tolerizing host tumor-specific CD8 T cells. *J Immunol*, 170, 4905-13 (2003)
223. Mullins, D. W., C. J. Burger & K. D. Elgert: Paclitaxel enhances macrophage IL-12 production in tumor-bearing hosts through nitric oxide. *J Immunol*, 162, 6811-8 (1999)
224. Byrd-Leifer, C. A., E. F. Block, K. Takeda, S. Akira & A. Ding: The role of MyD88 and TLR4 in the LPS-mimetic activity of Taxol. *Eur J Immunol*, 31, 2448-57 (2001)
225. Joo, H. G.: Altered maturation of dendritic cells by taxol, an anticancer drug. *J Vet Sci*, 4, 229-34 (2003)
226. Coral, S., L. Sigalotti, M. Altomonte, A. Engelsberg, F. Colizzi, I. Cattarossi, E. Maraskovsky, E. Jager, B. Seliger & M. Maio: 5-aza-2'-deoxycytidine-induced expression of functional cancer testis antigens in human renal cell carcinoma: immunotherapeutic implications. *Clin Cancer Res*, 8, 2690-5 (2002)
227. Ohtsukasa, S., S. Okabe, H. Yamashita, T. Iwai & K. Sugihara: Increased expression of CEA and MHC class I in colorectal cancer cell lines exposed to chemotherapy drugs. *J Cancer Res Clin Oncol*, 129, 719-26 (2003)
228. AbdAlla, E. E., G. E. Blair, R. A. Jones, H. M. Sue-Ling & D. Johnston: Mechanism of synergy of levamisole and fluorouracil: induction of human leukocyte antigen class I in a colorectal cancer cell line. *J Natl Cancer Inst*, 87, 489-96 (1995)
229. Rad, A. N., G. Pollara, S. M. Sohaib, C. Chiang, B. M. Chain & D. R. Katz: The differential influence of allogeneic tumor cell death via DNA damage on dendritic cell maturation and antigen presentation. *Cancer Res*, 63, 5143-50 (2003)
230. Vereecque, R., A. Saudemont & B. Quesnel: Cytosine arabinoside induces co-stimulatory molecule expression in acute myeloid leukemia cells. *Leukemia*, 18, 1223-30 (2004)
231. Do, Y., V. L. Hegde, P. S. Nagarkatti & M. Nagarkatti: Bryostatin-1 enhances the maturation and antigen-presenting ability of murine and human dendritic cells. *Cancer Res*, 64, 6756-65 (2004)
232. Bergmann-Leitner, E. S. & S. I. Abrams: Treatment of human colon carcinoma cell lines with anti-neoplastic agents enhances their lytic sensitivity to antigen-specific CD8⁺ cytotoxic T lymphocytes. *Cancer Immunol Immunother*, 50, 445-55 (2001)
233. Correale, P., M. T. Del Vecchio, G. Di Genova, G. G. Savellini, M. La Placa, C. Terrosi, M. Vestri, R. Urso, F. Lemonnier, A. Aquino, E. Bonmassar, G. Giorgi, G. Francini & M. G. Cusi: 5-fluorouracil-based chemotherapy enhances the antitumor activity of a thymidylate synthase-directed polyepitopic peptide vaccine. *J Natl Cancer Inst*, 97, 1437-45 (2005)
234. Yang, S. & F. G. Haluska: Treatment of melanoma with 5-fluorouracil or dacarbazine *in vitro* sensitizes cells to antigen-specific CTL lysis through perforin/granzyme- and Fas-mediated pathways. *J Immunol*, 172, 4599-608 (2004)
235. Frost, P. J., L. H. Butterfield, V. B. Dissette, J. S. Economou & B. Bonavida: Immunosenitization of melanoma tumor cells to non-MHC Fas-mediated killing by MART-1-specific CTL cultures. *J Immunol*, 166, 3564-73 (2001)
236. Machiels, J. P., R. T. Reilly, L. A. Emens, A. M. Ercolini, R. Y. Lei, D. Weintraub, F. I. Okoye & E. M. Jaffee: Cyclophosphamide, doxorubicin, and paclitaxel enhance the antitumor immune response of granulocyte/macrophage-colony stimulating factor-secreting whole-cell vaccines in HER-2/neu tolerized mice. *Cancer Res*, 61, 3689-97 (2001)
237. Pressley, J. S. & K. D. Elgert: Post-chemotherapeutic administration of interleukin-12 retards tumor growth and enhances immune cell function: combination therapy using paclitaxel and IL-12. *Cancer Invest*, 24, 351-9 (2006)
238. Allendorf, J. D., M. Bessler, R. L. Whelan, M. Trokel, D. A. Laird, M. B. Terry & M. R. Treat: Postoperative immune function varies inversely with the degree of surgical trauma in a murine model. *Surg Endosc*, 11, 427-30 (1997)
