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FOCUSSED RESEARCH REVIEW

Modulation of the myeloid compartment of the immune system by angiogenic- and kinase inhibitor-targeted anti-cancer therapies

Chiara Castelli · Licia Rivoltini · Monica Rodolfo · Marcella Tazzari · Cristina Belgiovine · Paola Allavena

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Abstract Targeted therapies were rationally designed to inhibit molecular pathways in tumor cells critically involved in growth and survival; however, many drugs used in targeted therapies may affect the immune system. In addition, selected conventional chemotherapeutic agents have also been reported to be endowed with direct or indirect effects on immunity, for instance via immunogenic death of tumors. Thus, cancer therapies may have off-target effects, some of which are directed to the immune system. Here, we will review some of these effects in specific therapeutic approaches. We will examine the modulation of the immune contexture in human sarcoma and melanoma induced by anti-angiogenic therapies and by BRAF inhibitors, respectively. We will then discuss how the anti-tumor agent trabectedin is selectively cytotoxic to cells of the monocytic-macrophage lineage and how these immune-related effects can be part of the response to treatment.

Keywords Targeted therapies · Immune responses · Tumor-associated myeloid cells · Anti-angiogenic therapies · BRAF inhibitors · NIBIT 2013

Abbreviations

ASPS	Alveolar soft part sarcoma
DC	Dendritic cells
GIST	Gastrointestinal stromal tumor
M/DSFT	Malignant and dedifferentiated solitary fibrous tumors
M-CSF	Monocyte-colony stimulating factor
MDSC	Myeloid-derived suppressive cells
PDGFR	Platelet-derived growth factor receptor
TAM	Tumor-associated macrophages
TRAIL	Tumor necrosis factor-related apoptosis-inducing ligand
Treg	Regulatory T cells
VEGFR	Vascular endothelial growth factor receptor

Introduction

Considering the intense interplay of the different cells harbored in the tumor micro-environment, it is not surprising that effects delivered to tumor cells may impact on normal host cells, especially those of the immune system. In the latest years, cancer treatment has gained new impetus due to the availability of new, more selective drugs, specifically designed to target pathways driving the survival and progression of cancer cells. In the scenario of these targeted therapies, the host immune system is emerging as a key component in the response to treatment. Data obtained both in humans and in a preclinical setting with dedicated mouse models, strongly demonstrate that these drugs possess immune-modulating activities [1]. These by-stander

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C. Castelli · L. Rivoltini · M. Rodolfo · M. Tazzari
Unit of Immunotherapy of Human Tumor, Department of Experimental Oncology and Molecular Medicine, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy

C. Belgiovine · P. Allavena (✉)
Department of Immunology and Inflammation, Clinical and Research Institute Humanitas, Via Manzoni 113, Rozzano, 20089 Milan, Italy
e-mail: Paola.Allavena@humanitasresearch.it

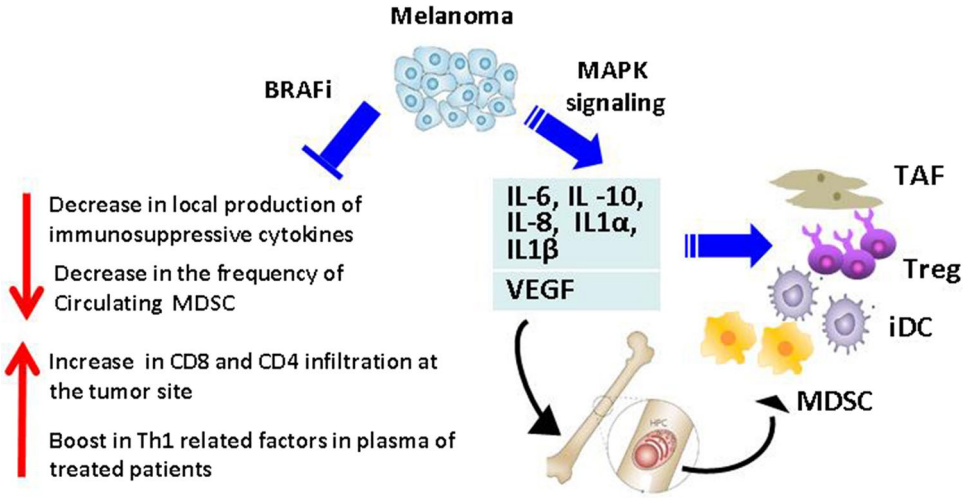

Fig. 1 Immunomodulating activities of BRAF inhibitors. In *melanoma* cells, *MAPK* signaling sustains the production of immunosuppressive and pro-inflammatory cytokines that promote the accumulation of immature myeloid suppressive cells (*MDSC*), immature dendritic cells (*iDC*), regulatory T cells, (*Treg*) and activated fibroblast stromal cells (*TAF*) by local and systemic mechanisms. Treatment with BRAF inhibitors (*BRAFi*) leads to a local re-shaping of the infiltrating lymphocytes with enrichment in $CD4^+$ $CD8^+$ activated T cells, while systemically it limits the frequency of MDCS and boosts the presence of Th1-related factors in the plasma of treated patients

