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FOCUSSED RESEARCH REVIEW

Myeloid-derived suppressor cells as intruders and targets: clinical implications in cancer therapy

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Abstract Chronic inflammation, typical of various diseases including cancer, is a "silent bomb within the body," leading to complications that are only evident in most cases upon their appearance, when disease is already deteriorated. Chronic inflammation is associated with accumulation of myeloid-derived suppressor cells (MDSCs), which lead to immunosuppression. MDSCs have numerous harmful effects as they support tumor initiation, tumor growth and spreading, which in turn, perpetuate the inflammatory and suppressive conditions, thus preventing anticancer responses. As the concept of the immune system combating many types of tumors was revived in recent years, immunotherapy has dramatically changed the view of cancer treatment, and numerous novel therapies have been developed and approved by the FDA. However, cumulative clinical data point at very limited success rates. It is most likely that the developing chronic inflammation and MDSC-induced immunosuppression interfere with responses to such treatments and hence are major obstacles in achieving higher response rates to immune-based therapies. Moreover, chemotherapies were shown to have adverse immunoregulatory effects, enhancing or decreasing MDSC levels and activity, thus affecting treatment success. Therefore, therapeutic manipulations of chronic inflammation and MDSCs during cancer development are likely to enhance efficacy of immune- and chemo-based treatments, switching chronic pro-cancer inflammatory environments to an anticancerous milieu. Based on the functional relevance of immune networking in tumors, it is critical to merge monitoring immune system biomarkers into the traditional patient's categorization and treatment regimens. This will provide new tools for clinical practice, allowing appropriate management of cancer patients toward a better-personalized medicine.

Keywords Cancer · Chronic inflammation · Immunotherapy · Chemotherapy · Immune system biomarkers · CITIM 2015

Abbreviations

ARG-1	Arginase-1
BM	Bone marrow
CP	Cyclophosphamide
CRC	Colorectal cancer
DCs	Dendritic cells
IBD	Inflammatory bowel diseases
iNOS	Inducible nitric oxide synthase
MDSCs	Myeloid-derived suppressor cells
NO	Nitric oxide
PFS	Progression-free survival
RAGE	Receptor for advanced glycation end products
ROS	Reactive oxygen species
TLR	Toll-like receptor
Tregs	Regulatory T cells

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Introduction

An array of non-cancerous pathologies, such as inflammatory bowel diseases (IBD), diabetes, multiple sclerosis and arthritis, as well as various types of cancer, are characterized by chronic inflammation. Unlike acute inflammatory response as in skin injuries, trauma, allergies and infections, which can heal within a short period of time, chronic inflammation is more systemic by nature and can become a repeating cycle of immune bursts over the years, due to the unresolved disease cause that stimulates the host's immune system continuously. Such inflammatory cycles generate harmful conditions due to the sustained activation of innate and adaptive immune systems and thus continuously damage the host's health.

It was shown by various groups including ours that during advanced stages of the above-mentioned diseases, chronic inflammation is exacerbated and associated with immunosuppression and deterioration of the hosts' health conditions [1, 2]. A decade ago we have shown that chronic inflammation is directly responsible for the observed immunosuppression, which is mediated primarily via MDSCs and their secreted compounds/factors. During the years, MDSCs were characterized in most diseases where chronic inflammation is evident, including under cancerous conditions. The observed MDSC-mediated immunosuppression is reflected in an impaired function of the adaptive (T cells) and innate (NK cells) immune systems, which under healthy conditions are critical for clearing pathogens and transformed cells. The combination between the generated chronic inflammatory and the immunosuppressive micro- (disease site) and macro- (periphery) environments leads to advanced complications such as opportunistic infections, tissue damage, unresponsiveness to immune-based therapies, all leading to disease progression. Thus, many of the non-cancerous pathologies can potentially become cancerous and in many cases result in death (Fig. 1).

Inflammation is rapidly becoming one of the most studied topics in the mainstream of health care, even featuring the cover of the February 2004 edition of *Time Magazine* as "*The Secret Killer*." Why secret? Because chronic inflammation in most cases develops "silently," with no clinical manifestations in most cases, until complications are evident. Since chronic inflammation is recognized as being the cause and a key player in different types of diseases, many healthcare researchers are now treating it as the primary disease cause and are searching for ways to combat the generated pro-inflammatory cells and compounds as therapeutic strategies. Moreover, as this field is developing, there is a tremendous need for new tools to evaluate/detect in advance the chronic inflammatory and immunosuppressive stage. Thus, monitoring of the immunological profile of the patient can be most useful in the direction of a given treatment, ensuring an optimal personalized medical care.

MDSC features

Cumulative evidence from various laboratories including ours indicates that the key players in turning off the immune response in chronic diseases are MDSCs, which are responsible for the suppression of an array of immune system activities. Under normal conditions, MDSCs are generated and retained in the bone marrow (BM) as non-polarized cells where they maintain a basal suppressive environment [3], possibly maintaining BM homeostasis. MDSCs migrate to the periphery and differentiate into mature macrophages, dendritic cells (DCs) and neutrophils while losing their suppressive phenotype and supporting normal immune functions. There are at least two MDSC subpopulations in humans and mice: immature monocytic and granulocytic cells [2]. In the normal mouse BM, 50–60 % of the resident cells are resting MDSCs with low suppressive activity. Mature T cells that are occasionally circulating through the normal BM become reversibly dysfunctional, similar to peripheral T cells during chronic inflammation [3]. Low levels (3–5 %) of MDSCs are also found under normal conditions in the mouse spleen and peripheral blood (~10 %) [4], with a basal immunosuppressive activity, most likely controlling peripheral homeostasis and resolving acute immune responses.

