Synergy of molecular targeted approaches and immunotherapy in melanoma: preclinical basis and clinical perspectives

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Introduction: Targeted therapy and immunotherapies are the novel pharmacologic treatment strategies for metastatic melanoma. BRAF and MEK inhibitors effectively block the hyperactivation of the MAPK pathway in BRAF mutant melanomas and also have several other effects on melanoma cells and on the immune response. The aim of this work is to discuss the rationale, evidence and perspectives of approaches combining target and immunotherapy against melanoma.

Areas covered: We first review the effects of BRAF and MEK inhibitors on melanoma cells and on the different components of the immune system. Afterwards, we summarize the results of the preclinical and clinical studies that have combined targeted therapy and immunotherapy for the treatment of melanoma.

Expert opinion: Clinical applications of immunotherapy strategies have recently changed the therapeutic mainstay for metastatic melanoma. Biologic and initial preclinical data support their integration with innovative molecular targeted therapies, opening enormous perspectives for researchers in the effort of finding a definitive cure. Main open challenges are the definition of reliable research models, assessment of effective schedules, safety issues and designing of personalized approaches.

Keywords: BRAF inhibitors, immunotherapy, melanoma, targeted therapy

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1. Introduction

The novel pharmacologic treatment strategies for metastatic melanoma are divided into two categories: i) targeted therapies that specifically block signaling pathways and ii) immunotherapies that boost the immune systems' response to tumor cells.

Approximately half of melanomas harbor a somatic point mutation of BRAF [1,2], which determines a constitutive activation of the MAPK pathway. This leads to tumor progression, evasion of senescence and apoptosis, unchecked replicative potential, angiogenesis, tissue invasion and metastasis as well as evasion of immune response [3].

The BRAF inhibitors (BRAFi) vemurafenib and dabrafenib and the MEK inhibitor (MEKi) trametinib are approved for the treatment of BRAF V600 mutant metastatic melanoma [4-6].

The different mechanisms of action and effects of target inhibitors and immunotherapies offer the rationale of combining these two approaches. Targeted therapies lead to quick cell death and release of tumor antigens that sensitize the immune system and can induce a stronger response [7]. The rapid tumor regression following targeted therapy decreases the tumor-associated immunosuppression typical of the tumors' microenvironment and offers a favorable window for immunotherapy. Moreover, targeted therapy can directly modulate the immune system components to boost the immune-mediated tumor destruction.

The aim of this work is to discuss the rationale, evidence and perspectives of approaches combining targeted and immunotherapy against melanoma. We first analyze the effects of the BRAFi and MEKi on both melanoma and immune system cells (Figure 1). We then evaluate preclinical and clinical data of combined treatments (Table 1).

2. Effects of BRAFi and MEKi on melanoma cells

BRAFi and MEKi specifically block the MAPK pathway and cause tumor growth arrest [8-10]. This direct effect on the signaling pathway leads to tumor cell death and rapid release of tumor antigens that could be picked up by antigen-presenting cells (APC), presented to T cells and could potentiate the immune system response. However, there are still no experimental data to support this hypothesis [7].

Beyond the direct effect of BRAFi and MEKi on the MAPK pathway, other effects were described on melanoma cells. In this paragraph, we review how BRAFi and MEKi could indirectly modulate the immune response through their effect on melanoma cells.

The expression of some surface molecules appears to change during target inhibitor treatment and this could affect the melanoma-specific immune response on the level of antigen-specific T-lymphocyte response.

Preclinical studies and patient specimen analysis showed an increased expression of melanocyte differentiation antigens following BRAFi and MEKi treatment [11-13]. It was hypothesized that target inhibitors could reverse the suppression of microphthalmia-associated transcription factor, a transcription factor essential for melanocyte differentiation, induced by the presence of the oncogenic BRAF mutation [14].

The expression of MHC class I and II in melanoma cells also increases during BRAFi treatment [15].

BRAF and MEK inhibition also resulted in reduced expression of CD200 mRNA in melanoma cells [16]. CD200, a type I glycoprotein, a member of the immunoglobulin superfamily, is highly expressed on melanoma cells. CD200 is regulated by ERK activation [17] and mediates an inhibitory signal interacting with its receptor on macrophages and dendritic cells (DCs) [16,18]. Both BRAFi and MEKi reduce CD200 levels in melanoma cells, prevent the inhibitory effect on DCs and therefore restore the ability of DCs to activate T cells [16].

