Chapter 8
Combination BRAF-Directed Therapy and Immunotherapy

Zachary A. Cooper, Zain Ahmed and Jennifer A. Wargo

Abstract There have been two major advances in the treatment of metastatic melanoma within the past several years, including immunotherapy and BRAF-directed therapy. Both of these classes of therapy demonstrate survival benefit, but also have limitations as monotherapy with regard to overall response rate and/or durability of response. We have gained significant insight into mechanisms of response to BRAF-directed therapy and to potential synergy between these two treatment modalities. This chapter focuses on the limitations of each of these strategies as monotherapy, and provides the rationale for combining these therapies. Importantly, ongoing clinical trials of combined BRAF-directed therapy and immunotherapy are discussed, as well as considerations and future directions for therapy.

Keywords Melanoma · BRAF · Immunotherapy · Immune checkpoint · Targeted therapy

8.1 Introduction

There have been significant advances in the past few years with regard to BRAF-directed therapy. Despite these advances, resistance to BRAF monotherapy develops in the majority of patients with most patients progressing within 6 to 7 months [1–3]. A better understanding of resistance mechanisms has led to therapeutic strategies that improve responses and enhance survival, including additional MAPK

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blockade via combination BRAF and MEK inhibition. Thus far, such combinations (e.g. BRAF + MEK inhibition) have yielded significant improvements in the durability of response, though most patients still progress within 10 months and only a small fraction of patients achieve a CR or prolonged PR [4]. More sustained responses are clearly needed, and other combinations are currently being tested in preclinical studies and in clinical trials.

In addition to advances in targeted therapy, significant headway has been made with regard to immunotherapy for melanoma. Several immunotherapy agents are currently approved by the US Food and Drug Administration (FDA) for the treatment of metastatic melanoma, including cytokine-based therapy with interleukin-2 (Aldesleukin) and the immune checkpoint inhibitor targeting Cytotoxic T-lymphocyte antigen 4 (CTLA-4) called ipilimumab. Several other agents are currently under investigation in the context of clinical trials (immune checkpoint inhibitors targeting programmed death receptor 1 [PD1] and its ligand [PDL1]), and have shown promise in early phase studies [5, 6]. The advantage of immunotherapy over BRAF-directed therapy is that responses are often durable, however the drawback is that overall response rates remain low (10–15 % in the case of Ipilimumab), with a minority of patients obtaining an objective response [7].

There is increasing evidence that BRAF-directed therapy may synergize with immunotherapy [8–13], with the potential to maintain high response rates while extending the durability of responses. Evidence regarding potential synergy is presented herein, and ongoing clinical trials combining these strategies are discussed. Finally, important questions are posed with regard to potential issues of toxicity, timing and sequence of the different strategies, and the duration of therapy.

8.2 Rationale for Combination BRAF-Directed Therapy and Immunotherapy

8.2.1 Limitations of BRAF-directed Therapy

Functional redundancy and compensatory activity through alternate signaling pathways might explain the emergence of resistance seen in patients treated with selective BRAF inhibitors. Intense research efforts are focused on resistance mechanisms, and several mechanisms have been identified [14–21]. To address these issues, combination of BRAF/MAPK-targeted therapy with other signal transduction inhibitors, or with conventional chemotherapy has been proposed.

Combination strategies to overcome resistance have gained traction, and the combination of dabrafenib (a BRAF inhibitor) with tremetinib (a MEK inhibitor) has been FDA-approved based on an improved progression free survival (PFS) benefit in comparison to either BRAF inhibitor alone. Specifically, median PFS was extended from under 6 months for BRAF inhibitor monotherapy to over 10 months with combination BRAF + MEK inhibition [4]. Perhaps more impressive is the percentage of patients alive without disease progression at 1 year, increasing from 10 %

for BRAF inhibitor monotherapy to 40% in the setting of combined BRAF inhibition and MEK inhibition [4]. Other strategies combining MAPK inhibition with blockade of additional signaling pathways are currently in clinical trials, however data regarding response rates and durability of response are not yet available.

Despite these advances, most patients progress within a year even with the best of these combination strategies [4]. Nonetheless this incremental benefit in survival provides a window of opportunity to offer novel agents and combination strategies, including combinations with immunotherapy. This strategy can be used on a backbone of BRAF inhibitor monotherapy or with combined BRAF and MEK inhibition, though there are important considerations with each which will be discussed herein.

8.2.2 Limitations of Immunotherapy

Several forms of immunotherapy are either FDA-approved or in clinical trials for the treatment of metastatic melanoma. High dose IL-2 was FDA-approved in 1998 based on its ability to produce durable responses in 6–10% of patients [22]. However, its application has been limited to a select group of patients treated in specialized centers due to its severe and unique acute toxicity [23].

Another form of immunotherapy that is currently FDA-approved for melanoma involves the use of a blocking antibody against the Cytotoxic T-Lymphocyte Antigen 4 (CTLA4) molecule on the surface of T lymphocytes. CTLA4 is an immunomodulatory molecule that functions to down-regulate an immune response [24]. Treatment with a monoclonal antibody that blocks this interaction (Ipilimumab) relieves cytotoxic T-lymphocytes from the inhibitory effects of CTLA4, resulting in an enhanced immune response. Treatment with ipilimumab has shown an overall survival advantage in patients with advanced melanoma in a randomized, placebo controlled trial [7] and received approval by the FDA in 2011. In this trial, patients with previously treated advanced melanoma were randomly assigned in a 3:1:1 ratio to ipilimumab plus a gp 100 vaccine, ipilimumab alone, or gp 100 alone. A significant improvement in median overall survival for patients receiving either ipilimumab containing regimen (median 10 months) relative to patients receiving the vaccine alone (6.4 months) was shown as well as a reduction of the risk of death (ipilimumab + vaccine or ipilimumab alone vs gp 100 vaccine; HR 0.68 or 0.66, respectively). Overall survival rates for the three groups were 44, 46 and 25% at 12 months and 22, 24 and 14% at 24 months, respectively [7].