Under chronic inflammatory conditions that develop when pathogenic or self-aberrant antigenic insults (e.g., cancer cells) are not resolved, an influx of pro-inflammatory innate and adaptive immune cells is evident. Such cells secrete various cytokines (TNF-α, IFN-γ, IL-1β, IL-6, TGF-β), chemokines (CXCL12, CCL2) and soluble factors [GM-CSF, VEGF] that accelerate MDSC expansion in the BM and accumulation in the periphery [5–7]. Eventually, micro- and macro-environments that support tumor initiation and development are created. Under chronic inflammatory conditions, the non-polarized MDSCs become polarized and highly suppressive, while their differentiation is arrested. Currently, initial evidence points at some growth factors and inflammatory mediators such as IFN-γ, IL-4, IL-13 and TNF-α, which are present in the tumor microenvironment, as inducers of MDSC polarization. IFN-γ induces iNOS expression, while IL-4 or IL-13 increases arginase expression in MDSCs. Activation via toll-like receptor (TLR), IFN-γR, IL-4R and IL-13R could modulate MDSC function [8]. Moreover, we have recently shown that TNF-α generated by a variety of immune and non-immune cells plays a critical role in MDSC polarization, leading to myeloid cell differentiation arrest, MDSC accumulation and enhanced suppressive function that

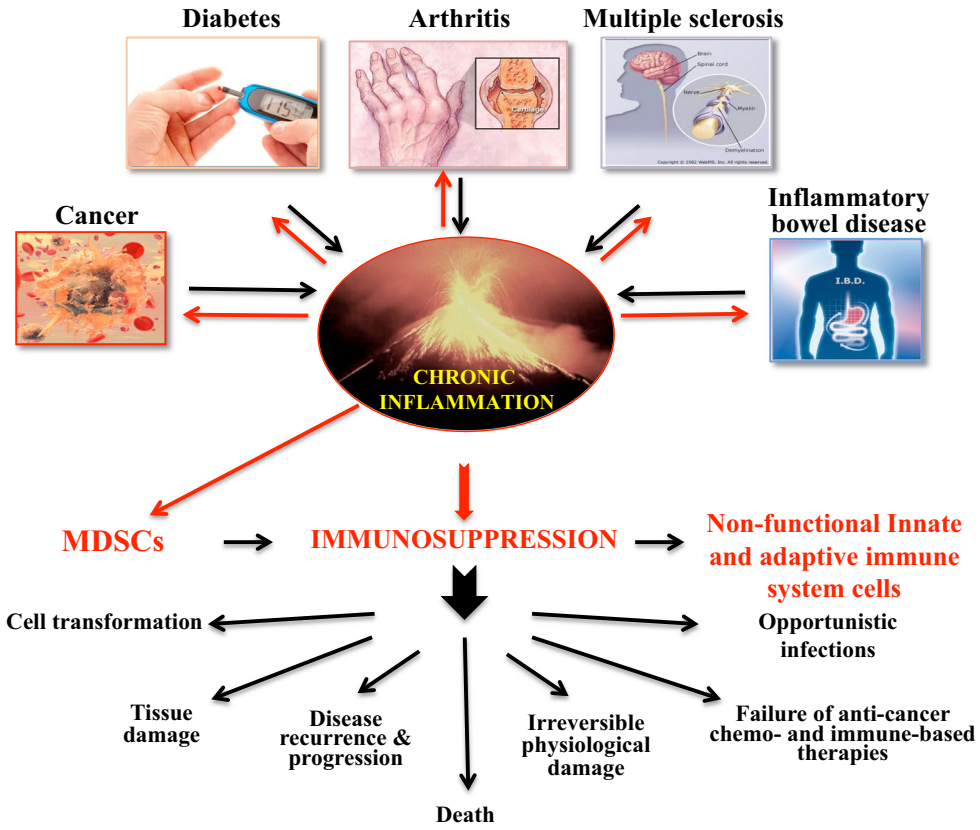

Fig. 1 Non-cancerous and cancerous diseases are characterized by chronic inflammation, which "silently" damages the host's homeostasis until complications are evident. Chronic inflammation induces MDSC-mediated immunosuppression, which is reflected by an impaired function of the adaptive (T cells) and innate (NK cells) immune systems. The combination between the generated chronic inflammatory and the immunosuppressive micro- (disease site) and macro- (periphery) environments leads to advanced complications such as cell transformation, opportunistic infections, tissue damage, unresponsiveness to immune-based therapies, all lead to disease progression. Thus, many of the non-cancerous pathologies can potentially become cancerous and in many cases result in death. Today, there is a critical need for biomarkers to identify the host's immune status and predict complications appearance and disease deterioration before they are evident, in order to direct optimal therapies. One can envision chronic inflammation as a volcano that its activity is silently increasing until its eruption, an event which is life threatening and leads to environmental destructions. As in the volcano cases that seismic waves are continuously measured and can predict eruptions and provide warning, monitoring immune status biomarkers that sense the severity of chronic inflammation is mandatory to sense health threats

affects effector immune cells [5]. MDSCs also produce high levels of TNF-α, operating in autocrine and paracrine loops and thus enhancing their own polarization and accumulation. Polarized MDSC suppressive features are characterized by elevated levels of arginase-1 (ARG-1) activity and inducible nitric oxide synthase (iNOS), associated with enhanced production of nitric oxide (NO) and reactive oxygen species (ROS). TNF-α intensifies these effects [5, 9]. The calcium binding pro-inflammatory proteins, primarily S100A8/9, and their corresponding receptor for advanced glycation end products (RAGE), are upregulated in MDSCs in response to TNF-α. These proteins regulate MDSC polarization, differentiation arrest in a STAT3-dependent mechanism and accumulation [5, 10].

It is evident that polarized MDSCs reach the blood stream, spleen and sites of inflammation, as evident in inflamed tissues such as in various types of IBD, bacterial infections such as Helicobacter pylori and in developing tumors. Interestingly, MDSCs do not enter peripheral lymph nodes, making them protected from the suppressive environment [3], unless metastases in the lymph nodes are evident [11].

Table 1 Detection of immunosuppression mediated by MDSCs in chronic diseases characterized by chronic inflammation

Cancerous settings	Non-cancerous settings
Renal	Diabetes
Pancreas	Ulcerative colitis
HNSCC	Crohn's disease
Colon cancer	Rheumatoid arthritis
Lung	Viral infection
Melanoma	Parasitic infection
HCC	Bacterial infection
Prostate	Sepsis
Breast	GVHD
Multiple myeloma	Traumatic stress

MDSCs as the intruders

MDSCs are activated and polarized toward suppressor cells under both non-cancerous and cancerous conditions (Table 1), intruding into the normal immune system and leading to unbalanced homeostatic conditions, thus affecting effector function of immune cells as well as of regulatory cells. Moreover, in the course of developing tumors, the tumor itself secretes a variety of factors (cytokines and chemokines) that directly affect MDSCs and, in parallel, perpetuate the inflammatory environment. These ensue in MDSC expansion and extended polarization, thus exacerbating the immunosuppression as well as affecting tumor growth and invasiveness.