The effect of BRAFi on the expression of programmed cell death ligand (PD-L)1 and PD-L2 is a matter of debate. PDL1 and PD-L2 are ligands for programmed cell death protein 1 (PD1), a co-stimulatory molecule that plays an inhibitory role in regulating T-cell activation in the periphery [19]. First preclinical and in vivo studies suggest an increase in PD-L1 tumor expression following treatment with BRAFi alone

and in combination with MEKi [13,20]. A more recent study described an initial transient in vitro inhibition of PD-L1 by BRAFi followed by steadily increased level of PD-L1 [21]. In cell lines made resistant to BRAFi, an increased expression of PD-L1 was described [20,21]. A possible relation between mechanisms of resistance and targeted therapy was also postulated: a resistance to BRAFi due to the activation of alternative signaling pathways was accompanied by the induction of PDL1 expression, whereas the resistance due to the reactivation of the MAPK pathway had no effect on PD-L1 expression [22]. The analysis of sequential biopsies taken prior to treatment, early during treatment and at time of progression from patients treated with BRAFi or combinations of BRAF and MEKi showed no difference in PD-L1 positivity rates. A different trend was noticed when patients were stratified on the basis of pre-treatment PD-L1 positivity. Patients' tumors that were PD-L1 positive at baseline showed a significant decrease in PD-L1 expression at progression, whereas patients' tumors that were PD-L1 negative at baseline showed a significant increase in PD-L1 expression at progression irrespective of treatment with BRAF or combination of BRAFi and MEKi [23].

Targeted therapy can also affect the tumor microenvironment and the immune response by modulating the tumor cell cytokine secretion. BRAF and MEK inhibition were reported to decrease the production of VEGF, IL-10 and IL-6 [24]. VEGF is an endothelial cell-specific growth factor and the principal regulator of angiogenesis under normal and pathological conditions in most organs [25]. Its downregulation during BRAFi treatment was confirmed in vitro and in melanoma patient biopsies before and during the treatment [26]. BRAFi inhibit VEGF production by reducing the binding of c-Myc to the VEGF promoter. This down-regulation of VEGF has a beneficial effect because the blockage of the interaction of VEGF with its receptor upregulates endothelial adhesion molecules in tumor vessels, which can in turn increase the number of leukocytes infiltrating the tumor [27]. Moreover, it can contribute to normalize tumor vessels and create a homogeneous distribution of perfused vessels throughout the tumor. This was shown to reprogram the tumor microenvironment away from immunosuppression toward potentiation of immunotherapy strategies [28].

3. Effects of BRAFi and MEKi on host immunity cells

In this section, we focus on the direct effect of BRAFi and MEKi on the different components of the immune system.

3.1 Tumor infiltrating lymphocytes

The number of tumor-infiltrating lymphocytes (TILs) within and around patients' metastases increases during BRAFi treatment [29]. CD8+ TILs were consistently increased during BRAFi treatment, while the increase in CD4+ TILs could not be confirmed in all studies [13,30]. The increase of CD8+ TILs during BRAFi treatment was correlated with decrease in tumor size and a correlation with patients' response was hypothesized: CD8+ amount increases after the beginning of BRAFi therapy and decreases at tumor progression [13,30]. However, a more recent study demonstrated that TILs increase in both responders and non-responders to BRAFi treatment. The difference between these two groups appeared to be related to the presence of a pre-existing population of tumor-infiltrating T-cell clones rather than to the infiltration of neoplastic lesions by new T-cell clones. In fact, 80% of the T-cells clones detected after initiation of BRAFi treatment are new clones, but only the pre-existing clones could predict a response to the treatment. Patients who had a higher proportion of pre-existing clones responded better to therapy than patients who had a low proportion of such pre-existing clones [31].