Other forms of immunotherapy are in clinical trials and have shown promising results. Blockade of the immune-modulatory molecule PD1 on the surface of T lymphocytes has shown significant promise in the treatment of metastatic melanoma with response rates approaching 40% in a phase II clinical trial [5]. Interestingly, responses were also seen in other solid tumors, including renal cell carcinoma and non small cell lung cancer [5]. Monoclonal antibodies blocking the immunosuppressive ligand PDL1 are also in clinical trials, though data regarding responses and durability are not yet mature [6].

Another area of great promise in immunotherapy involves the use of adoptive cell transfer, and includes the administration of autologous tumor infiltrating lymphocytes (TIL) or genetically-modified peripheral blood lymphocytes (PBL) to mediate an anti-tumor response. TIL-based approaches have been quite successful in expert hands [25–27], with response rates ranging from 30 to over 70% depending on the pre-conditioning regimen used [28]. However this therapy is still considered experimental and to date its use is limited to expert centers given the complexity and cost of generating this individualized form of treatment. Nonetheless, strategies are under development to optimize and standardize preparation of this type of product so that its use may be more generalizable. In addition, approaches using transduction of PBL with antigen-specific T cell receptors [29] and chimeric antigen receptors [30] are also underway and have shown some promising results.

The field of immunotherapy has certainly advanced the treatment of patients with metastatic melanoma, and treatment responses are often long-lasting. Unfortunately, only a minority of patients will ultimately benefit from these treatments. Thus a critical question is whether or not we can increase the durability of responses and/ or complete response rate by the addition of BRAF-directed therapy to immunotherapy regimens.

8.2.3 Effects of BRAF Inhibition on the Tumor Microenvironment and Immune System

8.2.3.1 Pre-Clinical Studies

Preliminary evidence suggests that oncogenic BRAF ($BRAF^{V600E}$) may contribute to immune escape in melanoma [31], and that blocking its activity via MAPK pathway inhibition leads to increased expression of melanocyte differentiation antigens (MDAs) [32]. We studied this extensively in the laboratory, and demonstrated that targeted inhibition of the MAPK pathway leads to up to a 100-fold increase in expression of MDAs in melanoma cell lines and fresh tumor digests (Fig. 8.1a) which is associated with significantly enhanced recognition by antigen-specific T lymphocytes (Fig. 8.1b) [9]. This appears to be mediated through microphthalmia-associated transcription factor (MITF), a master transcriptional regulator of melanocytes [9].

Importantly, BRAF-directed therapy does not appear to have deleterious effects on T lymphocytes [8, 9]. This is in contrast to MEK inhibitors, which demonstrate dose-dependent inhibition on T cell function *in vitro* [9]. This has relevance when contemplating combinations of BRAF-directed therapy with immunotherapy, as combination therapy including a MEK inhibitor may potentially have deleterious effects on T cells, which may abrogate any potential synergy.

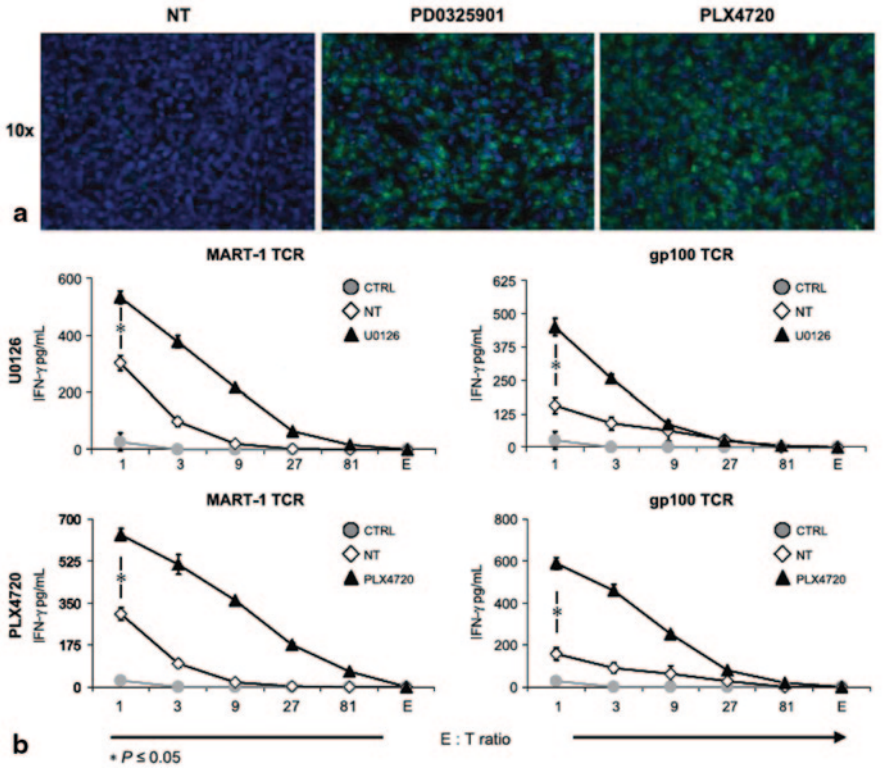

Fig. 8.1 MAPK pathway inhibition increases melanoma antigen expression. Expression of *MART-1* is increased with MEK inhibition and BRAF inhibition (**a**), which is associated with enhanced recognition by antigen-specific T lymphocytes (**b**), HLA-A2+UACC903 melanoma cells were treated as above with a MEK (*U0126*) or BRAF (*PLX4720*) inhibitor and cultured with CTL specific for *MART1* or gp100 versus control lymphocytes (GFP-transduced) at various E:T ratios. *IFN*γ release was measured by ELISA. (Adapted from Boni et al. [9])

8.2.3.2 Clinical Evidence

The first evidence that BRAF inhibition could result in increased immunogenicity in patients with metastatic melanoma was presented and published by several groups in 2012, demonstrating enhanced T cell infiltrates in tumors of patients with metastatic melanoma treated with BRAF inhibitors [33, 34] (Fig. 8.2a). Since these original reports, evidence regarding the immune effects of BRAF inhibition has mounted. In addition to an increase in CD8 T cell infiltrate, treatment with BRAF inhibitors is associated with a decrease in immunosuppressive cytokines IL-6, IL-8 [8] (Fig. 8.2b) and a decrease in vascular endothelial growth factor (VEGF) [12] (Fig. 8.2c). The tumor stroma appears to play a critical role, as stromal cell-mediated immunosuppression via interleukin 1 (IL-1) is induced by oncogenic BRAF and blocked with BRAF inhibitors [13].