MDSCs, intruders of the immune system: MDSCs impair $CD4^+$ and $CD8^+$ T cell and NK cell effector functions by affecting various cellular pathways. MDSCs produce peroxynitrites that cause nitration and nitrosylation of different amino acids on the T cell antigen receptor complex (TCR) and CD8 molecules on the surface of T cells, which lead to T cell unresponsiveness to antigen-specific stimulation [12]. One of the most prominent MDSC-mediated defects in T cells is the induced downregulation of CD247 (the ζ subunit of the TCR), a phenomenon also evident in NK cells under chronic inflammatory conditions and tumor settings. CD247 is the main signaling subunit of the TCR in T cells, and of the killing receptors NKp46, NKp30 and FcγRIII (CD16) in NK cells [13]. When CD247 is downregulated, the above-mentioned receptors become non-functional. One of the mechanisms affecting CD247 expression and T cell proliferation is MDSC-mediated depletion of L-arginine through ARG-1-dependent consumption [14] and L-cysteine deprivation via its consumption and sequestration [15]. Production of NO and ROS by MDSCs drive several molecular blockades in T cells, ranging from the loss of CD247 [16] and interference with IL-2 receptor signaling [17]. MDSCs can also impair migration of effector $CD8^+$ T cells to the tumor site [18]. Tumor-infiltrating DCs are also affected and found in an immature phenotype and tolerogenic features to tumor antigens [19]. VEGF and IL-10 secreted in the tumor microenvironment by MDSCs activate STAT3 in DCs, which prevents execution of an optimal DC-mediated immune response [20]. Furthermore, MDSCs (monocytic) from CIA mice also inhibited autologous B cell proliferation and antibody production [21]. MDSCs also support the expansion and recruitment of regulatory T cells (Tregs), resulting in tumor progression [22]. There are assumptions that in vivo, Tregs are functional primarily at early stages of the inflammatory disease, while in advanced stages, the major immunosuppressive function is mediated by MDSCs. MDSCs as intruders of the immune system indirectly support tumor growth by enabling tumor escape from host immunity and by blocking a variety of anticancer therapies, which are based on a functional host's immune response and optimal immune environment for adoptively transferred cells. MDSCs also operate directly, supporting the tumor via a variety of secreted factors.

It is important to stress that the immunosuppression mediated by MDSCs is bystander, causing dysfunction of the general T cell population in an antigen nonspecific manner. This phenomenon is reversible, as elimination of the chronic stimulus and/or the MDSCs or neutralization of the inflammatory environment allows recovery of CD247 and T cell and NK cell functions, leading to tumor regression [1].

MDSCs, supporters of tumor initiation, growth and invasion: MDSCs could be considered as intruders, not only of the immune system, but also due to their ability to directly support tumor initiation, growth and invasion. The highly reactive oxidative agents ROS and NO, produced by MDSCs and additional innate immune system cells in the inflammatory settings, have been shown to cause genomic instability and induce somatic mutations as shown in the cases of IBD and Helicobacter pylori infections [23, 24]. These effects are greatly enhanced when chronic inflammation develops and increasing numbers of NO/ROS producing MDSCs accumulate. The inflammatory environment generated by MDSCs provides an optimal niche for cancer initiation, tumor microenvironment enrichment and support of malignant progression. Furthermore, inflammation also modulates expression of miRNAs and induces epigenetic changes that not only regulate the expression of tumor-related proteins, but also enhance the tumor-promoting inflammation-associated carcinogenesis [25, 26]. Moreover, MDSCs have the potential to secrete high levels of proteolytic enzymes such as MMPs, facilitating cancer cell invasion and spreading [27]. It was also proposed that MDSCs support trapping of metastatic cells in unique metastatic niches [28]. Moreover, infiltrating and circulating

MDSCs also play a role in tumor angiogenesis and vasculogenesis [29, 30].

It is important to remember that an internal loop of activation processes operates between the arising tumors and the infiltrating MDSCs; MDSCs secrete factors that indirectly or directly support tumor initiation and growth, and the developing tumors in turn, secrete factors that perpetuate MDSC expansion and suppressive activity. The key role of MDSCs in this loop was shown by in vivo depletion experiments using mouse model systems for various types of cancer such as melanoma [31] and breast [32]. Upon MDSC depletion in tumor-bearing mice, immune system recuperation was observed, associated with a significant tumor regression. These findings strengthen the key role of MDSCs in cancer development and spreading, and highlight the necessity to design therapies combining anti-MDSC modalities.

MDSC-mediated interference of immune function and responses to anticancer therapies

Data gathered over the years highlight the unique MDSC plasticity as reflected by their wide range of intruding properties affecting both the host's immune system and organ/physiology homeostasis. As long as MDSCs are present, effector immune responses mediated via the host's immune system or adoptively transferred cells are significantly weakened and unable to respond to in vivo antigen-induced stimulation, vaccination or infections. Such immune abnormalities could be recuperated following MDSC depletion [5, 33].

The following paragraphs will describe the role of MDSCs in the outcome of growing human tumors and immune- and chemo-based therapies' consequences, focusing on melanoma and colorectal cancer, as both are characterized by chronic inflammation, MDSCs accumulation and immunosuppression.

Melanoma and immune-based therapy

Melanoma is a lethal form of skin cancer. Although it comprises less than 5 % of skin cancer cases, it accounts for the great majority of skin cancer-related deaths. Melanoma initiation and progression in many cases involves a developing chronic inflammation [34]. In advanced stages of the disease, immunosuppression is evident and correlates with elevated levels of MDSCs and disease progression [[35, 36], (Sade-Feldman unpublished data)]. Over the past few years, immunotherapy has dramatically changed the view of melanoma treatment. The current immune-based therapies for melanoma patients are focused on: 1) strategies that use the patient's own immune endogenous system to act against the tumor such as in cancer vaccines, oncolytic virus therapies, cytokine treatments and the use of inhibitors of key immune system checkpoints as Yervoy (ipilimumab; anti-CTLA4), Keytruda (pembrolizumab; anti-PD1) and Opdivo (nivolumab; anti-PD1) and 2) the use of exogenous immune cells to combat the tumor such as different combinations of adoptive T cell therapies. In both cases, it is imperative that a functional host's immune system should operate alongside with a supportive immune environment to enable the treatment's action. However, if an immunosuppressive environment exists, it could affect both strategies and reduce therapy efficacies. Indeed, despite the recent FDA-approved immune-based treatments for melanoma patients, most patients with advanced metastatic melanoma still have a significant risk of mortality. For example, in a phase III study, pembrolizumab treatment demonstrated significantly longer progression-free survival (PFS) and improved overall survival compared with ipilimumab as immunotherapy in patients with advanced melanoma [37]. A phase II trial directly comparing ipilimumab and nivolumab combination to ipilimumab alone showed that the combination had an objective response rate of 61 % compared to 11 % for ipilimumab alone in patients without a BRAF mutation, with 2 years survival [38]. As to tumor-infiltrating lymphocyte (TIL)-mediated therapy, it was reported by the Melanoma International Foundation that the responses are "amazing" but only in a very small fraction of patients. Moreover, adoptive T cell therapies have been dampened by the tumor cells' undergoing clonal evolution and modifications, rendering them invisible to a TCR-mediated T cell attack [39]. Although clonal evolution of tumor cells can potentially generate immunogenic mutations that could be targets of many immunotherapies, including checkpoint inhibitors and TIL therapies, it is a matter of a race between the effectiveness of a given therapy versus the tumor growth and evolution rates, which will dictate the therapy outcome.