BRAFi specifically target the mutant form of BRAF harbored by melanoma cells. The mechanism of action of BRAFi appears to be different in cells that are wild type for BRAF. Studies performed in RAF wild-type tumors proposed that under physiological conditions BRAFwt maintains itself in an inactive conformation through its own kinase activity. BRAFi, interfering with BRAF activity, allow BRAF to escape from this auto-inhibited state and to be recruited to the plasma membrane by RAS where it forms a stable complex with CRAF. In these BRAF-CRAF dimers, BRAF seems to act as a scaffold whose function is to enhance CRAF activation that leads to hyperactivation of the MAPK pathway. This CRAF-mediated paradoxical activation is RASdependent [32-34]. The effect of BRAFi on non-neoplastic BRAFWT cells, as T cells, is matter of debate. Different studies reported no impact on the function of T cells during BRAFi treatment [26,35,36]. However, a more recent work proposed that BRAFi potentiate T-cell activation in vitro and in vivo in a dose-dependent manner. This activation requires a T-cell activating stimulus, such as engagement of the T-cell receptor (TCR), which triggers ERK signaling through RAS. The same effect was observed also using a pan-RAF inhibitor. This suggests that the CRAF-mediated hyperactivation cannot entirely explain the paradoxical activation of the MAPK pathway [37].

The effect of MEKi on T cells is also not clear. In vitro MEKi had an inhibitory effect on T lymphocytes [12], but no difference was observed in the absolute number of CD8+ TILs comparing patients receiving BRAFi alone or a BRAFi plus a MEKi [13,23]. It has been hypothesized that CD8+ TILs may be insensitive to MEK inhibition since they consist mostly of antigen-experienced memory cells and MEKi may have a less pronounced effect on memory T cells than on their naı¨ve counterparts [38].

BRAFi appear to affect not only the amount of TILs, but also their phenotype. In a mouse model, BRAFi promoted the expression of CD40L and IFN-g on intratumoral CD4+ TILs and reduced the accumulation of regulatory T cells (Tregs). These changes lead to the development of a more immune stimulatory microenvironment [39]. The number of PD1+ TILs increased early during BRAFi treatment compared with pre-treatment patients' biopsies, while this was not observed in patients treated with a combination of BRAFi and MEKi. This could be due to a defect in T-cell differentiation when the patient is receiving both BRAFi and MEKi, but could also be due to the lack of statistical power of the study [23].

3.2 Peripheral blood cells

A drug-specific effect rather than a class-specific effect was postulated for BRAFi on peripheral blood cells. No changes in the absolute number of T-cells (both CD4+ and CD8+), B-cells, natural killer (NK) cells, DCs, monocytes and Tregs or the ex vivo functionality of T cells were found following dabrafenib treatment [35,40]. Meanwhile, a decline in CD4+ T cells and an increase in circulating NK cells were observed in patients treated with vemurafenib [41]. The MEKi trametinib alone or in combination with dabrafenib suppressed T-lymphocyte proliferation, cytokine production and antigen-specific expansion [40].

3.3 Dendritic cells

DCs are a sentinel component of the innate immune system and can function as APCs to induce adaptive responses by processing and presenting antigens to naive T-lymphocytes in lymphoid organs [42]. Melanoma cells have an inhibitory effect on DCs function. Lysates of primary melanoma cells and cell lines suppress the IL-12 secretion of autologous patients' and healthy donor DCs, subsequently limiting the ability of DCs to initiate a Th1 response [43]. This inhibitory effect can be reversed by both BRAFi and MEKi [24,43]. BRAFi showed no direct effect on DCs function [44] and their numbers in peripheral blood [35]. The direct effect of MEKi on DCs is controversial.

Some studies described an enhanced DC maturation [40,45,46], others reported no or minimal impact on DC function [47,48]. One study found an inhibition of DCs maturation [44] during MEKi treatment. The discrepant results could be due to the different maturation stimuli used and investigated in each of the above-mentioned studies. An enhanced maturation of DCs would implicate a loss in DCs ability to endocytose, capture and internalize immune complexes resulting in reduced cross-presentation of tumor antigens [40].

3.4 Natural killer cells

NK cells play critical roles in immunity against cancer. NK cells recognize the tumor cells via stress or danger signals. Activated NK cells directly kill target tumor cells and act as regulatory cells when interacting with DCs, improving their antigen uptake and presentation and facilitating the generation of an antigen-specific T-cell response [49]. A preclinical study found that NK cells are also crucial for the therapeutic effect of BRAFi. BRAFi increase the proliferation of mouse and human NK cells in vitro (with increased levels of p-ERK and CD69) and enhance the frequencies of NK cells in lung metastases of a mouse model. In the same model, depletion of NK cells significantly impaired the antimetastatic effects of BRAFi treatment [50].