immune-related effects stem from the capacity of targeted drugs to directly affect signaling pathways regulating the functional activities or the differentiation/maturation programs of immune cells and/or from their ability to modulate the immune-related features of tumor cells. Imatinib, a drug inhibiting the c-kit tyrosine kinase receptor, is a paradigm for this double activity. As shown in animal models and in humans, Imatinib directly induces the host DCs to promote NK activation, and this immunological effect was associated with prolonged disease-free survival in Imatinib-treated GIST patients [2]. Simultaneously, Imatinib strongly reduces the release of the immunosuppressive enzyme indoleamine 2,3-dioxygenase by tumor cells [3] and Imatinib-sensitive, but not Imatinib-resistant GIST, drive intratumoral macrophage polarization [4].

The active interplay between the immune system and anti-cancer therapies occurs also for conventional chemotherapy. Established evidence demonstrates that some chemotherapeutic treatments may induce immunogenic cell death in cancer cells. The signals released by dying tumor cells activate immune effectors and have an impact on the anti-tumor immune response and long-term success of anti-cancer therapies. Indeed, the anti-tumor activity of some drugs is greatly reduced in immune deficient mice, demonstrating that immune cells are required for a successful therapeutic outcome [5]. Furthermore, some anti-tumor agents, like gemcitabine, doxorubicin and 5-fluorouracile, kill MDSC or block their immunosuppressive function, diminishing the tumor-mediated immunesuppression and likely favoring the setting of a more active anti-tumor response [6, 7].

Interaction of anti-cancer therapies with host defense mechanisms occurs at different levels and with multiple mechanisms (Fig. 1) whose dissection is an area of great interest with important implications for the future design of more effective combination therapies. This review will focus on selected anti-tumor strategies pointing specifically to immunity.

Anti-angiogenic drugs are active modulators of the immune contexture in cancer patients

Targeted agents include several drugs with anti-angiogenic properties such as the tyrosin kinase inhibitors sunitinib, pazopanib or axitinib, and they all inhibit the signaling activities of VEGFR, PDGFR, c-KIT, although with different affinity [8]. Neo-angiogenic processes are key events in tumor development and progression. The angiogenic switch occurring at the tumor site results in the formation of new, highly abnormal blood vessels displaying a heterogeneous distribution, irregular blood flow and increased permeability [9, 10]. In addition, 'endothelial cell anergy' induced by pro-angiogenic factors, strongly limits the leukocyte–endothelial interaction and the subsequent extravasation of effector cells into tumor sites [11]. As a result, the tumor microenvironment displays poor effector T-cell infiltration and is characterized by hypoxia and acidity, conditions known to foster immunosuppressive cells, including cells of the myeloid lineage and regulatory T cells [12]. Certainly, tumor cells directly drive the cellular events supporting neo-angiogenesis and the expression of pro-angiogenic

factors is controlled by oncogene activation. However, considerable evidence has now emerged for the key role played by the resident or newly recruited tumor—infiltrating myeloid cells in the phenomenon of tumor neo-angiogenesis [13].