These reports and the obtained results represent the tip of the iceberg of what has still to be done in order to optimize anticancer therapies, and raising major questions of why immunotherapy, if it is so promising, works for some cancer patients, but not for others? More importantly, how could they be improved? The possible solutions will be discussed below.

CRC and chemotherapy

Colorectal cancer is the third most common type of cancer among both men and women in the USA and is the second most deadly. CRC, as other tumors, is characterized by chronic inflammation and immunosuppression mediated mainly by MDSCs, which subvert the outcome of anticancer therapy [1, [33]. The most common treatment for CRC is surgery, which may completely eliminate the cancer in

the case of localized tumors. When the cancer has invaded the bowel wall or the lymph nodes, chemotherapy (sometimes in combination with radiation therapy) is administered before or after surgery. Standard first-line chemotherapy for metastatic CRC consists of one of two regimens: FOLFOX [5-fluorouracil (5-FU), leucovorin and oxaliplatin] or FOLFIRI (5-FU, leucovorin and irinotecan). Chemotherapeutic drugs commonly used to treat cancer including CRC affect not only the tumor but also the immune system, having a crucial impact on anti-tumor responses and disease outcome. We had recently shown that different chemotherapeutic agents have adverse immunoregulatory effects, affecting MDSCs and thus are beneficial or harmful to anticancer immune responses and tumor recurrence [33]. It is important to note that currently, the effect of FOLFIRI and FOLFOX when treating CRC patients is not tested as to their effects on the immune system and therefore, such parameters are not taken into account when choosing between these two treatments. We have shown that stage IV CRC patients, displaying elevated levels of MDSCs and downregulated expression of CD247 in peripheral blood T cells before treatment initiation, upon treatment with FOLFIRI (irinotecan in combination with 5-FU), showed a further increased accumulation of MDSCs and downregulation of CD247. In contrast, the FOLFOX regimen, which contains oxaliplatin instead of irinotecan, reduces MDSC levels and leads to recovery of CD247, suggesting a functional recovery of the patients' immune system. Moreover, MDSCs in FOLFIRI-treated patients are more active, showing increased NO and ROS production. These results were verified using a mouse model of CRC, demonstrating that in contrast to the harmful effects of irinotecan or irinotecan and 5-FU therapies, treatment with 5-FU only resulted in a positive outcome as reflected by the restoration of the immune function, the regression of the tumors and longer survival rates [33]. The underlying mechanisms for the adverse effects of 5-FU and irinotecan on MDSCs were defined [33]. The effect of chemotherapy on the immune system was also shown in melanoma cases; low-dose cyclophosphamide (CP) therapy that induces immunogenic tumor cell death and decreases Treg levels had no beneficial anti-melanoma effects. Instead, it increased accumulation of MDSCs with elevated suppressive activity. Thus, melanoma therapy with low-dose CP could be efficient only when combined with the neutralization of MDSCs' immunosuppressive function and the chronic inflammatory microenvironment [40]. These data suggest a significant impact of a given chemotherapeutic protocol on both the tumor and its immunosuppressive environment. In recent years, CRC became one of the major cancer types for which new immune-based cancer treatments are developed, which fall into the same categories as described above for anti-melanoma immune-based therapies. However, such therapies are still in early phase clinical testing (phase I and II) for colorectal cancer.

We propose that the efficacy of a given chemotherapy could be accurately evaluated and modified by monitoring the host's immune function along with certain tumor parameters.

Improving success rates of immune- and chemo-based therapies in patients with melanoma, CRC and other cancer types

As each of the above-described immune-based therapies is tremendously costly, some key changes in the concept of anticancer treatments must be made in order to avoid continuous failure of treatments, disease deterioration, patients' disappointment, unnecessary treatments and hospitalization expenses. These unmet needs are also relevant to cancer patients treated with chemo- and targeted therapies.

When focusing on immune-based therapies, patients are treated with the hope that their endogenous immune system will operate upon treatment with check point inhibitors (anti-CTLA4 and anti-PD1) and tumor vaccinations, or enable the response of adoptively transferred T lymphocytes directed against the tumor. However, it is well established that in cases of melanoma, a chronic inflammatory environment develops within the tumor site and in the periphery that leads to immune suppression, which will most likely have a negative effect on any immune-based treatment.

When focusing on chemotherapy or targeted therapies, it should be remembered that the immune system is directly or indirectly affected as well. While development of new drugs and therapy strategies is primarily oriented at eliminating the tumor load and spreading, attention should also be directed toward understanding their effects on the host's immune system. As discussed above, chemotherapeutic drugs and/or their combined regimens can display positive or negative effects toward the immune system. Such discrepancies between the immunoregulatory effects of chemotherapies could explain why even though tumors regress during treatment, the disease reoccurs eventually. As the immune system in most tumors cases plays a key role in eliminating residual cells after the major destruction by chemotherapy, if an immunosuppressed environment persists, it could prevent the critical action of the immune system to clear remaining cancer cells.

Monitoring the immune status

We have shown that the immune status of a patient can be a major consideration for the ultimate success of any immune-based therapy. Today, there are several biomarkers that could be used to detect the patients' immune status in a