3.5 Myeloid-derived suppressor cells

Myeloid-derived suppressor cells (MDSC) suppress T- and NK-cell function by increasing the expression of inflammatory mediators [51]. Patients with metastatic melanoma had a higher number of circulating MDSC compared with healthy donors and patients with localized disease [52]. The amount of MDSC in the blood of melanoma patients declined during BRAFi treatment in individuals achieving clinical responses. This seemed to rely on the decreased release of soluble factors that induce MDSC, such as IL-6 from melanoma cells, more than on the direct effect of BRAFi on MDSC. In fact, the conditioned medium from untreated melanoma cells induced MDSC that exerted robust immunosuppressive effects on T cells. In contrast, the conditioned medium from BRAFitreated melanoma cells did not [53].

4. Preclinical and clinical data on target and immunotherapy combinatory regimens

In this section, we will review the preclinical and clinical data already available on treatment regimens which combined BRAFi and/or MEKi with an immunotherapy strategy

(Table 1).

4.1 BRAFi combined with IFN-a-2b

The overexpression of MHC class I and II molecules on melanoma cells during BRAFi treatment, which leads to a more favorable melanoma-specific immune response, is mediated by IFN [15]. Therefore, the combination of BRAFi and IFN was first investigated in preclinical studies. In a mouse model, it was able to prolong rodent survival [54]. This led to the design of clinical trails, which test the safety and efficacy of such combinations (NCT01959633, NCT01943422). The trials are currently ongoing and preliminary data are at present not available.

However, a possible concern could raise from the recent evidence of the crucial role of NK cells for the antimetastatic activity of BRAFi [50]. In fact, the MHC class I overexpression in tumor cells induced by IFN-a-2b can trigger an inhibitory signal in NK cells.

4.2 BRAFi combined with IL-2

IL-2 was the first immunotherapy approved for the treatment of metastatic melanoma. High-dose (HD) IL-2 demonstrated a complete response rate of 6% and partial response rate of 10% in patients with advanced melanoma, with some longlasting responses [55]. However, due to its significant acute toxicity (severe hypotension, pulmonary edema, systemic edema with significant weight gain and renal insufficiency, rash and fatigue), this regimen requires hospitalization and it is reserved for patients in a good performance status and without involvement of the CNS [56,57].

The rationale of combining IL-2 with BRAFi comes from the observation that BRAFi increase the expression of tumor-specific antigens leading to an enhanced T-cell recognition [11-13], which could be sustained by IL-2 that plays a central role in the activation and stimulation of T-lymphocytes. Moreover, IL-2 could boost the NK response, which was shown to be crucial for the antimetastatic activity of BRAFi [50]. However, HD IL-2 was shown to enhance the expansion of highly suppressive Tregs more than anyother lymphocyte subsets, and this increased level of Treg in blood following the first cycle of HD IL-2 was also correlated with worse outcomes [58].

Clinical trails investigating the combination of a BRAFi with IL-2 are currently ongoing (NCT01683188, NCT01603212).

4.3 BRAFi and/or MEKi combined with adoptive T-cells transfer

Adoptive T-cells transfer (ACT) involves the direct administration of autologous ex vivo expanded tumor reactive T-lymphocytes to preconditioned recipients. Several of the aforementioned effects of targeted therapy lead to the hypothesis of their effective combination with ACT. The tumor antigens released from dead melanoma cells during targeted therapy can provide an 'endogenous' vaccine-like stimulus, which could enhance the ACT effectiveness. In fact, in a mouse model ACT was enhanced by vaccination. The up-regulation of melanoma antigens during both BRAFi and MEKi treatment [11,12] can also improve tumor cell recognition by ACT. The increased T-cell infiltrate, which was observed during targeted therapy [13,23,30], and the observed inhibition of MDSC [53] could also favor the outcome of an ACT therapy.

In a mouse model, BRAFi treatment was shown to increase tumor infiltration of adoptively transferred gp100-specific T cells and to improve antitumor responses [26]. In another mouse model of syngeneic BRAF(V600E)-driven melanoma, SM1, ACT based on both T cells with a TCR recognizing chicken ovalbumin expressed by the tumor or on pmel-1 TCR transgenic lymphocytes recognizing gp100 endogenously expressed by the tumor, resulted in superior antitumor responses when combined with BRAFi treatment [59]. In the same mouse model, SM1, the combination of BRAFi and MEKi with pmel-1 ACT showed complete tumor regression, increased T-cell infiltration into tumors and improved in vivo cytotoxicity [60]. Clinical trials investigating the combination of ACT and targeted therapy are currently ongoing (NCT01585415, NCT02354690, NCT01659151) [61].