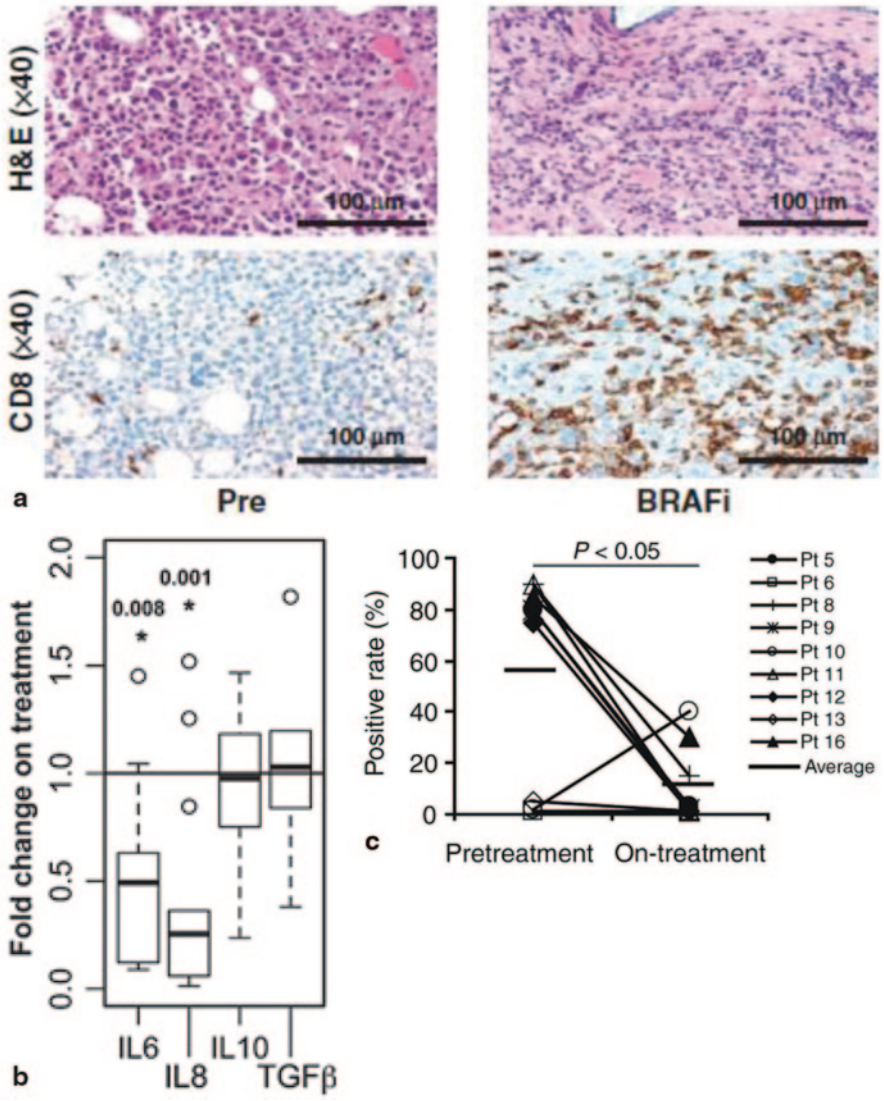

Fig. 8.2 *BRAF* inhibition is associated with increased CD8+ T-cell infiltrate, decreased immunosuppressive cytokines and VEGF in tumors of patients with metastatic melanoma. Patients with metastatic melanoma were treated with *BRAF* inhibitor +/− MEK inhibitor and tumor biopsies were performed before treatment and within 1–2 weeks of initiation of therapy. *CD8*+ T cell infiltrate was assayed via immunohistochemistry (IHC) showing a significant increase of *CD8*+ T cells on therapy (**a**), This was associated with a decrease in IL-6 and IL-8 (**b**), as well as a decrease in VEGF (**c**). (Adapted from Frederick et al. [8] and Liu et al. [12])

An additional piece of evidence supporting the hypothesis that T cells play an important role in response to BRAF-targeted therapy and that BRAF-directed therapy may synergize with immunotherapy comes from analysis of melanoma antigen expression and CD8+ T cell infiltrate in lesions of patients who have progressed on