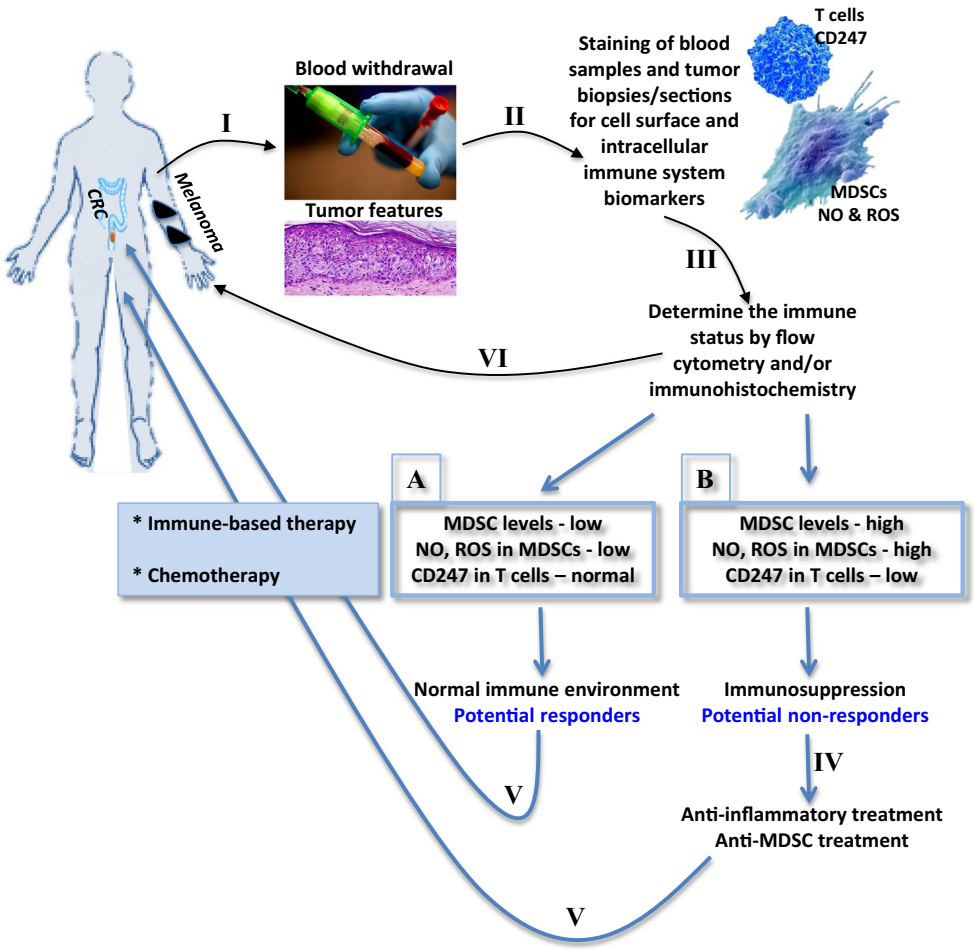

Fig. 2 Monitoring and treatment strategies for cancer patients. Patients must be monitored for their peripheral and tumor site immune status, and for tumor features prior to applying anticancer therapy to allocate responders versus non-responders (*I–III*). The potential responders (*III-A*), showing an active immune system, will be treated as planned with immune-based and/or chemotherapy treatments (*V*). The non-responders (*III-B*), which show a suppressed immune status, will be first subjected to modalities combating the generated inflammatory/immunosuppressive environment (*IV*) to ensure the next step of immune-based and/or chemotherapy treatments (*V*) that should result in effective anti-tumor immune responses. All patients must undergo a routine follow-up monitoring during and posttreatment, testing the tumor features as well as their immune status (*VI*) to ensure disease regression

simple blood test by using flow cytometry and immunohistochemistry on biopsies and tumor sections. As shown by numerous groups, including ours, such biomarkers include: 1) MDSC levels based on their phenotype; $CD11b^{+}CD33^{+}HLADR^{low/-}CD14^{+}$ for monocytic MDSCs and or $CD11b^{+}CD33^{+}HLADR^{low/-}CD15^{+}$ for granulocytic MDSCs, 2) their suppressive features measured by NO and ROS and 3) expression levels of CD247 in T cells. Low MDSC levels in the peripheral blood with low NO and ROS levels, and normal CD247 expression levels in T cells, indicate normal immune status with no chronic inflammation. Such patients are good candidates and are most likely to benefit from immune-based therapy. In contrast, patients showing an opposite pattern of these markers most likely display an immunosuppressive environment due to a developing chronic inflammation. In such cases, it is suggested that these patients will undergo a pretreatment to "suppress the immunosuppression" by neutralizing the chronic inflammation and/or directly combating MDSCs (described below). These approaches are expected to restore the potency of the immune system and increase the probability of responding to immune-based therapies. The relief of chronic inflammation and immunosuppression, upon applied pretreatment regimens, could be monitored as well by the same biomarkers. This strategy has been proven to be effective in various mouse models and in humans [[33, 35, 36, 41] and Sade-Feldman unpublished]. Moreover, these biomarkers could also serve to monitor therapy efficacy and disease recurrence or progression (Fig. 2).

As to patients undergoing chemotherapy, it is mandatory to follow up their immune status prior to and in the course of treatment. First, it is preferable to use drugs that would be beneficial for the immune system. However, if this is limited and drugs negatively affecting the immune system must be used, than monitoring the immune status is a must. If during treatment immunosuppression mediated by MDSCs and chronic inflammation are induced, actions must be taken to neutralize the immunosuppression, thus allowing the chemotherapeutic drug to destroy the tumor and the immune cells to attack tumor cell escapees. Interestingly, a combination of chemotherapy and immunotherapy is already applied in the clinic. However, immune status parameters must be measured to increase success rates.

In conclusion, biomarkers that provide an indication of the patients' immune status are expected to provide invaluable input in accurately tailoring anticancer therapies. Such

biomarkers will help in monitoring therapy efficacies and disease regression and recurrence. As more biomarkers that provide an indication of the patient's immune status are discovered an array of immune status sensors could be developed and used for a cross-validation indication to enable optimal personalized treatments.

Combating chronic inflammation-induced immunosuppression

As immunosuppression induced by chronic inflammation is a reversible phenomenon, eliminating MDSCs by inducing their differentiation, inhibiting signals that lead to their accumulation or blocking their suppressive activity, are expected to generate an environment that no longer nourishes the tumor, and restore immune functions that will lead to optimal anticancer responses. Various drugs and compounds, some FDA approved, have been shown to directly or indirectly target MDSCs and chronic inflammation in mouse models and in some cases of cancer patients, resulting in tumor regression. Along this line, several strategies could be undertaken:

(1) *Blocking various inflammatory cytokines, chemokines and cellular/secreted compounds prevalent in the chronically inflamed environment.* Some were shown to be effective in reducing accumulation of MDSCs and decreasing tumor load in mouse models and in some cases of cancer patients. For example: (a) TNF-α: TNF-α antagonist could directly affect MDSCs, leading to their differentiation and loss of immunosuppressive activity and in parallel, prevent prosperity of the tumor cells and culminating in tumor regression [5, 42 Sade-Feldman et al. unpublished]. (b) GM-CSF: among patients with unresectable stage III or IV melanoma, treatment with ipilimumab plus sargramostim (GM-CSF) versus ipilimumab alone resulted in longer overall survival and lower toxicity [43], but no difference in progression-free survival [44]. GM-CSF may enhance MDSC differentiation to macrophages and DCs [5], resulting in the loss of immunosuppression due to the decrease in MDSC load, enabling T cells to operate and execute the anticancer response in the course of the ipilimumab treatment. These effects must be further studied. (c) COX-2: inhibition of COX-2 with celecoxib [45] can potentially be used with immune-based therapy as it was shown in mouse models that treatment of tumor-bearing mice with dietary celecoxib prevented the local and systemic expansion of all MDSC subtypes. MDSC function was impaired as indicated by the reduced ROS and NO levels and the reversal of T cell tolerance, resulting in refinement of immunotherapy. (d) VEGF: Renal cell carcinoma patients treated with Bevacizumab (Avastin) to reduce VEGF-mediated angiogenesis also had reduced numbers of MDSCs in the tumors [46] as VEGF directly induces MDSC accumulation. (e) Several drugs have been tested to target MDSC suppressive activity. Nitroaspirins were shown to inhibit ARG-1, reduce NO production and arginine consumption, thus correcting immune dysfunction and promoting tumor eradication in mice [47]. Sildenafil inhibits cyclic GMP-specific phosphodiesterase type 5 (PDE-5) in MDSCs and reduces their suppressive activity, thus augmenting an anti-tumor response in mice in vivo [11]. Although various modalities capable of combating MDSCs are already FDA approved and show great promise in mouse models, substantial data regarding the effects of such treatments on MDSCs and chronic inflammation in cancer patients is still missing.

(2) *The use of chemotherapeutic drugs as immunoregulators:* Gemcitabine and 5-FU have been shown to specifically eliminate MDSCs by inducing apoptosis and/or differentiation in mouse models and patients [4, 33]. It was also suggested that low doses of 5-FU can affect the high proliferative potential of the MDSC subset, $CD11b^{+}Gr\text{-}1^{int}Ly6C^{hi}$, a treatment which could be exploited as an adjuvant to passive immunotherapy [48]. Therefore, a combination of chemotherapy and immunotherapy could be optimal in cases where the chemodrug will affect the tumor and MDSCs; the first inducing immunogenic cell death and the latter recuperating the immune response, thus enabling anticancer responses to operate optimally.

(3) *Compounds that affect MDSC differentiation:* In addition to the above-mentioned anti-TNF-α, G-MCSF and 5-FU that affect MDSC differentiation, all-trans retinoic acid (ATRA) can potently induce MDSC differentiation into mature myeloid cells, which lose the harmful suppressive phenotype and improve immune responses in cancer patients [4, 49]. In patients with late-stage small cell lung cancer, ATRA improved the immune response to vaccination by significant reduction of MDSCs and consequently restored antigen-specific T cell responses [50]. Elimination of MDSCs: MDSC elimination is easily done in mice by using anti-Gr1 (a mouse MDSC marker) monoclonal antibody [5], a treatment which leads to immune system recuperation from it suppressive stage, as reflected by recovered responses to vaccination, operating adoptively T cells, which result in tumor regression. However, no specific markers in human MDSCs are currently known to serve as targets for their elimination. This is a very important issue that clinical research laboratories must focus on. We have to remember that MDSC serve on the one hand as biomarkers together with their suppressive features and their outcome, the affected CD247 expression in T cells, and on the other hand MDSCs could serve as targets for treating immunosuppression. It is important to stress that some of the anti-MDSC treatments can change the balance of mature myeloid cells, which are the differentiating outcomes of MDSCs. However, we have to remember that as long as this imbalance exists as a transient solution

until the immune system recuperates and forms the optimal ground for immune-based therapy or any other anticancer responses; such treatments are envisioned to be beneficial.

Conclusion

Taken together, based on the cumulative data pointing at the critical role of immune networking in tumor development and dictating efficacies of anticancer therapies, it is undoubtedly clear that the immunosuppression generated under chronic inflammatory conditions in the course of developing tumors is a serious obstacle in the therapeutic area that includes functional anti-tumor immune response. There is already convincing evidence showing that the selection of an appropriate and effective therapy must take into consideration not only tumor parameters or its type and stage, but also the host's immune status. Thus, monitoring patients' immune status by testing blood samples and when available, tumor biopsies/sections for immune biomarkers, combined with changes in the tumor features before applying any anticancer therapy, responders versus non-responders patients could be determined (Fig. 2I–III) and accordingly, optimal therapies could be designed. The potential responders (Fig. 2, III-A), showing an active immune system, will be treated as planned with immune-based and/or chemotherapy treatments (Fig. 2V). The non-responders (Fig. 2III-B), which showed a suppressed immune status, will be first subjected to modalities combating the generated inflammatory/immunosuppressive environment (Fig. 2IV), aiming at eradicating the suppressive mediators. This will ensure the next step of immune-based and/or chemotherapy treatments (Fig. 2V) that should result in effective anti-tumor immune responses. All patients must undergo a routine follow-up during and posttreatment, testing the tumor features as well as their immune status (Fig. 2VI). If their immune status becomes impaired during treatment, this will indicate harmful effects of the given therapy or an unresolved disease. If the immune status becomes impaired after treatment was terminated and disease had already regressed, this will suggest disease recurrence. As more biomarkers that provide an indication of the patient's immune status are discovered, an array of immune status sensors could be developed for a cross-validation clue and in combination with genetic and biologic tumor parameters, optimal personalized treatments could be designed.

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Compliance with ethical standards

Conflict of interest There is no conflict of interest in publishing this review.