We explored the presence and the localization of cells expressing myeloid markers in the inflammatory infiltrate of metastatic alveolar soft part sarcoma (ASPS) [14]. In the metastatic form, ASPS expresses an array of angiogenesis-related molecules and this tumor is characterized by a peculiar tumor-associated vasculature [15]. We found that myeloid cells expressing CD14 and CD163 markers constitute the prominent cells in the inflammatory infiltrate. In the ASPS environment, $CD14^+$ $CD163^+$ cells are structurally organized in two distinct localizations. $CD14^+$ $CD163^+$ cells form a network surrounding the endothelial cells or, as single cells, they are interspersed in tumor nests, keeping deep contact with tumor cells. In the perivascular region, $CD163^+$ cells are aligned to $VEGFR2^+$ $CD31^+$ cells of endothelial nature. Of note, this same distribution of immunoreactivity is also typical of the M-CSF receptor, the major regulator of survival, proliferation and functional differentiation of macrophages. Our observations established the presence of M2-like, $CD163^+$ $CD14^+$ macrophages in the tumor microenvironment of naive ASPS. These myeloid cells are active inflammatory components that may promote VEGF-mediated vasculogenesis and, although not physically part of the vasculature, they are thought to provide trophic support to the characteristic ASPS vascular network. These immunophenotypic ASPS signatures, together with the known positivity of ASPS cells for the expression of pro-angiogenic factors [15, 16] provide the rationale for the usage of different anti-angiogenic targeted therapies for this sarcoma. Indeed, bevacizumab, sunitinib and more recently cediranib have been reported to induce durable responses in metastatic ASPS patients [16–19]. Molecular analysis of ASPS after cediranib treatment showed a strong modulation of transcripts related to angiogenesis/vasculogenesis. Of note, genes encoding for markers of inflammatory myeloid cells were also affected, thus indicating the tumor-infiltrating myeloid cells as potential targets of cediranib and their numeric or functional modulation as part of the response to treatment.

In a soft tissue sarcoma of different histology, namely in malignant and dedifferentiated solitary fibrous tumors (M/DSFT), we observed that another anti-angiogenic therapy based on sunitinib malate treatment also induced a profound remodeling in the myeloid infiltrate. At the tumor site, this myeloid shift favored the acquisition of a new immune contexture displaying features of the adaptive immune response enriched with a strong T-cell infiltration (Tazzari M, personal communication).

Several pro-angiogenic factors, in addition to exert their activity on endothelial cells, also possess immunosuppressive functions. VEGF plays key regulatory roles on the adaptive and innate immunity directly inhibiting DC maturation and fostering the accumulation of immature, tolerogenic DCs at the tumor site [20, 21]. Moreover, VEGF promotes the systemic accumulation of MDSC. Since immature DC and MDSC are strong inducers of regulatory T cells (Treg), VEGF is also indirectly involved in boosting Tregs; more recent evidence also indicates that VEGF directly induces Treg proliferation in a VEGFR2-dependent manner in tumor-bearing mice and in metastatic colorectal cancer patients [22]. Thus, drugs inhibiting VEGF-mediated signaling affect the balance of these various cell subsets and impact on the anti-tumor immune response [23]. Sunitinib and Bevacizumab are first-line standard of care in the treatment of renal cancer patients [24]; several data showed that the frequency of circulating Tregs and the different subsets of MDSC, including monocytic MDSC ($CD11b^+$ $CD14^+$ $DR^{neg/low}$), MDSC defined as $CD33^+DR^-$ and as $CD15^+$ $CD14^-$, are down-modulated in the blood of renal cancer patients receiving sunitinib treatment [25–28]. Furthermore, in the subset of patients experiencing tumor regression, sunitinib induced the reacquisition of a normal frequency of CD1c/BDCA-1^+ myeloid DC [29]. Normalization in the levels of immune-suppressive cells, both Tregs and MDSC, paralleled a regained Th1 function by peripheral $CD3^+$ T cells [26]. Of note, we recently confirmed that the down-modulation of Treg and monocytic MDSC also occurs in the blood of patients with solitary fibrous tumors treated with sunitinib, and by ex vivo analysis we showed that the modulation of these suppressive cells correlates with a regained capacity of T cells to produce Th1-related cytokines (Tazzari M, personal communications).

BRAF inhibitors and immunity: an on-going cross-talk

Melanoma is an immunogenic tumor for which lymphocytic infiltration, defined as brisk, non brisk or absent, has long been known to have prognostic significance [30, 31]. Recently, new studies on a large series of melanoma cases strongly indicated that tumor grade and distribution of lymphocyte infiltration predicted survival, independently of age, sex, tumor site and stage [32]. Furthermore, independent lines of evidence also confirmed that melanoma in its metastatic form is highly suppressive and that several and multi-levels mechanisms of immune evasion are actively operated by melanoma cells [33]. From in vitro and in vivo studies using targeted specific drugs, the emerging concept is that the immunosuppressive ability of melanoma cells is dependent on gene and signaling