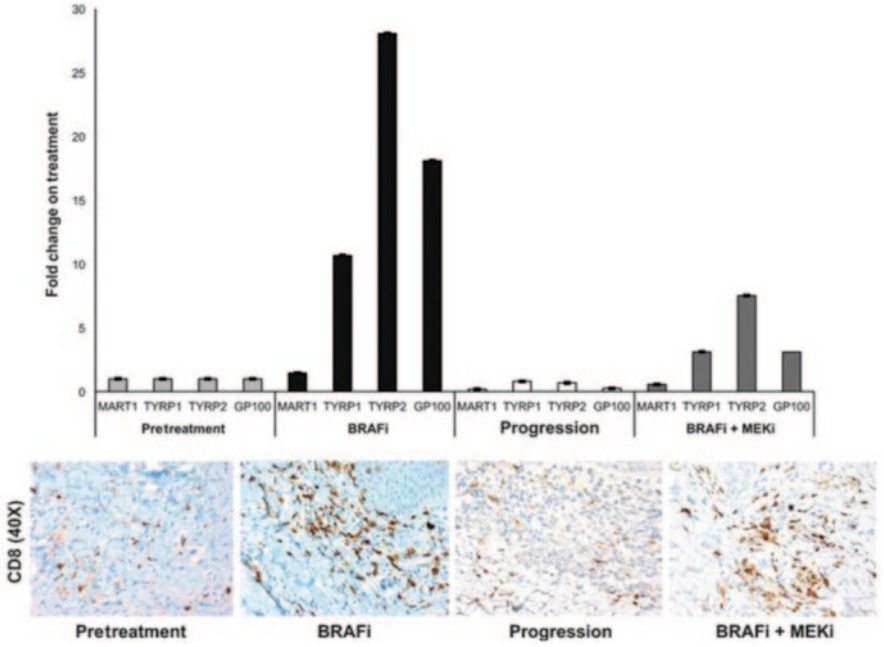

Fig. 8.3 Melanoma antigen expression and CD8+ T-cell infiltrate are decreased at time of progression and restored through MEK inhibition. Tumors were harvested pre-treatment, 10–14 days after *BRAFi* initiation, at time of progression and at time of treatment with combined BRAF inhibition and MEK inhibition for a patient. mRNA levels of the melanoma antigens gp100, MART-1, TYRP-1, and TYRP-2 were assayed. Immunohistochemistry (IHC) was conducted for CD8+ T cells on patient tumor samples. (Adapted from Frederick et al. [8])

BRAF-directed therapy [8]. Based on our initial data, we would expect that resistance to therapy would be associated with a decrease in melanoma antigen expression and a decrease in CD8 T cell infiltrate. We tested this by analyzing melanoma antigen expression and CD8+ T cells in lesions of patients who progressed on therapy and we found exactly what we expected (Fig. 8.3), namely reduced melanoma antigen expression and CD8+ T cell infiltrate at time of progression. Interestingly, if you treat with additional MAPK blockade you can potentially restore antigen expression and T cell infiltrate (Fig. 8.3) [8].

Another insight into tumor—stromal—T cell interactions came with the observation that the infiltrating T cells in tumors of patients treated with BRAF inhibitors demonstrate an activated phenotype and express high levels of PD-1 (Fig. 8.4a) [8]. The PD-1 molecule is an immunomodulatory molecule that serves to down-regulate an immune response after an initial period of activation, functioning normally to prevent autoimmunity. However another critical finding in patients treated with BRAF inhibitors is that the tumor cells themselves express high levels of PD-L1 within 2 weeks of initiation of BRAF inhibitor therapy (Fig. 8.4b) [8]. This may represent a mechanism of resistance, and is corroborated by in vitro work demonstrating high PD-L1 expression in melanoma cell lines resistant to BRAF inhibition [35]. Inter-