239. Ordemann, J., C. A. Jacobi, W. Schwenk, R. Stosslein & J. M. Muller: Cellular and humoral inflammatory response after laparoscopic and conventional colorectal resections. *Surg Endosc*, 15, 600-8 (2001)
240. Whelan, R. L., M. Franklin, S. D. Holubar, J. Donahue, R. Fowler, C. Munger, J. Doorman, J. E. Balli, J. Glass, J. J. Gonzalez, M. Bessler, H. Xie & M. Treat: Postoperative cell mediated immune response is better preserved after laparoscopic vs open colorectal resection in humans. *Surg Endosc*, 17, 972-8 (2003)
241. Bolla, G. & G. Tuzzato: Immunologic postoperative competence after laparoscopy versus laparotomy. *Surg Endosc*, 17, 1247-50 (2003)
242. Leung, K. L., K. S. Tsang, M. H. Ng, K. J. Leung, P. B. Lai, J. F. Lee & W. Y. Lau: Lymphocyte subsets and natural killer cell cytotoxicity after laparoscopically assisted resection of rectosigmoid carcinoma. *Surg Endosc*, 17, 1305-10 (2003)
243. Wichmann, M. W., T. P. Huttel, H. Winter, F. Spelsberg, M. K. Angele, M. M. Heiss & K. W. Jauch: Immunological effects of laparoscopic vs open colorectal surgery: a prospective clinical study. *Arch Surg*, 140, 692-7 (2005)
244. Whelan, R. L.: Laparotomy, laparoscopy, cancer, and beyond. *Surg Endosc*, 15, 110-5 (2001)
245. Wouters, B. G., M. Koritzinsky, R. K. Chiu, J. Theys, J. Buijsen & P. Lambin: Modulation of cell death in the tumor microenvironment. *Semin Radiat Oncol*, 13, 31-41 (2003)
246. Crockart, N., K. Radermacher, B. F. Jordan, C. Baudalet, G. O. Cron, V. Gregoire, N. Beghein, C. Bouzin, O. Feron & B. Gallez: Tumor radiosensitization by antiinflammatory drugs: evidence for a new mechanism involving the oxygen effect. *Cancer Res*, 65, 7911-6 (2005)
247. Hur, E., H. H. Kim, S. M. Choi, J. H. Kim, S. Yim, H. J. Kwon, Y. Choi, D. K. Kim, M. O. Lee & H. Park: Reduction of hypoxia-induced transcription through the repression of hypoxia-inducible factor-1 α /aryl hydrocarbon receptor nuclear translocator DNA binding by the 90-kDa heat-shock protein inhibitor radicicol. *Mol Pharmacol*, 62, 975-82 (2002)
248. Mabeesh, N. J., D. Escuin, T. M. LaVallee, V. S. Pribluda, G. M. Swartz, M. S. Johnson, M. T. Willard, H. Zhong, J. W. Simons & P. Giannakakou: 2ME2 inhibits

The tumor microenvironment and immunity

- tumor growth and angiogenesis by disrupting microtubules and dysregulating HIF. *Cancer Cell*, 3, 363-75 (2003)
249. Fang, J., Z. Cao, Y. C. Chen, E. Reed & B. H. Jiang: 9-beta-D-arabinofuranosyl-2-fluoroadenine inhibits expression of vascular endothelial growth factor through hypoxia-inducible factor-1 in human ovarian cancer cells. *Mol Pharmacol*, 66, 178-86 (2004)
250. Isaacs, J. S., Y. J. Jung, E. G. Mimnaugh, A. Martinez, F. Cuttitta & L. M. Neckers: Hsp90 regulates a von Hippel Lindau-independent hypoxia-inducible factor-1 alpha-degradative pathway. *J Biol Chem*, 277, 29936-44 (2002)
251. Jones, M. K., I. L. Szabo, H. Kawanaka, S. S. Husain & A. S. Tarnawski: von Hippel Lindau tumor suppressor and HIF-1alpha: new targets of NSAIDs inhibition of hypoxia-induced angiogenesis. *Faseb J*, 16, 264-6 (2002)
252. Rapisarda, A., R. H. Shoemaker & G. Melillo: Targeting topoisomerase I to inhibit hypoxia inducible factor 1. *Cell Cycle*, 3, 172-5 (2004)
253. Liu, X. H., A. Kirschenbaum, M. Lu, S. Yao, A. Dosoretz, J. F. Holland & A. C. Levine: Prostaglandin E2 induces hypoxia-inducible factor-1alpha stabilization and nuclear localization in a human prostate cancer cell line. *J Biol Chem*, 277, 50081-6 (2002)
254. Rapisarda, A., B. Uranchimeg, D. A. Scudiero, M. Selby, E. A. Sausville, R. H. Shoemaker & G. Melillo: Identification of small molecule inhibitors of hypoxia-inducible factor 1 transcriptional activation pathway. *Cancer Res*, 62, 4316-24 (2002)