alterations that drive their transformation [34]. In melanoma, the release of immunosuppressive cytokines, such as IL-6, VEGF, IL-10 and of pro-inflammatory cytokines such as IL-1α and IL-1β, known to aberrantly stimulate stromal cells at the tumor site, is driven by the activated MAPK signaling and abrogated by the treatment of melanoma cells with BRAF or MEK inhibitors or by silencing of the mutated BRAF (V600E) [35–37]. These data, together with the observation that mutated Ras is crucial in sustaining CXCL8 secretion [38], provide a strong rationale for considering the drugs targeting these signaling pathways as endowed with strong immunomodulating capacity. Thus, smoldering of an immunosuppressive tumor microenvironment and reactivation of the host immune system are likely taking part in the response to treatment. Indeed, in patients treated with the BRAF inhibitors vemurafenib or dabrafenib, several immunological effects have been described as correlated/associated with clinical responses. Tumors surgically removed after short term treatment with vemurafenib, displayed enhanced infiltration with activated $CD4^+$ and $CD8^+$ lymphocytes [39]. Increased intratumoral $CD8^+$ cells correlated with the dimensional response to therapy; moreover, post-treatment biopsies displayed an increased degree of necrosis [39]. Of note, T cells infiltrating tumors post BRAF treatment displayed an increased clonality, thus suggesting the expansion of tumor-specific T-cell clones [40].

All together these results indicate that BRAF treatment has unleashed or has newly promoted a T-cell-mediated response to autologous melanoma in treated patients, suggesting that a relieve in the local and/or systemic immunosuppression might have occurred upon drug treatment.

Indeed, in BRAF inhibitor-treated patients, enhanced T-cell infiltration correlated at the tumor site with a decrease in the local production of IL-6 and CXCL8 [36].

At systemic level, we have shown that melanoma patients display enhanced frequency of monocytic MDSC defined as $CD11b^+$ $CD14^+$ $DR^{low/neg}$; immunological monitoring of immune cells in the blood of patients at different time points during treatments indicates that vemurafenib reversed MDSC accumulation and decreased immune suppression in patients with advanced melanoma [41, 42]. In agreement with this finding, the profile of chemokines and cytokines in the sera of melanoma patients before and early during treatment with dabrafenib and vemurafenib, indicates that BRAF inhibition leads to a significant decrease in the serum levels of the pro-inflammatory, suppressive CXCL8, while it induced a boost of the Th1-related factors IFNγ, CCL4 and TNFα. Furthermore, these systemic changes correlated with the modulation occurring at the tumor site: the decrease in CXCL8 levels was associated with reduction of the proliferation marker Ki67 in melanoma cells and with an increase in tumor-infiltrating cytotoxic T cells in the corresponding tumor biopsies [43].

On the other hand, the ability of cancer therapies to modulate tumor–host interactions, raises additional crucial questions on the role of immune-related factors in directing the resistance to treatment. In this perspective, we recently found that cytokine/chemokine secretion is altered in BRAF-induced-resistant cell lines as compared to their BRAF-susceptible pairs, and that these altered profiles paralleled those found in the sera of melanoma patients under treatment with Vemurafenib. Our data indicate, in patient settings, the relevance of CCL2, a chemokine previously found to be crucially involved in the host response to BRAF treatment in animal models [44].

Therapeutic effects on monocytes/macrophages: the case of the marine-derived compound trabectedin

It is now established that Tumor-Associated Macrophages (TAM) and related myeloid cells infiltrating the tumor micro-environment promote tumor progression and are associated with poor patient prognosis. In fact, in most established tumors, incoming monocytes are conditioned by the tumor micro-environment and acquire an M2-like functional polarization, displaying a number of pro-tumoral functions, e.g., increase of tumor cell proliferation and survival, tumor dissemination, promotion of angiogenesis and matrix remodeling [45–48]. Strategies to deplete TAM or to inhibit their recruitment in tumors have been successful in experimental settings and are now considered in oncology as promising therapeutic approaches. Indeed, a number of recent studies have demonstrated that inhibitors of the M-CSF receptor are effective in inhibiting macrophage recruitment and/or pro-tumoral differentiation [49, 50]. We recently reported that monocytes and macrophages are susceptible to the cytotoxic effect of the anti-tumor agent trabectedin, a compound originally extracted from a marine organism, the Tunicate Ecteinascidia, and now synthetically produced [51].

Trabectedin is the first marine anti-tumor agent to have reached the market. It is registered in Europe and in several other countries, for second-line treatment of soft tissue sarcoma and for ovarian cancer, in combination with liposomal doxorubicin [52, 53]. Trabectedin binds the minor groove of DNA and blocks cell cycle and proliferation in tumor cells. Other recognized effects on cancer cells are its interference on DNA repair mechanisms and on selected transcription factors.