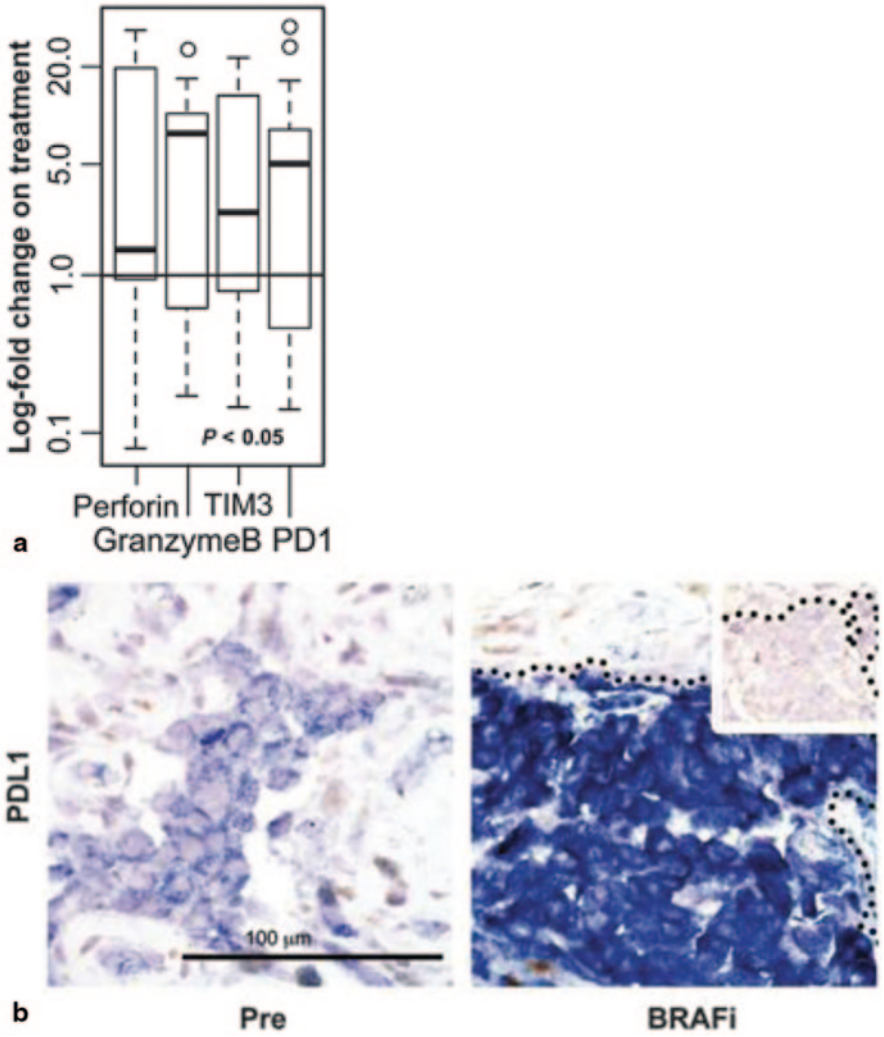

Fig. 8.4 BRAF inhibition is associated with decreased markers of T-cell cytotoxicity but increased T-cell exhaustion markers and PDL1 in tumors of patients with metastatic melanoma. Tumors were harvested and mRNA levels perforin ($n=11$), Granzyme B ($n=11$), TIM-3 ($n=14$) and PD1 ($n=14$; (**a**), in patients with metastatic melanoma undergoing treatment with a selective inhibitor of $BRAF^{V600E}$ were assayed. All patients are expressed in a box and whiskers plot. *Open circles* represent data points greater than 1.5 times the interquartile range. *P* values indicated are from a 2-tailed Student *t* test with a μ of 1, which represents no change in mRNA value with respect to the pretreatment value. *, $P \leq 0.05$. Immunohistochemistry ($\times 40$ magnification) for PDL1 in a representative pretreatment and on-treatment biopsy (**b**). *The dotted line* = tumor–stroma interface and the inset is the isotype-specific control. (Adapted from Frederick et al. [8])

estingly, the addition of MEK inhibition may abrogate the up-regulation of PD-L1 in these cell lines in vitro, which has significant translational implications [35]. Taken together, these data suggest that addition of an immune checkpoint inhibitor to a regimen of BRAF inhibition may augment responses to therapy (Fig. 8.5) [36].

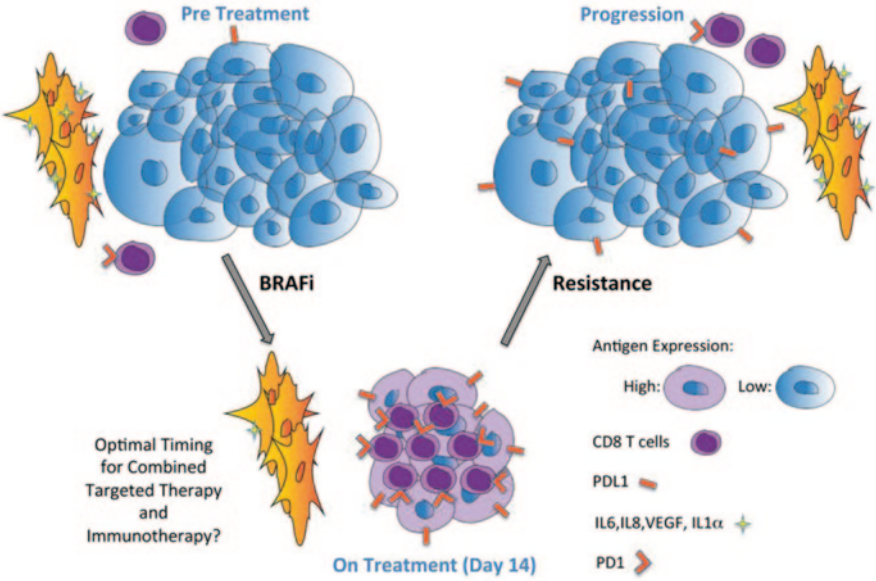

Fig. 8.5 Oncogenic *BRAF* contributes to immune escape through the down-regulation of melanoma-differentiation antigens and by establishing an immunosuppressive tumor microenvironment. The administration of a *BRAF* inhibitor promotes clinical responses along with an increased expression of melanoma-differentiation antigens by malignant cells, an increased tumor infiltration by CD8+T cells, and a decreased production of immunosuppressive cytokines such as *IL-6*, *IL*-8 and *IL-1* as well as of the angiogenic mediator vascular endothelial growth factor (VEGF). This phenotype is reverted at time of disease progression. Importantly, the expression of immunomodulatory molecules on T cells (e.g., *PD1*) and on tumor cells (e.g., *PDL1*) is also increased within 14 d of BRAF-targeted therapy initiation. Taken together, these data suggest that the therapeutic potential of BRAF-targeted agents may be significantly improved by the early blockade of immune checkpoints. (Adapted from Cooper et al. [36])

8.2.3.3 Murine Models

Mouse models have provided important insights into cancer development, progression, therapy, and resistance. Recent melanoma models have incorporated interactions of several signature mutations found in human melanoma, enabling the generation of a mouse that recapitulates hallmark features of the disease. To date, several studies have been published showing synergy of BRAF-directed therapy in murine models [10–12, 37] and one study has shown no synergy [38].

The first model demonstrating synergy was published by Koya, et al. and utilized a $BRAF^{V600E}$-driven murine model of melanoma, SM1, which is syngeneic to fully immunocompetent mice. In this mouse model of $BRAF^{V600E}$ melanoma, Koya et al. showed improved anti-tumor activity, in vivo cytotoxic activity, and intratumoral cytokine secretion by adoptively transferred cells in combination with a BRAF inhibitor [10]. However, T cell analysis also showed that BRAF inhibition did not alter the expansion, distribution or tumor accumulation of adoptively transferred T cells [10].

Another model demonstrating synergy between BRAF-directed therapy and immunotherapy was published by Liu, et al. In this manuscript, the authors used melanoma cells transduced with gp100 and H-2Db in a xenograft model on pmel-1 TCR transgenic mice on a C57BL/6 background and found an increase in tumor infiltrate and anti-tumor activity of adoptively transferred cells after BRAF inhibition (Fig. 8.6a) [12]. In this model, BRAF inhibition induced T-cell infiltration that was associated with a decrease in VEGF (Fig. 8.6b). In this paper they also found that VEGF overexpression in melanoma cells abrogates T cell infiltration [12]. This corroborates what is seen in patients treated with BRAF-directed therapy, as down regulation of intratumoral VEGF correlates with increased T-cell infiltration when melanoma patients are treated with a BRAF inhibitor [12].