255. Chau, N. M., P. Rogers, W. Aherne, V. Carroll, I. Collins, E. McDonald, P. Workman & M. Ashcroft: Identification of novel small molecule inhibitors of hypoxia-inducible factor-1 that differentially block hypoxia-inducible factor-1 activity and hypoxia-inducible factor-1alpha induction in response to hypoxic stress and growth factors. *Cancer Res*, 65, 4918-28 (2005)
256. Tan, C., R. G. de Noronha, A. J. Roecker, B. Pyrzynska, F. Khwaja, Z. Zhang, H. Zhang, Q. Teng, A. C. Nicholson, P. Giannakakou, W. Zhou, J. J. Olson, M. M. Pereira, K. C. Nicolaou & E. G. Van Meir: Identification of a novel small-molecule inhibitor of the hypoxia-inducible factor 1 pathway. *Cancer Res*, 65, 605-12 (2005)
257. Kong, D., E. J. Park, A. G. Stephen, M. Calvani, J. H. Cardellina, A. Monks, R. J. Fisher, R. H. Shoemaker & G. Melillo: Echinomycin, a small-molecule inhibitor of hypoxia-inducible factor-1 DNA-binding activity. *Cancer Res*, 65, 9047-55 (2005)
258. Kurabayashi, A., M. Furihata, M. Matsumoto, H. Hayashi & Y. Ohtsuki: Distribution of tumor-infiltrating dendritic cells in human non-small cell lung carcinoma in relation to apoptosis. *Pathol Int*, 54, 302-10 (2004)
259. Gan, Y., J. L. Au, J. Lu & M. G. Wientjes: Antiproliferative and cytotoxic effects of geldanamycin, cytochalasin E, suramin and thiocetazone in human prostate xenograft tumor histocultures. *Pharm Res*, 15, 1760-6 (1998)
260. Lin, S., S. C. Tsai, C. C. Lee, B. W. Wang, J. Y. Liou & K. G. Shyu: Berberine inhibits HIF-1alpha expression via enhanced proteolysis. *Mol Pharmacol*, 66, 612-9 (2004)
261. Kojima, H., H. Gu, S. Nomura, C. C. Caldwell, T. Kobata, P. Carmeliet, G. L. Semenza & M. V. Sitkovsky: Abnormal B lymphocyte development and autoimmunity in hypoxia-inducible factor 1alpha -deficient chimeric mice. *Proc Natl Acad Sci U S A*, 99, 2170-4 (2002)
262. Kojima, H., M. V. Sitkovsky & M. Cascalho: HIF-1 alpha deficiency perturbs T and B cell functions. *Curr Pharm Des*, 9, 1827-32 (2003)
263. Makino, Y., H. Nakamura, E. Ikeda, K. Ohnuma, K. Yamauchi, Y. Yabe, L. Poellinger, Y. Okada, C. Morimoto & H. Tanaka: Hypoxia-inducible factor regulates survival of antigen receptor-driven T cells. *J Immunol*, 171, 6534-40 (2003)
264. Oda, T., K. Hirota, K. Nishi, S. Takabuchi, S. Oda, H. Yamada, T. Arai, K. Fukuda, T. Kita, T. Adachi, G. L. Semenza & R. Nohara: Activation of hypoxia-inducible factor 1 during macrophage differentiation. *Am J Physiol Cell Physiol*, 291, C104-13 (2006)
265. Hopfl, G., O. Ogunshola & M. Gassmann: HIFs and tumors--causes and consequences. *Am J Physiol Regul Integr Comp Physiol*, 286, R608-23 (2004)
266. Hellwig-Burgel, T., D. P. Stiehl, A. E. Wagner, E. Metzgen & W. Jelkmann: Review: hypoxia-inducible factor-1 (HIF-1): a novel transcription factor in immune reactions. *J Interferon Cytokine Res*, 25, 297-310 (2005)
267. Cramer, T., Y. Yamanishi, B. E. Clausen, I. Forster, R. Pawlinski, N. Mackman, V. H. Haase, R. Jaenisch, M. Corr, V. Nizet, G. S. Firestein, H. P. Gerber, N. Ferrara & R. S. Johnson: HIF-1alpha is essential for myeloid cell-mediated inflammation. *Cell*, 112, 645-57 (2003)
268. Albina, J. E., B. Mastrofrancesco, J. A. Vessella, C. A. Louis, W. L. Henry, Jr. & J. S. Reichner: HIF-1 expression in healing wounds: HIF-1alpha induction in primary inflammatory cells by TNF-alpha. *Am J Physiol Cell Physiol*, 281, C1971-7 (2001)
269. Sun, X., J. R. Kanwar, E. Leung, K. Lehnert, D. Wang & G. W. Krissansen: Gene transfer of antisense hypoxia inducible factor-1 alpha enhances the therapeutic efficacy of cancer immunotherapy. *Gene Ther*, 8, 638-45 (2001)