4.4 BRAFi combined with immune-checkpoint modulators (Ab anti-CTLA-4 and anti-PD1)

Ipilimumab is an inhibitory monoclonal antibody directed against cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4). The resulting blockade of CTLA-4 signaling prolongs T-cell activation, restores T-cell

proliferation and thus amplifies T-cell-mediated immunity, which enhances the patient's capacity to mount an antitumor immune response [62]. The observation that BRAFi promote the development of a more immune stimulatory microenvironment by increasing intratumoral cytotoxic T cells suggests a possible synergistic effect with ipilimumab [13,30,31].

The first clinical trial which explored the concurrent administration of ipilimumab and vemurafenib was interrupted because of hepatotoxicity [63], but the combination of the other BRAFi, dabrafenib, with ipilimumab showed a more favorable safety profile [64] and is currently under investigation in patients (NCT01767454, NCT01940809, NCT02200562) [61].

Sequential treatment has also been evaluated. Ipilimumab does not appear to negatively influence response to BRAFi, but ipilimumab therapy following BRAFi treatment was associated with low responses and poor survival [65,66]. These results could be due to the fact that patients who discontinue the BRAFi usually have rapid progression and therefore have insufficient lifespan to benefit from ipilimumab, which typically requires weeks or months to show a response. The low response to ipilimumab after BRAFi resistance can also be due to the changes in tumor antigen expression and tumor microenvironment over time. The favorable tumor microenvironment characterized by increased tumor melanocytic antigen expression and CD8+ T cells, typical of the beginning of BRAFi treatment, is no longer evident in tumor samples collected at time-of-progression [13,30].

PD1 and its ligands, PD-L1 and PD-L2 play an important role in regulating immune response through various mechanisms [19]. In a BRAF(V600E)/Pten(-/-) syngeneic tumor graft immunocompetent mouse model, the administration of anti-PD1 or anti-PD-L1 with a BRAFi led to an enhanced response, a prolonged survival and slower tumor growth. Moreover, the combinatory regimen increased the number and activity of TILs [67].

In another mouse model of syngeneic BRAF(V600E)- driven melanoma, SM1, the combination of BRAFi, MEKi and anti PD1 therapy showed a superior antitumor effect compared with anti-PD1 plus either therapy alone or isotope control with both BRAFi and MEKi [60].

In clinical trials, anti-PD1 antibodies showed a higher efficacy in patients with PD-L1-positive tumors [68,69]. The increased expression of PD-L1 in tumors treated with a BRAFi can explain the benefit of a combination with anti-PD1 or PD-L1 antibodies. Such combinatory regimens are currently under investigation (NCT02130466, NCT02027961, NCT02357732, NCT02224781, NCT01656642) [61].

5. Expert opinion

In this review, we discuss the rationale supporting potential synergisms between various immunotherapy and targeted therapy approaches for melanoma. We discuss goals, perspectives, critical aspects and limitations in research models that need to be faced by scientists in the field. In the previous paragraphs, we emphasized the possible positive correlation between target therapies and antitumor immune response. However, we also need to consider and explore potential downsides of these treatments, such as bystander effects of targeted therapy on tumor microenvironment and 'negative regulators' of immune response.

The development of new therapies without the consideration of the intrinsic biological variability of tumors and patients is a methodological weakness. For example, tumor immune infiltration is highly variable among patients. The amount and quality of tumor immune infiltration is a prerequisite for the

effectiveness of immuno-modulatory therapies [70]. This could also influence the outcomes of possible combination therapies and should be taken into account in the upfront selection of patients and in the data interpretation. Similarly, the tumor microenvironment can also affect the response to treatment. The predictive role and the potential susceptibility to treatments of negative regulatory elements such as MDSC and Treg is an important end point to evaluate.

The first goal in order to rationally elucidate the basis for experimental treatments that involve immunotherapy is the investigation of the tumor 'neo-antigen landscape'. In fact, the presence of relevant and immunogenic antigens is mandatory for the ultimate efficacy of adaptive immune responses. The genetic instability and the mutational load of tumors might positively correlate with the generation of immunogenic neo-epitopes [71,72]. From a practical point of view, the incorporation of such analyses in future studies requires the introduction of translational end points and a tight collaboration of clinical and preclinical researchers. In order to achieve this, deep sequencing data and prediction of immunogenic molecules should be done on tumor samples; whenever possible, longitudinal tumor biopsies should be scheduled to collect tumor samples representative of relapse or chemo-resistance conditions.