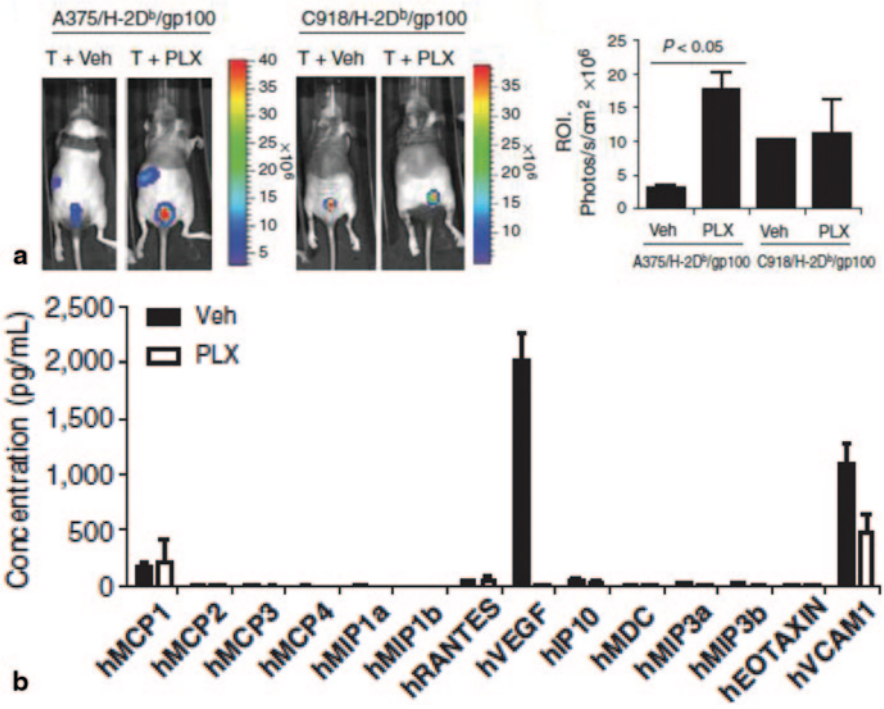

Fig. 8.6 PLX4720 increases infiltration of adoptively transferred T cells only in tumors containing $BRAF^{V600E}$. B6 nude mice (5 mice/group) bearing $BRAF^{V600E}$ A375/H-2D^b/gp 100 and BRAFT WT C918/H-2D^b/gp 100 tumors were treated with OFL-expressing pmel-1 T cells, along with gp100 peptide-pulsed dendritic cells, by intravenous injection on day seven after tumor inoculation. 2 days after T-cell transfer, PLX4720 or vehicle alone was administered by oral gavage daily for 3 days. Luciferase imaging showing *in vivo* trafficking of OFL-expressing pmel-1 T cells on day five after T-cell transfer. Quantitative imaging analysis of transferred T cells at the tumor sites is summarized and expressed as the average of photon flux within ROI (**a**), Data shown are expressed as mean + SEM and are representative of two independent experiments with similar results. In addition, BRAF mutant A375 tumor-bearing mice were sacrificed 3 days after oral gavage of PLX4720, and tumors were resected and weighed. Tumors were homogenized and sonicated in lysis buffer containing protease inhibitors. Cleared tumor lysates after centrifugation were tested using protein array analysis (**b**). (Adapted from Liu et al. [12])

Additionaly, Knight et al. utilized two relatively resistant syngeneic variants of $BRAF^{V600E}$-driven mouse melanoma, SM1 and SM1WT1, and a transgenic mouse model of melanoma to illustrate the ability of the BRAF inhibitor, PLX4720, to reduce melanoma CCL2 production Interestingly, host CCR2 was demonstrated in the antitumor activity of PLX4720. While there was no obvious target molecules influenced with in the SM1WT1 tumor, there was an increase in the CD8/Treg ratio in the TILs with PLX4720 treatment. In addition, depleting CD8 + T cells, but not NK cells, were partially required for the therapeutic activity of PLX4720. Combination therapy of BRAF-directed therapies and anti-CCL2 or anti-CD137 antibodies demonstrated significant antitumor activity in these models supporting the therapeutic potential of combining BRAF inhibitors with immunotherapy [11].

Recently, a BRAF(V600E)/$Pten^{-/-}$ syngeneic tumor graft immunocompetent mouse model showed synergy of adding immune checkpoint blockade to BRAF inhibition [37]. In this model, BRAF inhibition leads to a significant increase in intratumoral CD8+ T cell density and cytokine production, similar to effects of BRAF inhibition in patients. Furthermore, administration of anti-PD-1 or anti-PD-L1 blockade together with BRAF inhibitor led to an enhanced response, significantly prolonging survival and slowing tumor growth, as well as significantly increasing the number and activity of tumor infiltrating lymphocytes [37].

One manuscript has been published disputing possible synergy between BRAF-directed therapy and immunotherapy [38]. This manuscript described work utilizing a murine model with conditional melanocyte-specific expression of $BRAF^{V600E}$ combined with Pten gene silencing which leads to development of melanoma with 100% penetrance, short latency, and lung and lymph node metastases. The mice are responsive to BRAF and MEK inhibition. In this paper, primary melanoma tumors were induced via topical Tamoxifen and were then treated with BRAF-directed therapy alone or in combination with immune checkpoint blockade. Of note, the induced melanomas showed histological and immune cell compartment similarities to human melanomas [38]. However, unlike in humans [8, 34], there is a decrease in tumor resident lymphocytes in the setting of BRAF-directed therapy [38]. Furthermore, the addition of CTLA4 blockade did not improve tumor growth control [38].

It is important to note that tumors generated in this model may be implanted into syngeneic C57BL/6 mice, suggesting a potential for a syngeneic subcutaneous tumor model [38]. This is relevant as other groups (including our own) have used this approach with syngeneic subcutaneously implanted tumors and have demonstrated synergy with BRAF-directed therapy and immunotherapy [37]. The syngeneic subcutaneously implanted tumor model in C57BL/6 may better recapitulate metastatic disease, though this is a hypothesis that clearly needs to be tested.