270. Lewis, C. & C. Murdoch: Macrophage responses to hypoxia: implications for tumor progression and anti-cancer therapies. *Am J Pathol*, 167, 627-35 (2005)
271. Peyssonnaud, C., V. Datta, T. Cramer, A. Doedens, E. A. Theodorakis, R. L. Gallo, N. Hurtado-Ziola, V. Nizet & R. S. Johnson: HIF-1alpha expression regulates the bactericidal capacity of phagocytes. *J Clin Invest*, 115, 1806-15 (2005)
272. Lahat, N., M. A. Rahat, M. Ballan, L. Weiss-Cerem, M. Engelmayer & H. Bitterman: Hypoxia reduces CD80 expression on monocytes but enhances their LPS-stimulated TNF-alpha secretion. *J Leukoc Biol*, 74, 197-205 (2003)
273. Bosco, M. C., M. Puppo, S. Pastorino, Z. Mi, G. Melillo, S. Massazza, A. Rapisarda & L. Varesio: Hypoxia selectively inhibits monocyte chemoattractant protein-1 production by macrophages. *J Immunol*, 172, 1681-90 (2004)
274. Rahat, M. A., B. Marom, H. Bitterman, L. Weiss-Cerem, A. Kinarty & N. Lahat: Hypoxia reduces the output of matrix metalloproteinase-9 (MMP-9) in monocytes by inhibiting its secretion and elevating membranal association. *J Leukoc Biol*, 79, 706-18 (2006)
275. Schlichting, C. L., W. D. Schareck & M. Weis: Renal ischemia-reperfusion injury: new implications of dendritic

- cell-endothelial cell interactions. *Transplant Proc*, 38, 670-3 (2006)
276. Meehan, R., U. Duncan, L. Neale, G. Taylor, H. Muchmore, N. Scott, K. Ramsey, E. Smith, P. Rock, R. Goldblum & *et al.*: Operation Everest II: alterations in the immune system at high altitudes. *J Clin Immunol*, 8, 397-406 (1988)
277. Maurus, C. F., M. K. Schneider, D. Schmidt, G. Zund & J. D. Seebach: Activation of human microvascular endothelial cells with TNF-alpha and hypoxia/reoxygenation enhances NK-cell adhesion, but not NK-Cytotoxicity. *Transplantation*, 81, 1204-11 (2006)
278. Helmlinger, G., F. Yuan, M. Dellian & R. K. Jain: Interstitial pH and pO₂ gradients in solid tumors *in vivo*: high-resolution measurements reveal a lack of correlation. *Nat Med*, 3, 177-82 (1997)
279. Fukumura, D., L. Xu, Y. Chen, T. Gohongi, B. Seed & R. K. Jain: Hypoxia and acidosis independently up-regulate vascular endothelial growth factor transcription in brain tumors *in vivo*. *Cancer Res*, 61, 6020-4 (2001)
280. Mekhail, K., M. Khacho, L. Gunaratnam & S. Lee: Oxygen sensing by H⁺: implications for HIF and hypoxic cell memory. *Cell Cycle*, 3, 1027-9 (2004)
281. Cecchi, A., A. Hulikova, J. Pastorek, S. Pastorekova, A. Scozzafava, J. Y. Winum, J. L. Montero & C. T. Supuran: Carbonic anhydrase inhibitors. Design of fluorescent sulfonamides as probes of tumor-associated carbonic anhydrase IX that inhibit isozyme IX-mediated acidification of hypoxic tumors. *J Med Chem*, 48, 4834-41 (2005)
282. Cairns, R., I. Papandreou & N. Denko: Overcoming physiologic barriers to cancer treatment by molecularly targeting the tumor microenvironment. *Mol Cancer Res*, 4, 61-70 (2006)
283. Lardner, A.: The effects of extracellular pH on immune function. *J Leukoc Biol*, 69, 522-30 (2001)
284. Heming, T. A., S. K. Dave, D. M. Tuazon, A. K. Chopra, J. W. Peterson & A. Bidani: Effects of extracellular pH on tumour necrosis factor-alpha production by resident alveolar macrophages. *Clin Sci (Lond)*, 101, 267-74 (2001)
285. Harhaji, L., D. Popadic, D. Miljkovic, I. Cvetkovic, A. Isakovic & V. Trajkovic: Acidosis affects tumor cell survival through modulation of nitric oxide release. *Free Radic Biol Med*, 40, 226-35 (2006)
286. Fischer, B., B. Muller, P. Fisch & W. Kreutz: An acidic microenvironment inhibits antitumoral non-major histocompatibility complex-restricted cytotoxicity: implications for cancer immunotherapy. *J Immunother*, 23, 196-207 (2000)