Another ambitious goal for new experimental treatments is to identify and eradicate cancer stem cells (CSC), which are considered responsible for disease relapse and chemo-resistance. Some preclinical evidence supports the possibility that immunotherapy may be effective against CSC in solid tumors including melanoma [73-75] and potential synergisms with targeted therapy in this direction are an intriguing field to explore.

Another important factor for the future preclinical research is the definition and availability of appropriate in vivo models to investigate synergisms of immunotherapy and molecular targeted approaches. Murine immune-competent models may be indicative but not entirely representative of the complex and patient-specific interactions between immune system and tumors. Tumor xenografts in immunodeficient animals, on the other hand, are very useful to assess the activity of targeted therapy but miss the potential to explore functions and modulations of the immune response.

Researchers are currently exploring the possibility to generate 'humanized mice', where immunodeficient animals are engrafted with human hematopoietic stem cells (HSC) capable of durable immune reconstitution [76,77]. This system would allow exploring the effect of molecular targeted drugs on immune effectors. However, it still holds important limitations given by the HLA-mismatch between the engrafted immune system and the implanted tumor. In theory, it would be ideal to reconstitute mice with autologous HSC collected from cancer patients, but this is obviously not practicable outside exceptional situations.

These limitations may be partially overcome for studies exploring adoptive cell therapy (ACT) approaches. In this case, it is feasible to collect adoptive immune effectors (e.g., TIL, NK, LAK, CIK cells) and infuse them into immunodeficient mice engrafted with autologous tumors.

However, these models would still be incomplete. They would not represent potential negative modulations operated by the tumor-conditioned immune system, as seen in patients, but could enable a transitory platform to investigate synergism with molecular targeted approaches.

While the association of targeted therapy with ACT is intriguing and supported by the reported biologic evidences, the optimal schedule is still to be defined.

We speculate that a sequential treatment may benefit from the immunogenic effect of targeted therapy on tumor cells followed by an augmented antigen presentation, while a concomitant administration might exploit the reported activation exerted by RAFi on circulating lymphocytes. Even when animal models should be available, unpredictable safety issues should always be considered, and 'first-in-human' studies should consider progressive dose-escalation schedules and accurate monitoring for undesired effects.

It has to be considered that combinatorial approaches of molecular targeted drugs with immune checkpoint modulators have logistic advantages over ACT. The ACT therapies require dedicated facilities and procedures compliant with rigorous good manufacturing procedures requirements, currently limiting its application to a few specialized centers.

An intriguing evolution of ACT includes the possibility to genetically redirect T lymphocytes against specific tumor antigens. Redirected specificity may be conferred by transferring genes encoding for either a tumor-antigen-specific TCR or an antibody-based chimeric receptor. Initial promising clinical results against melanoma have been reported with the infusion of TCR-engineered T cells against MART-1, GP-100 and NY-ESO antigens [78-80]. Clinical data of synergism between engineered ACT and molecular targeted therapies have not been reported yet. However, positive premises are in place and it is conceivable to expect interesting studies to come in the next future.

Overall, we are experiencing exciting times in melanoma research, which will hopefully lead the way to biologic knowledge and innovative treatments exportable to other tumor models. The central point of this process is immunotherapy and its possible integration with innovative molecular targeted approaches. From a 'clinical practice' point of view, the melanoma treatment is moving towards a 'personalized' approach based on biologic basis. The field will have to find a balance between the ever-increasing treatment possibilities generated by translational research and the desire of the clinician for easily understandable and manageable approaches with the ultimate goal of finding the best cost--effectiveness in curing melanoma.

Declaration of interests

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(□□) to readers.