References

1. Baniyash M, Sade-Feldman M, Kanterman J (2014) Chronic inflammation and cancer: suppressing the suppressors. Cancer Immunol Immunother 63(1):11–20. doi:10.1007/s00262-013-1468-9
2. Talmadge JE, Gabrilovich DI (2013) History of myeloid-derived suppressor cells. Nat Rev Cancer 13(10):739–752. doi:10.1038/nrc3581
3. Ezernitchi AV, Vaknin I, Cohen-Daniel L, Levy O, Manaster E, Halabi A et al (2006) TCR zeta down-regulation under chronic inflammation is mediated by myeloid suppressor cells differentially distributed between various lymphatic organs. J Immunol 177(7):4763–4772. doi:10.4049/jimmunol.177.7.4763
4. Kanterman J, Sade-Feldman M, Baniyash M (2012) New insights into chronic inflammation-induced immunosuppression. Semin Cancer Biol 22(4):307–318. doi:10.1016/j.semcancer.2012.02.008
5. Sade-Feldman M, Kanterman J, Ish-Shalom E, Elnekave M, Horwitz E, Baniyash M (2013) Tumor necrosis factor-alpha blocks differentiation and enhances suppressive activity of immature myeloid cells during chronic inflammation. Immunity 38(3):541–554. doi:10.1016/j.immuni.2013.02.007
6. Obermajer N, Wong JL, Edwards RP, Odunsi K, Moysich K, Kalinski P (2012) PGE(2)-driven induction and maintenance of cancer-associated myeloid-derived suppressor cells. Immunol Invest 41(6–7):635–657. doi:10.3109/08820139.2012.695417
7. Huang B, Lei Z, Zhao J, Gong W, Liu J, Chen Z et al (2007) CCL2/CCR2 pathway mediates recruitment of myeloid suppressor cells to cancers. Cancer Lett 252(1):86–92. doi:10.1016/j.canlet.2006.12.012
8. Yang WC, Ma G, Chen SH, Pan PY (2013) Polarization and reprogramming of myeloid-derived suppressor cells. J Mol Cell Biol 5(3):207–209. doi:10.1093/jmcb/mjt009
9. Gabrilovich DI, Nagaraj S (2009) Myeloid-derived suppressor cells as regulators of the immune system. Nat Rev Immunol 9(3):162–174. doi:10.1038/nri2506
10. Ostrand-Rosenberg S, Sinha P (2009) Myeloid-derived suppressor cells: linking inflammation and cancer. J Immunol 182(8):4499–4506. doi:10.4049/jimmunol.0802740
11. Meyer C, Sevko A, Ramacher M, Bazhin AV, Falk CS, Osen W et al (2011) Chronic inflammation promotes myeloid-derived suppressor cell activation blocking antitumor immunity in transgenic mouse melanoma model. Proc Natl Acad Sci USA 108(41):17111–17116. doi:10.1073/pnas.1108121108
12. Nagaraj S, Schrum AG, Cho HI, Celis E, Gabrilovich DI (2010) Mechanism of T cell tolerance induced by myeloid-derived

suppressor cells. J Immunol 184(6):3106–3116. doi:10.4049/jimmunol.0902661
13. Baniyash M (2004) TCR zeta-chain downregulation: curtailing an excessive inflammatory immune response. Nat Rev Immunol 4(9):675–687. doi:10.1038/nri1434
14. Rodriguez PC, Zea AH, Culotta KS, Zabaleta J, Ochoa JB, Ochoa AC (2002) Regulation of T cell receptor CD3zeta chain expression by L-arginine. J Biol Chem 277(24):21123–21129. doi:10.1074/jbc.M110675200
15. Srivastava MK, Sinha P, Clements VK, Rodriguez P, Ostrand-Rosenberg S (2010) Myeloid-derived suppressor cells inhibit T-cell activation by depleting cystine and cysteine. Cancer Res 70(1):68–77. doi:10.1158/0008-5472.CAN-09-2587
16. Schmielau J, Nalesnik MA, Finn OJ (2001) Suppressed T-cell receptor zeta chain expression and cytokine production in pancreatic cancer patients. Clin Cancer Res 7(3 Suppl):933s–939s
17. Mazzoni A, Bronte V, Visintin A, Spitzer JH, Apolloni E, Serafini P et al (2002) Myeloid suppressor lines inhibit T cell responses by an NO-dependent mechanism. J Immunol 168(2):689–695. doi:10.4049/jimmunol.168.2.689
18. Molon B, Ugel S, Del Pozzo F, Soldani C, Zilio S, Avella D et al (2011) Chemokine nitration prevents intratumoral infiltration of antigen-specific T cells. J Exp Med 208(10):1949–1962. doi:10.1084/jem.20101956
19. Almand B, Resser JR, Lindman B, Nadaf S, Clark JI, Kwon ED et al (2000) Clinical significance of defective dendritic cell differentiation in cancer. Clin Cancer Res 6(5):1755–1766
20. Wang T, Niu G, Kortylewski M, Burdelya L, Shain K, Zhang S et al (2004) Regulation of the innate and adaptive immune responses by Stat-3 signaling in tumor cells. Nat Med 10(1):48–54. doi:10.1038/nm976
21. Crook KR, Jin M, Weeks MF, Rampersad RR, Baldi RM, Glekas AS et al (2015) Myeloid-derived suppressor cells regulate T cell and B cell responses during autoimmune disease. J Leukoc Biol 97(3):573–582. doi:10.1189/jlb.4A0314-139R
22. Zhang Y, Liu Q, Zhang M, Yu Y, Liu X, Cao X (2009) Fas signal promotes lung cancer growth by recruiting myeloid-derived suppressor cells via cancer cell-derived PGE2. J Immunol 182(6):3801–3808. doi:10.4049/jimmunol.0801548
23. Ullman TA, Itzkowitz SH (2011) Intestinal inflammation and cancer. Gastroenterology 140(6):1807–1816. doi:10.1053/j.gastro.2011.01.057
24. Kidane D, Murphy DL, Sweasy JB (2014) Accumulation of abasic sites induces genomic instability in normal human gastric epithelial cells during Helicobacter pylori infection. Oncogenesis 3:e128. doi:10.1038/oncsis.2014.42
25. Chiba T, Marusawa H, Ushijima T (2012) Inflammation-associated cancer development in digestive organs: mechanisms and roles for genetic and epigenetic modulation. Gastroenterology 143(3):550–563. doi:10.1053/j.gastro.2012.07.009
26. Ma Y, Han W, Yang L, He L, Wang H (2015) The regulation of miRNAs in inflammation-related carcinogenesis. Curr Pharm Des 21(21):3023–3031. doi:10.2174/1381612821666150514105606#sthash.8nPnrzyJ.dpuf
27. Katoh H, Watanabe M (2015) Myeloid-derived suppressor cells and therapeutic strategies in cancer. Mediat Inflamm 2015:159269. doi:10.1155/2015/159269
28. Sceneay J, Parker BS, Smyth MJ, Moller A (2013) Hypoxia-driven immunosuppression contributes to the pre-metastatic niche. Oncoimmunology 2(1):e22355. doi:10.4161/onci.22355
29. Yang L, DeBusk LM, Fukuda K, Fingleton B, Green-Jarvis B, Shyr Y et al (2004) Expansion of myeloid immune suppressor Gr + CD11b + cells in tumor-bearing host directly promotes tumor angiogenesis. Cancer Cell 6(4):409–421. doi:10.1016/j.ccr.2004.08.031
30. Kitamura T, Fujishita T, Loetscher P, Revesz L, Hashida H, Kizaka-Kondoh S et al (2010) Inactivation of chemokine (C-C motif) receptor 1 (CCR1) suppresses colon cancer liver metastasis by blocking accumulation of immature myeloid cells in a mouse model. Proc Natl Acad Sci USA 107(29):13063–13068. doi:10.1073/pnas.1002372107
31. Mauti LA, Le Bitoux MA, Baumer K, Stehle JC, Golshayan D, Provero P et al (2011) Myeloid-derived suppressor cells are implicated in regulating permissiveness for tumor metastasis during mouse gestation. J Clin Invest 121(7):2794–2807. doi:10.1172/JCI41936
32. Liu Y, Kosaka A, Ikeura M, Kohanbash G, Fellows-Mayle W, Snyder LA et al (2013) Premetastatic soil and prevention of breast cancer brain metastasis. Neuro Oncol 15(7):891–903. doi:10.1093/neuonc/not031
33. Kanterman J, Sade-Feldman M, Biton M, Ish-Shalom E, Lasry A, Goldshtein A et al (2014) Adverse immunoregulatory effects of 5FU and CPT11 chemotherapy on myeloid-derived suppressor cells and colorectal cancer outcomes. Cancer Res 74(21):6022–6035. doi:10.1158/0008-5472.CAN-14-0657
34. Maru GB, Gandhi K, Ramchandani A, Kumar G (2014) The role of inflammation in skin cancer. Adv Exp Med Biol 816:437–469. doi:10.1007/978-3-0348-0837-8_17
35. Gebhardt C, Sevko A, Jiang H, Lichtenberger R, Reith M, Tarnanidis K et al (2015) Myeloid cells and related chronic inflammatory factors as novel predictive markers in melanoma treatment with ipilimumab. Clin Cancer Res 21(24):5453–5459. doi:10.1158/1078-0432.CCR-15-0676
36. Kitano S, Postow MA, Ziegler CG, Kuk D, Panageas KS, Cortez C et al (2014) Computational algorithm-driven evaluation of monocytic myeloid-derived suppressor cell frequency for prediction of clinical outcomes. Cancer Immunol Res 2(8):812–821. doi:10.1158/2326-6066.CIR-14-0013
37. Robert C, Schachter J, Long GV, Arance A, Grob JJ, Mortier L et al (2015) Pembrolizumab versus ipilimumab in advanced melanoma. N Engl J Med 372(26):2521–2532. doi:10.1056/NEJMoa1503093
38. Postow MA, Chesney J, Pavlick AC, Robert C, Grossmann K, McDermott D et al (2015) Nivolumab and ipilimumab versus ipilimumab in untreated melanoma. N Engl J Med 372(21):2006–2017. doi:10.1056/NEJMoa1414428
39. Makalowski J, Abken H (2013) Adoptive cell therapy of melanoma: the challenges of targeting the beating heart, Melanoma - From Early Detection to Treatment. Dr Ht Duc (Ed), ISBN: 978-953-51-0961-7, InTech. www.intechopen.com/books/melanoma-from-early-detection-to-treatment/adoptive-cell-therapy-of-melanoma-the-challenges-of-targeting-the-beating-heart. Accessed January 30, 2013. doi:10.5772/53619
40. Sevko A, Sade-Feldman M, Kanterman J, Michels T, Falk CS, Umansky L et al (2013) Cyclophosphamide promotes chronic inflammation-dependent immunosuppression and prevents antitumor response in melanoma. J Invest Dermatol 133(6):1610–1619. doi:10.1038/jid.2012.444
41. Boniface JD, Poschke I, Mao Y, Kiessling R (2012) Tumor-dependent down-regulation of the zeta-chain in T-cells is detectable in early breast cancer and correlates with immune cell function. Int J Cancer 131(1):129–139. doi:10.1002/ijc.26355
42. Draghiciu O, Lubbers J, Nijman HW, Daemen T (2015) Myeloid derived suppressor cells-An overview of combat strategies to increase immunotherapy efficacy. Oncoimmunology 4(1):e954829. doi:10.4161/21624011.2014.954829
43. Kapanadze T, Gamrekelashvili J, Ma C, Chan C, Zhao F, Hewitt S et al (2013) Regulation of accumulation and function of myeloid derived suppressor cells in different murine models of hepatocellular carcinoma. J Hepatol 59(5):1007–1013. doi:10.1016/j.jhep.2013.06.010