287. Vermeulen, M., M. Giordano, A. S. Trevani, C. Sedlik, R. Gamberale, P. Fernandez-Calotti, G. Salamone, S. Raiden, J. Sanjurjo & J. R. Geffner: Acidosis improves uptake of antigens and MHC class I-restricted presentation by dendritic cells. *J Immunol*, 172, 3196-204 (2004)
288. Joyce, J. A.: Therapeutic targeting of the tumor microenvironment. *Cancer Cell*, 7, 513-20 (2005)
289. Jain, R. K.: Tumor angiogenesis and accessibility: role of vascular endothelial growth factor. *Semin Oncol*, 29, 3-9 (2002)
290. Willett, C. G., Y. Boucher, E. di Tomaso, D. G. Duda, L. L. Munn, R. T. Tong, D. C. Chung, D. V. Sahani, S. P. Kalva, S. V. Kozin, M. Mino, K. S. Cohen, D. T. Scadden, A. C. Hartford, A. J. Fischman, J. W. Clark, D. P. Ryan, A. X. Zhu, L. S. Blaszkowsky, H. X. Chen, P. C. Shellito, G. Y. Lauwers & R. K. Jain: Direct evidence that the VEGF-specific antibody bevacizumab has antivascular effects in human rectal cancer. *Nat Med*, 10, 145-7 (2004)
291. Jain, R. K.: Normalizing tumor vasculature with anti-angiogenic therapy: a new paradigm for combination therapy. *Nat Med*, 7, 987-9 (2001)
292. Sun, X., J. R. Kanwar, E. Leung, K. Lehnert, D. Wang & G. W. Krissansen: Angiostatin enhances B7.1-mediated cancer immunotherapy independently of effects on vascular endothelial growth factor expression. *Cancer Gene Ther*, 8, 719-27 (2001)
293. Clauss, M., M. Gerlach, H. Gerlach, J. Brett, F. Wang, P. C. Familletti, Y. C. Pan, J. V. Olander, D. T. Connolly & D. Stern: Vascular permeability factor: a tumor-derived polypeptide that induces endothelial cell and monocyte procoagulant activity, and promotes monocyte migration. *J Exp Med*, 172, 1535-45 (1990)
294. Ramanathan, M., A. Giladi & S. J. Leibovich: Regulation of vascular endothelial growth factor gene expression in murine macrophages by nitric oxide and hypoxia. *Exp Biol Med (Maywood)*, 228, 697-705 (2003)
295. Leek, R. D., N. C. Hunt, R. J. Landers, C. E. Lewis, J. A. Royds & A. L. Harris: Macrophage infiltration is associated with VEGF and EGFR expression in breast cancer. *J Pathol*, 190, 430-6 (2000)
296. Leek, R. D., C. E. Lewis, R. Whitehouse, M. Greenall, J. Clarke & A. L. Harris: Association of macrophage infiltration with angiogenesis and prognosis in invasive breast carcinoma. *Cancer Res*, 56, 4625-9 (1996)
297. Tsutsui, S., K. Yasuda, K. Suzuki, K. Tahara, H. Higashi & S. Era: Macrophage infiltration and its prognostic implications in breast cancer: the relationship with VEGF expression and microvessel density. *Oncol Rep*, 14, 425-31 (2005)
298. Kaplan, R. N., R. D. Riba, S. Zacharoulis, A. H. Bramley, L. Vincent, C. Costa, D. D. MacDonald, D. K. Jin, K. Shido, S. A. Kerns, Z. Zhu, D. Hicklin, Y. Wu, J. L. Port, N. Altorki, E. R. Port, D. Ruggero, S. V. Shmelkov, K. K. Jensen, S. Rafii & D. Lyden: VEGFR1-positive haematopoietic bone marrow progenitors initiate the pre-metastatic niche. *Nature*, 438, 820-7 (2005)
299. Rafii, S.: Vaccination against tumor neovascularization: Promise and reality. *Cancer Cell*, 2, 429-31 (2002)
300. Li, Y., M. N. Wang, H. Li, K. D. King, R. Bassi, H. Sun, A. Santiago, A. T. Hooper, P. Bohlen & D. J. Hicklin: Active immunization against the vascular endothelial growth factor receptor flk1 inhibits tumor angiogenesis and metastasis. *J Exp Med*, 195, 1575-84 (2002)
301. Dong, Y., J. Qian, R. Ibrahim, J. A. Berzofsky & S. N. Khleif: Identification of H-2Db-specific CD8⁺ T-cell epitopes from mouse VEGFR2 that can inhibit angiogenesis and tumor growth. *J Immunother*, 29, 32-40 (2006)
302. Zhou, H., Y. Luo, M. Mizutani, N. Mizutani, R. A. Reisfeld & R. Xiang: T cell-mediated suppression of angiogenesis results in tumor protective immunity. *Blood*, 106, 2026-32 (2005)