8.3 Ongoing Clinical Trials

Based on promising results from pre-clinical and clinical studies demonstrating potential synergy between immunotherapy and targeted therapy for melanoma, clinical trials are underway to investigate the efficacy and safety of combining targeted therapy and immunotherapy in patients with melanoma positive for BRAF mutations (Table 8.1).

There are several clinical trials studying the combination of BRAF-directed therapy with the FDA-approved agent aldesleukin (interleukin-2). The first of these trials was developed at the Massachusetts General Hospital (Boston, Massachusetts) and is a phase II trial (NCT01754376) of BRAF-directed therapy (vemurafenib) and immunotherapy using aldesleukin (IL-2) in patients with metastatic melanoma harboring a BRAFV600E mutation. In this trial, patients receive a 2 week "lead-in" with vemurafenib and then receive high dose IL-2. The primary endpoints for this trial include efficacy (as measured by progression-free survival and durable response rate) and toxicity and comparisons will be made to historic controls of vemurafenib alone and aldesleukin alone. Importantly, this trial also includes correlative studies to assess for treatment response and immunologic parameters. The target accrual for this clinical trial is 42 patients over a 2 year time period.

A similar study is being run by the cytokine working group (CWG) (NCT01683188), examining the complete response rate to combination therapy of vemurafenib and high dose IL-2 in two cohorts: (1) BRAF V600 mutation-positive metastatic melanoma patients ($n=135$) who receive vemurafenib < 7 weeks before treatment with high dose IL-2 and (2) BRAF V600 mutation-positive metastatic melanoma patients ($n=50$) who receive vemurafenib > 7–18 weeks before treatment with IL-2.

In addition to IL-2 and vemurafenib combination strategies, clinical trials are also assessing the investigational use of adoptive cell therapy (ACT) in patients with metastatic melanoma. A pilot trial (NCT01585415) at the National Cancer Institute (Bethesda, Maryland) is investigating the safety of vemurafenib in combination with the investigational use of ACT of autologous tumor-infiltrating lymphocytes (TILs) in patients with metastatic melanoma. In this interventional study, investigators will first biopsy or resect melanoma tumors from patients ($n=25$) in order to generate and expand autologous TILs ex vivo. Patients will first undergo non-myeloablative lymphocyte depletion by chemotherapeutic agents: cyclophosphamide (60 mg/kg/day IV) on days seven and six and fludarabine (25 mg/day IV) on days five until one. On day zero, patients will receive up to 10^{11} TILs and aldesluekin (a total of 15 doses of 720,000 IU/kg IV every 8 hours). Patients will then start vemurafenib (960 mg) regimen on day one. Similarly, a single-center, Phase II Trial (NCT01659151) has commenced at H. Lee Moffitt Cancer Center and Research Institute (Tampa, Florida) to improve: (1) drop-out rates from ACT and (2) 12 month- PR and CR in patients with metastatic melanoma ($n=60$) that receive a combination of vemurafenib, lymphodepletion using cyclophosphamide and fludarabine plus adoptive cell transfer and high dose IL-2.

Table 8.1 Clinical trials investigating the combination of targeted therapy and immunotherapy

Official title of study	Clinical trials.gov identifier	Study type	Cohorts	Intervention	End-points	Institution/sponsor
COMBAT 1: A Phase II Trial of Combined BRAF-Targeted Therapy and Immunotherapy for Melanoma	NCT01754376	Phase II clinical trial	*N*=49 BRAFV600 mutation positive metastatic melanoma patients	Vemurafenib+aldesleukin	Primary end-point: Efficacy of combination of vemurafenib and aldesleukin as measured by progression-free survival compared to historic controls Secondary end-points: (1) complete, partial and durable response rates (2) overall survival (3) toxicity and safety (4) confirm pre-clinical data that BRAF inhibitors increase immunogenicity of melanoma (5) exploration of biomarkers	Massachusetts General Hospital
A Multi-Center Study of High Dose Aldesleukin (Interleukin-2)+Vemurafenib Therapy in Patients With BRAFV600 Mutation Positive Metastatic Melanoma	NCT01683188	Open-label, uncontrolled, nonrandomized two-arm, multicenter, Phase IV clinical trial	Arm 1: BRAFV600 mutation positive metastatic melanoma patients (n=135) who receive vemurafenib< 7 weeks before treatment with IL-2 Arm 2: BRAFV600 mutation positive metastatic melanoma patients (n=50) who receive vemurafenib> 7–18 weeks before treatment with IL-2	BRAF Inhibitor: Vemurafenib High Dose Interleukin-2: Aldesleukin	Primary end-point: Complete response (CR) rate in BRAFV600 mutation positive metastatic melanoma patients who receive vemurafenib plus IL-2 at 10 (±3) weeks and 26 (±3) weeks from the start of IL-2	Prometheus Laboratories

Table 8.1 (continued)

Official title of study	Clinical trials.gov identifier	Study type	Cohorts	Intervention	End-points	Institution/sponsor
A Pilot Trial of the Combination of Vemurafenib With Adoptive Cell Therapy in Patients With Metastatic Melanoma	NCT01585415	Open label, single group, Phase I clinical trial	*N*=26 BRAFV600 mutation positive metastatic melanoma patients	Combination of non-myeloablative lymphodepletion, vemurafenib, ACT of autologous TIL, and high dose aldesleukin	Primary end-point: safety of combination of non-myeloablative lymphodepletion, vemurafenib, ACT of autologous TIL, and high dose aldesleukin Secondary: Tumor regression and effect of vemurafenib on tumor infiltrating lymphocytes	National Cancer Institute
A Phase II Clinical Trial of Vemurafenib With Lymphodepletion Plus Adoptive Cell Transfer and High Dose IL-2 in Patients With Metastatic Melanoma	NCT01659151	Open-label, Phase II, single-center clinical trial	*N*=60 BRAFV600 mutation positive metastatic melanoma patients	Vemurafenib followed by lymphodepletion with fludarabine and cyclophosphamide chemotherapy, adoptive cell therapy (ACT) with tumor infiltrating lymphocytes (TIL) infusion, and high dose IL-2	Primary end-point: Overall response & drop-out rate Secondary end-point: progression free survival	H. Lee Moffitt Cancer Center and Research Institute
A Phase I/II Trial of Vemurafenib and Ipilimumab in Subjects With V600 BRAF Mutation-positive Metastatic Melanoma	NCT01400451	Phase I clinical trial Phase II clinical trial	*N*=50 BRAFV600 mutation positive metastatic melanoma patients	Ipilimumab (CTLA-4 inhibitor)+vemurafenib	Primary end-point of Phase I trial: Safety and tolerability of ipilimumab and vemurafenib combination Primary end-point of Phase II trial: Overall survival	Bristol-Myers Squibb and Roche-Genentech