By treating non-activated resting leukocytes, we demonstrated that trabectedin induces apoptosis selectively on monocytes and macrophages, but not in neutrophils and lymphocytes. We further demonstrated that the drug rapidly

triggers the activation of caspase 8 downstream of TRAIL receptors; among leukocyte subsets only monocytes/macrophages express appreciable levels of signaling TRAIL-R, while neutrophils and T lymphocytes preferentially express the non-signaling decoy receptor. When used in vivo in different mouse tumor models, trabectedin was effective in significantly decreasing the number of blood monocytes, spleen and tumor macrophages, but had no effect on neutrophils and lymphocytes [51].

We then asked the question whether the macrophage-depleting effect of trabectedin was relevant for its anti-tumor efficacy. Treatment of mice bearing a trabectedin-resistant tumor cell line resulted in slowed tumor growth, in spite of confirmed resistance of cancer cells to the drug. The hypothesis was that by targeting tumor macrophages trabectedin inhibited the pro-tumoral effects of TAM. In line with this interpretation, the adoptive transfer of macrophages to treated mice significantly reinstated tumor growth. Therefore, macrophage targeting in vivo is a key component of the anti-tumor activity of trabectedin. Effects other than macrophage depletion may also account for its efficacy. Pathological examination of tumor sections revealed that in treated tumors the vessel network, the angiogenic factor VEGF and the chemokine CCL2 were significantly down-modulated. Thus, in addition to direct cytotoxic activity on mononuclear phagocytes, trabectedin may reduce the recruitment of circulating monocytes into tumors and may affect angiogenesis [51].

Patients with soft tissue sarcoma receiving trabectedin as single treatment were studied for blood monocyte counts: a decrease in monocytes occurred within few days after injection of trabectedin in most patients. Furthermore, in tumor sections collected before and after neo-adjuvant therapy, a dramatic decrease of macrophage infiltration and reduction of the vessel network were observed, confirming also in cancer patients that this compound is able to target both the neoplastic compartment and the tumor micro-environment (Fig. 2).

Trabectedin is currently used in a limited number of tumors and as second line of treatment; these findings open interesting perspectives for the rational exploitation of this peculiar property in cancer therapy in a wider range of tumors.

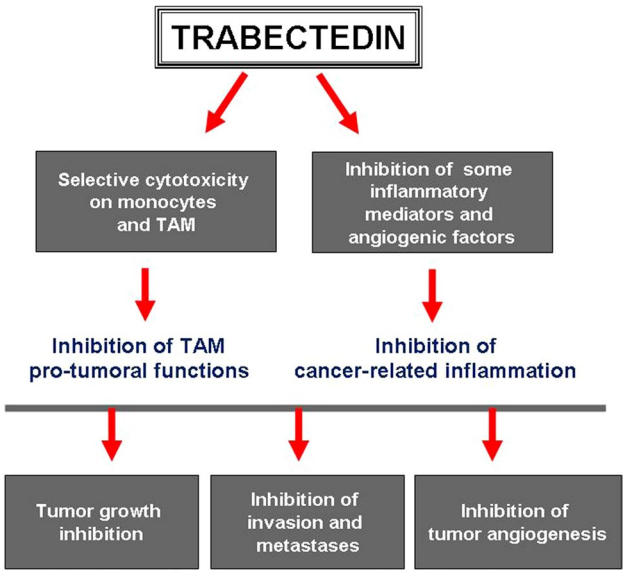

Fig. 2 Multiple effects of the anti-tumor agent trabectedin on the tumor micro-environment. In addition to blocking tumor cell proliferation, the anti-tumor agent trabectedin is selectively cytotoxic to cells of the monocyte-macrophage lineage, including Tumor-Associated Macrophages (TAM). Trabectedin also inhibits the production of selected inflammatory mediators such as the chemokines CCL2 and CXCL8, the cytokine IL-6 and the angiogenic factor VEGF. These mechanisms of action impact on the pro-tumoral role of TAM and on the cancer-related inflammation, thus augmenting its anti-tumor activity

Concluding notes

Immune implication of novel-targeted therapies in cancer is extending very far away from what could be expected on the basis of our current knowledge of the molecular events regulating cancer. It is now clear that the immunological status/response of cancer patients is strongly relevant, not only in those patients receiving immune-based therapies. The immune system is indeed potentially affecting the clinical efficacy of treatments previously considered devoid of any immunological implication. This evidence is reinforcing the notion that a broad immunological monitoring of treated cancer patients is a worthwhile effort potentially providing clues and rationale for discontinuing or further sustaining a given treatment, thus possibly ensuring a better standard of care. Moreover, the new accumulating knowledge on the immunological relevance of these new drugs may also provide rationale for the design of novel, previously unforeseen combinations of drug-based therapies with immune-related approaches.

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Conflict of interest No conflict of interest to declare.

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