303. Niethammer, A. G., R. Xiang, J. C. Becker, H. Wodrich, U. Pertl, G. Karsten, B. P. Eliceiri & R. A.

The tumor microenvironment and immunity

- Reisfeld: A DNA vaccine against VEGF receptor 2 prevents effective angiogenesis and inhibits tumor growth. *Nat Med*, 8, 1369-75 (2002)
304. Wada, S., T. Tsunoda, T. Baba, F. J. Primus, H. Kuwano, M. Shibuya & H. Tahara: Rationale for antiangiogenic cancer therapy with vaccination using epitope peptides derived from human vascular endothelial growth factor receptor 2. *Cancer Res*, 65, 4939-46 (2005)
305. Nair, S., D. Boczkowski, B. Moeller, M. Dewhirst, J. Vieweg & E. Gilboa: Synergy between tumor immunotherapy and antiangiogenic therapy. *Blood*, 102, 964-71 (2003)
306. Coussens, L. M., B. Fingleton & L. M. Matrisian: Matrix metalloproteinase inhibitors and cancer: trials and tribulations. *Science*, 295, 2387-92 (2002)
307. Overall, C. M. & O. Kleinfeld: Tumour microenvironment - opinion: validating matrix metalloproteinases as drug targets and anti-targets for cancer therapy. *Nat Rev Cancer*, 6, 227-39 (2006)
308. Parks, W. C., C. L. Wilson & Y. S. Lopez-Boado: Matrix metalloproteinases as modulators of inflammation and innate immunity. *Nat Rev Immunol*, 4, 617-29 (2004)
309. McQuibban, G. A., J. H. Gong, J. P. Wong, J. L. Wallace, I. Clark-Lewis & C. M. Overall: Matrix metalloproteinase processing of monocyte chemoattractant proteins generates CC chemokine receptor antagonists with anti-inflammatory properties *in vivo*. *Blood*, 100, 1160-7 (2002)
310. Baratelli, F. E., N. Heuze-Vourc'h, K. Krysan, M. Dohadwala, K. Riedl, S. Sharma & S. M. Dubinett: Prostaglandin E2-dependent enhancement of tissue inhibitors of metalloproteinases-1 production limits dendritic cell migration through extracellular matrix. *J Immunol*, 173, 5458-66 (2004)
311. Mantena, S. K., A. M. Roy & S. K. Katiyar: Epigallocatechin-3-gallate inhibits photocarcinogenesis through inhibition of angiogenic factors and activation of CD8+ T cells in tumors. *Photochem Photobiol*, 81, 1174-9 (2005)
312. Sheu, B. C., S. M. Hsu, H. N. Ho, H. C. Lien, S. C. Huang & R. H. Lin: A novel role of metalloproteinase in cancer-mediated immunosuppression. *Cancer Res*, 61, 237-42 (2001)
313. Davis, G. E., K. J. Bayless, M. J. Davis & G. A. Meininger: Regulation of tissue injury responses by the exposure of matricryptic sites within extracellular matrix molecules. *Am J Pathol*, 156, 1489-98 (2000)
314. Schenk, S. & V. Quaranta: Tales from the crypt[ic] sites of the extracellular matrix. *Trends Cell Biol*, 13, 366-75 (2003)
315. Jin, H. & J. Vamser: Integrins: roles in cancer development and as treatment targets. *Br J Cancer*, 90, 561-5 (2004)
316. Rosenberg, S. A.: Immunologic and therapeutic evaluation of a synthetic peptide vaccine for the treatment of patients with metastatic melanoma. *Nature Med.*, 4, 321-327 (1998)
317. Johnson, T. W., K. E. Anderson, D. Lazovich & A. R. Folsom: Association of aspirin and nonsteroidal anti-inflammatory drug use with breast cancer. *Cancer Epidemiol Biomarkers Prev*, 11, 1586-91 (2002)
318. Pollard, M. & P. H. Luckert: Effect of piroxicam on primary intestinal tumors induced in rats by N-methylnitrosourea. *Cancer Lett*, 25, 117-21 (1984)
319. Thun, M. J., M. M. Namboodiri, E. E. Calle, W. D. Flanders & C. W. Heath, Jr.: Aspirin use and risk of fatal cancer. *Cancer Res*, 53, 1322-7 (1993)
320. Jacobs, E. J., C. J. Connell, C. Rodriguez, A. V. Patel, E. E. Calle & M. J. Thun: Aspirin use and pancreatic cancer mortality in a large United States cohort. *J Natl Cancer Inst*, 96, 524-8 (2004)
321. Jacobs, E. J., C. Rodriguez, A. M. Mondul, C. J. Connell, S. J. Henley, E. E. Calle & M. J. Thun: A large cohort study of aspirin and other nonsteroidal anti-inflammatory drugs and prostate cancer incidence. *J Natl Cancer Inst*, 97, 975-80 (2005)