Table 8.1 (continued)

Official title of study	Clinical trials.gov identifier	Study type	Cohorts	Intervention	End-points	Institution/sponsor
A Single Arm Open-Label Phase II Study of Vemurafenib Followed by Ipilimumab in Subjects With Previously Untreated V600 BRAF Mutated Advanced Melanoma	NCT01673854	Phase II clinical trial	*N*=45 BRAFV600E mutation positive previously untreated metastatic melanoma patients	Vemurafenib followed by Ipilimumab (CTLA-4 inhibitor)		Bristol-Myers Squibb
A Phase Ib, Open-Label Study of The Safety and Pharmacology of MPDL3280A Administered in Combination With Vemurafenib (Zelboraf®) in Patients With Previously Untreated BRAFV600-Mutation Positive Metastatic Melanoma	NCT01656642	Phase 1 clinical trial	*N*=44 BRAFV600E mutation positive previously untreated metastatic melanoma patients	Concomitant MPDL3280A (PDL1 Blockade)+ Vemurafenib	Primary end-point of Phase I trial: Safety and tolerability of MPDL3280A and vemurafenib combination	Genentech
A Sequential Safety and Biomarker Study of BRAF-MEK Inhibition on the Immune Response in the Context of CTLA-4 Blockade for BRAF Mutant Melanoma	NCT01940809	Phase 1 clinical trial	*N*=40 BRAFV600E mutation positive metastatic melanoma that is surgically unresectable	A 25 day lead in of dabrafenib, trametinib or both followed by ipilimumab or ipilimumab as monotherapy	Primary endpoint: Safety and tolerability of ipilimumab following lead-in of BRAF or MEK inhibitors, either alone or in combination, in patients with BRAFV600 mutant melanoma Secondary end-point: Disease-control rate and response rate for the total treatment period (4 weeks after completion of study treatment)	National Cancer Institute

There are also several clinical trials of BRAF-directed therapy in combination with immune checkpoint inhibitors. The first of these trials was a phase I/II trial of vemurafenib and ipilimumab given concurrently in patients with BRAF mutant melanoma (NCT01400451). This trial involved a run-in of 1 month of BRAF-directed therapy (vemurafenib) alone followed by four infusions of ipilimumab. The primary goal of this trial was to assess safety and to define a schedule that could be used for further clinical trials. The target accrual for this trial was 50 patients, though the trial was stopped early due to toxicity (see discussion below). After the trial was stopped, another trial was opened with sequential (i.e. non-overlapping) administration of these agents. The target accrual for this trial is 45 patients.

Another trial is currently underway investigating the combination of BRAF-directed therapy with immune checkpoint blockade using anti-PD-L1 (NCT01656642). This trial aims to enroll 44 patients with BRAF-mutant melanoma with the primary endpoint of safety and tolerability.

Given the encouraging findings of combined BRAF-directed therapy with MEK inhibition, efforts are also underway to use combined BRAF + MEK inhibition with immune checkpoint blockade using ipilimumab (anti-CTLA-4). A phase I trial is currently underway and involves a 25 day lead-in of dabrafenib, trametinib, or both followed by ipilimumab (NCT01940809). The primary endpoint of this study is safety and tolerability, with a secondary endpoint of disease control rate and response rate. Importantly, biomarkers will also be studied with the goal of identifying potential predictors of response.

8.4 Summary of Responses and Toxicity to Date

Clinical trials investigating combination BRAF inhibitors and immunotherapeutic strategies to address metastatic melanoma remain in the early stages of patient accrual, and mature response and toxicity data are not yet available. However, some interesting data has emerged regarding toxicity with the combination of BRAF-directed therapy (Vemurafenib) and anti-CTLA-4 (Ipilimumab). Specifically, hepatotoxicity was observed in a phase 1 study of the concurrent administration of these two agents leading to closure of the trial to further accrual. Of note, the grade two or three elevations in liver function tests were completely asymptomatic, and resolved after the therapy was discontinued or with the systemic steroid administration [39]. Nonetheless this highlights the potential for unexpected toxicity in these trials, and suggests the need for well-controlled clinical trials, even when combining FDA-approved agents.

8.5 Future Directions

As a classic example of a bedside-to-bench-to-bedside paradigm, results from these trials will set the foundation for future clinical and translational studies to elucidate potential synergistic effects of combined BRAF-directed therapy and immunotherapy in patients with BRAF-mutant melanoma. Important questions remain and need to be answered. Will there be synergy between these two strategies? Namely, will the combination increase durable response rates and lead to more complete responses? Will there be increased toxicity with these combinations?

Additional questions regarding timing of therapy and duration of therapy also remain. What is the appropriate sequence and timing, and does therapy need to be continued even in the setting of a complete response or prolonged partial response?

There is some question as to whether or not other not synergy will be seen when immunotherapy is combined with other forms of MAPK pathway blockade (e.g. MEK inhibitors), as MAPK pathway activity is critical to T cell activation and may abrogate T cell responses [9]. These questions all beg answers, which will be provided in the context of translational research and carefully planned clinical trials with appropriate correlative studies.

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