44. Hodi FS, Lee S, McDermott DF, Rao UN, Butterfield LH, Tarhini AA et al (2014) Ipilimumab plus sargramostim vs ipilimumab alone for treatment of metastatic melanoma: a randomized clinical trial. JAMA 312(17):1744–1753. doi:10.1001/jama.2014.13943
45. Veltman JD, Lambers ME, van Nimwegen M, Hendriks RW, Hoogsteden HC, Aerts JG et al (2010) COX-2 inhibition improves immunotherapy and is associated with decreased numbers of myeloid-derived suppressor cells in mesothelioma. Celecoxib influences MDSC function. BMC Cancer 10:464. doi:10.1186/1471-2407-10-464
46. Kusmartsev S, Eruslanov E, Kubler H, Tseng T, Sakai Y, Su Z et al (2008) Oxidative stress regulates expression of VEGFR1 in myeloid cells: link to tumor-induced immune suppression in renal cell carcinoma. J Immunol 181(1):346–353. doi:10.4049/jimmunol.181.1.346
47. De Santo C, Serafini P, Marigo I, Dolcetti L, Bolla M, Del Soldato P et al (2005) Nitroaspirin corrects immune dysfunction in tumor-bearing hosts and promotes tumor eradication by cancer vaccination. Proc Natl Acad Sci USA 102(11):4185–4190. doi:10.1073/pnas.0409783102
48. Ugel S, Peranzoni E, Desantis G, Chioda M, Walter S, Weinschenk T et al (2012) Immune tolerance to tumor antigens occurs in a specialized environment of the spleen. Cell Rep 2(3):628–639. doi:10.1016/j.celrep.2012.08.006
49. Mirza N, Fishman M, Fricke I, Dunn M, Neuger AM, Frost TJ et al (2006) All-trans-retinoic acid improves differentiation of myeloid cells and immune response in cancer patients. Cancer Res 66(18):9299–9307. doi:10.1158/0008-5472.CAN-06-1690
50. Iclozan C, Antonia S, Chiappori A, Chen DT, Gabrilovich D (2013) Therapeutic regulation of myeloid-derived suppressor cells and immune response to cancer vaccine in patients with extensive stage small cell lung cancer. Cancer Immunol Immunother 62(5):909–918. doi:10.1007/s00262-013-1396-8