322. Lee, J., B. M. Fenton, C. J. Koch, J. G. Frelinger & E. M. Lord: Interleukin 2 expression by tumor cells alters both the immune response and the tumor microenvironment. *Cancer Res*, 58, 1478-85 (1998)
323. Bonvalot, S., A. Laplanche, F. Lejeune, E. Stoeckle, C. Le Pechoux, D. Vanel, P. Terrier, J. Lumbroso, M. Ricard, G. Antoni, A. Cavalcanti, C. Robert, N. Lassau, J. Y. Blay & A. Le Cesne: Limb salvage with isolated perfusion for soft tissue sarcoma: could less TNF-alpha be better? *Ann Oncol*, 16, 1061-8 (2005)
324. Grunhagen, D. J., J. H. de Wilt, W. J. Graveland, C. Verhoef, A. N. van Geel & A. M. Eggermont: Outcome and prognostic factor analysis of 217 consecutive isolated limb perfusions with tumor necrosis factor-alpha and melphalan for limb-threatening soft tissue sarcoma. *Cancer*, 106, 1776-84 (2006)
325. Stolina, M., S. Sharma, Y. Lin, M. Dohadwala, B. Gardner, J. Luo, L. Zhu, M. Kronenberg, P. W. Miller, J. Portanova, J. C. Lee & S. M. Dubinett: Specific inhibition of cyclooxygenase 2 restores antitumor reactivity by altering the balance of IL-10 and IL-12 synthesis. *J Immunol*, 164, 361-70 (2000)
326. Sharma, S., M. Stolina, S. C. Yang, F. Baratelli, J. F. Lin, K. Atianzar, J. Luo, L. Zhu, Y. Lin, M. Huang, M. Dohadwala, R. K. Batra & S. M. Dubinett: Tumor cyclooxygenase 2-dependent suppression of dendritic cell function. *Clin Cancer Res*, 9, 961-8 (2003)
327. Hahn, T., I. Alvarez, J. J. Kobbie, L. Ramanathapuram, S. Dial, A. Fulton, D. Besselsen, E. Walker & E. T. Akporiaye: Short-term dietary administration of celecoxib enhances the efficacy of tumor lysate-pulsed dendritic cell vaccines in treating murine breast cancer. *Int J Cancer*, 118, 2220-31 (2006)
328. Kundu, N., T. C. Walser, X. Ma & A. M. Fulton: Cyclooxygenase inhibitors modulate NK activities that control metastatic disease. *Cancer Immunol Immunother*, 54, 981-7 (2005)
329. Haas, A. R., J. Sun, A. Vachani, A. F. Wallace, M. Silverberg, V. Kapoor & S. M. Albelda: Cyclooxygenase-2 inhibition augments the efficacy of a cancer vaccine. *Clin Cancer Res*, 12, 214-22 (2006)
330. Basu, G. D., T. L. Tindler, J. M. Bradley, T. Tu, C. L. Hattrup, B. A. Pockaj & P. Mukherjee: Cyclooxygenase-2 Inhibitor Enhances the Efficacy of a Breast Cancer Vaccine: Role of IDO. *J Immunol*, 177, 2391-402 (2006)
331. Karin, M.: NF-kappaB and cancer: mechanisms and targets. *Mol Carcinog*, 45, 355-61 (2006)
332. Karin, M., Y. Yamamoto & Q. M. Wang: The IKK NF-kappa B system: a treasure trove for drug development. *Nat Rev Drug Discov*, 3, 17-26 (2004)

The tumor microenvironment and immunity

333. Luo, J. L., S. Maeda, L. C. Hsu, H. Yagita & M. Karin: Inhibition of NF-kappaB in cancer cells converts inflammation- induced tumor growth mediated by TNFalpha to TRAIL-mediated tumor regression. *Cancer Cell*, 6, 297-305 (2004)
334. Hodge, J. W., S. Abrams, J. Schlom & J. A. Kantor: Induction of antitumor immunity by recombinant vaccinia viruses expressing B7-1 or B7-2 co-stimulatory molecules. *Cancer Res*, 54, 5552-5 (1994)
335. Kaufman, H. L., G. Deraffle, J. Mitcham, D. Moroziewicz, S. M. Cohen, K. S. Hurst-Wicker, K. Cheung, D. S. Lee, J. Divito, M. Voulo, J. Donovan, K. Dolan, K. Manson, D. Panicali, E. Wang, H. Horig & F. M. Marincola: Targeting the local tumor microenvironment with vaccinia virus expressing B7.1 for the treatment of melanoma. *J Clin Invest*, 115, 1903-12 (2005)
336. Kudo-Saito, C., J. W. Hodge, H. Kwak, S. Kim-Schulze, J. Schlom & H. L. Kaufman: 4-1BB ligand enhances tumor-specific immunity of poxvirus vaccines. *Vaccine*, 24, 4975-86 (2006)
337. Vianello, F., N. Papeta, T. Chen, P. Kraft, N. White, W. K. Hart, M. F. Kircher, E. Swart, S. Rhee, G. Palu, D. Irimia, M. Toner, R. Weissleder & M. C. Poznansky: Murine B16 melanomas expressing high levels of the chemokine stromal-derived factor-1/CXCL12 induce tumor-specific T cell chemorepulsion and escape from immune control. *J Immunol*, 176, 2902-14 (2006)