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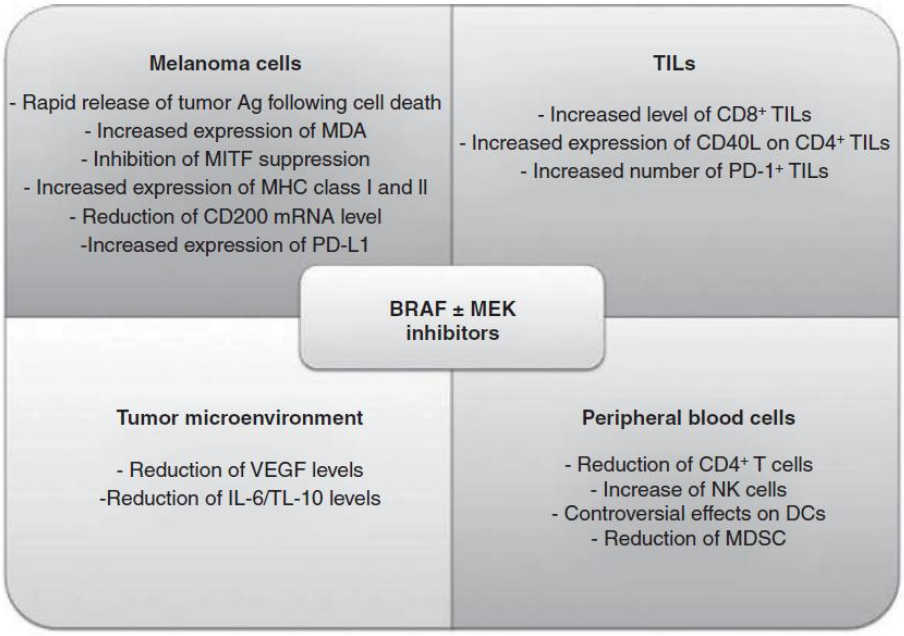

Figure 1. Main effects of BRAF and MEK inhibitors in melanoma and immune system cells.

DCs: Dendritic cells; MDA: Melanocyte differentiation antigens; MDSC: Myeloid-derived suppressor cells; MITF: Microphthalmia-associated transcription factor;

NK: Natural killer; TILs: Tumor-infiltrating lymphocytes.

Table 1. Clinical trials in melanoma exploring BRAF inhibitors (with/without MEK inhibitors) in combination with cancer immunotherapies (June 2015).

Clinical trial number	Phase	Intervention – Schedule	Status of the trial
NCT01959633	I/II	Vemurafenib + Peg-IFN IFN treatment should start after 15 days of vemurafenib	Recruiting
NCT01943422	I/II	Vemurafenib + high-dose IFN-α-2b IFN-α is administered intravenously for 5 consecutive days every week for 4 weeks	Recruiting
NCT01683188	IV	Vemurafenib + high-dose IL-2	Terminated
NCT01603212	I/II	Vemurafenib + IFN-α-2b + IL-2 Vemurafenib (21days cycle) + IL-2 (days 2 – 5) + IFN-α (days 1 – 5)	Active, not recruiting
NCT01585415	I	Vemurafenib + white blood cell therapy After 2 weeks of vemurafenib and a lymphocyte depleting preparative regimen (cyclophosphamide and fludarabine), young TILs are infused and followed by IL-2	Active, not recruiting
NCT02354690	I/II	Vemurafenib + TIL therapy After 2 weeks of vemurafenib, the tumor is harvested; TILs are isolated and expanded in the lab. After lymphodepleting chemotherapy the patient receives TILs and IL-2	Recruiting
NCT01659151	II	Vemurafenib + lymphodepletion + adoptive cell transfer & high-dose IL-2 After 2 weeks of vemurafenib, the tumor is harvested; TILs are isolated and expanded in the lab. After lymphodepleting chemotherapy the patient receives ACTs and IL-2	Recruiting
NCT01767454	I	Dabrafenib + ipilimumab ± trametinib	Recruiting
NCT01940809	I	Ipilimumab ± dabrafenib, ± trametinib	Recruiting
NCT02200562	I/II	Ipilimumab + dabrafenib	Recruiting
NCT02130466	I/II	Pembrolizumab ± trametinib ± dabrafenib	Recruiting
NCT02027961	I/II	MEDI4736 (anti PD-L1) + trametinib ± dabrafenib	Recruiting
NCT02357732	I	Nivolumab + dabrafenib ± trametinib	Active, not recruiting
NCT02224781	III	Dabrafenib + trametinib followed by ipilimumab + nivolumab versus ipilimumab + nivolumab followed by dabrafenib + trametinib	Active, not recruiting
NCT01656642	I	MPDL3280A (anti PD-L1) + vemurafenib ± cobimetinib	Recruiting

ACT: Adoptive T-cells transfer; PD-L1: Programmed cell death ligand 1; Peg-IFN: Pegylated-IFN; TIL: Tumor-infiltrating lymphocyte.