338. Dudley, M. E., J. R. Wunderlich, P. F. Robbins, J. C. Yang, P. Hwu, D. J. Schwartzentruber, S. L. Topalian, R. Sherry, N. P. Restifo, A. M. Hubicki, M. R. Robinson, M. Raffeld, P. Duray, C. A. Seipp, L. Rogers-Freezer, K. E. Morton, S. A. Mavroukakis, D. E. White & S. A. Rosenberg: Cancer regression and autoimmunity in patients after clonal repopulation with antitumor lymphocytes. *Science*, 298, 850-4 (2002)
339. Morgan, R. A., M. E. Dudley, J. R. Wunderlich, M. S. Hughes, J. C. Yang, R. M. Sherry, R. E. Royal, S. L. Topalian, U. S. Kammula, N. P. Restifo, Z. Zheng, A. Nahvi, C. R. de Vries, L. J. Rogers-Freezer, S. A. Mavroukakis & S. A. Rosenberg: Cancer Regression in Patients After Transfer of Genetically Engineered Lymphocytes. *Science* (2006)
340. Shevach, E. M.: Fatal attraction: tumors beckon regulatory T cells. *Nature Med.*, 10, 900-901 (2004)
341. Wei, W. Z., G. P. Morris & Y. C. Kong: Anti-tumor immunity and autoimmunity: a balancing act of regulatory T cells. *Cancer Immunol Immunother*, 53, 73-8 (2004)
342. Turk, M. J., J. A. Guevara-Patino, G. A. Rizzuto, M. E. Engelhorn & A. N. Houghton: Concomitant tumor immunity to a poorly immunogenic melanoma is prevented by regulatory T cells. *J. Exp. Med.*, 200, 771-782 (2004)
343. Nagai, H., T. Horikawa, I. Hara, A. Fukunaga, S. Oniki, M. Oka, C. Nishigori & M. Ichihashi: *In vivo* elimination of CD25+ regulatory T cells leads to tumor rejection of B16F10 melanoma, when combined with interleukin-12 gene transfer. *Exp Dermatol*, 13, 613-20 (2004)
344. Uyttenhove, C.: Evidence for a tumoral immune resistance mechanism based on tryptophan degradation by indoleamine 2,3-dioxygenase. *Nature Med.*, 9, 1269-1274 (2003)

345. Muller, A. J., J. B. DuHadaway, P. S. Donover, E. Sutanto-Ward & G. C. Prendergast: Inhibition of indoleamine 2,3-dioxygenase, an immunoregulatory target of the cancer suppression gene Bin1, potentiates cancer chemotherapy. *Nat Med*, 11, 312-9 (2005)
346. Webster, W. S., E. J. Small, B. I. Rini & E. D. Kwon: Prostate cancer immunology: biology, therapeutics, and challenges. *J Clin Oncol*, 23, 8262-9 (2005)

Abbreviations: bFGF: Basic fibroblast growth factor, CT: Chemotherapy, CTLs: Cytolytic T cells, COX-2: Cyclooxygenase 2, DCs: Dendritic cells, DMXAA: 5,6-dimethylxanthenone-4-acetic acid, ECM: Extracellular matrix, GITR: Glucocorticoid-inducible TNF receptor, HIF-1: Hypoxia-inducible factor, Hsp: Heat shock protein, iDC: Immature dendritic cell, IDO: Indoleamine-2,3-deoxygenase, IL-: Interleukin-, IL-1Ra: Interleukin-1 receptor antagonist, iNOS: Inducible nitric oxide synthase, IT: Immunotherapy, LAK: Lymphokine-activated killer cells, LOX: Lipoxygenase, LTB4: Leukotriene B4, mDCs: Myeloid DCs, MHC: Major histocompatibility complex, MMP: Matrix-metalloprotease, 1MT: 1-methyl-tryptophan, NF-kappaB: Nuclear factor kappa B, NK cells: Natural killer cells, NO: Nitric oxide, NSAIDs: Non-steroidal anti-inflammatory drugs, pDCs: Plasmacytoid DCs, PDGF: Platelet-derived growth factor, PGE2: Prostaglandin E2, RT: Radiation therapy, TAAs: Tumor-associated antigens, TAMs: Tumor-associated macrophages, TCR: T cell receptor, TIDCs: Tumor-infiltrating DCs, TILs: Tumor-infiltrating T cells, TIMPs: Tissue inhibitors of MMPs, TGF-beta: Transforming growth factor beta, TNF-alpha: Tumor necrosis factor alpha, TRAIL: Tumor-necrosis-factor related apoptosis inducing ligand, Treg: T regulatory (suppressor) cells, VEGF: Vascular endothelial growth factor

Key Words: Tumor, Microenvironment, Neoplasia, Cancer, Immune cells, Immunity, Immune system, Review

Send correspondence to: Dr. William H. McBride, Department of Radiation Oncology, UCLA David Geffen School of Medicine, B3-109 CHS, 10833 Le Conte Avenue, Los Angeles, CA 90095-1714, Tel: 310-794-7051, Fax: 310-206-1260, E-mail: wmcbride@mednet.ucla.edu

<http://www.bioscience.org/current/vol12.htm>