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Adoptive T-cell transfer therapy and oncogene targeted therapy for melanoma: The search for synergy

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Abstract

The clinical strengths of immunotherapy and small molecule inhibitors targeting the MAPK pathway appear to be largely complementary for the treatment of advanced melanoma. In current practice, most patients with BRAF V600 mutant melanomas will see both modalities. Several in vitro and in vivo studies suggest that combining immunotherapy with MAPK inhibition may have synergistic effects. First, mouse models show that adoptive cell therapy (ACT) can be enhanced by vaccination. Rapid tumor destruction by vemurafenib could provide a vaccine-like stimulus to adoptively transferred T-cells. Second, both in mice and in early clinical trials, melanoma metastases treated with MAPK inhibitors appear to display increased T-cell infiltrates. Third, MAPK inhibition upregulates the expression of some melanoma antigens and therefore may enhance T-cell recognition of vemurafenib treated melanomas. Fourth, vemurafenib may sensitize tumor cells to immune destruction. Lastly, some investigators have found that an optimal anti-tumor effect from MAPK inhibition is dependent on an intact host immune response. Currently, the Surgery Branch of the National Cancer Institute has initiated a phase II trial combining the BRAF inhibitor vemurafenib with ACT using tumor infiltrating lymphocytes (TIL) in patients with BRAF mutant tumors to investigate the safety and efficacy of this combination. The proposed mechanisms for synergy between these two modalities can be complex and their optimal combination may require testing a variety of sequences and schedules.

Keywords

vemurafenib; BRAF; MAPK; T-cell; Adoptive cell therapy

INTRODUCTION

In the last decade there has been a surge in new treatments for patients with metastatic melanoma. These have fallen into two main categories; immunotherapies and targeted therapies. A simplified characterization of these two approaches is that immune-based therapies such as interleukin-2 or ipilimumab (a CTLA-4 blocking antibody) have low response rates, but can achieve long-term disease control and even curative outcomes in some patients, while treatment with small molecules targeting activated oncogenes within the MAPK-pathways (such as BRAF V600-, NRAS-, or cKIT mutated melanoma) can show dramatic initial response rates but achieve few if any durable regressions. Therefore, there has been considerable interest in rationally combining or sequencing these treatments to

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overcome their individual shortcomings. More detailed information about CTLA-4, anti-PD-1/PD-L1, inhibitions of CDK4, senescence induction, MEK inhibition, as well as targeting the PI3K-AKT pathway will be reviewed elsewhere in this edition (1, 2, 3).

As noted, the strength of immunotherapy is its potential for curative outcomes in patients with widely disseminated metastatic melanoma. This has been true from the first use of recombinant interleukin-2 (IL-2) in 1984; with some patients from that early experience remaining in complete response to this day (4). This also appears to be the case for newer agents such as ipilimumab, with ongoing complete responses documented in excess of 5 years (5). Nevertheless, the frequency of such outcomes with either agent is generally 5% or less. Perhaps the most promising immunotherapy, although not FDA-approved, is adoptive cell therapy (ACT). ACT involves the direct administration of autologous ex-vivo expanded tumor reactive T-lymphocytes to properly preconditioned recipients. The largest clinical experience used tumor infiltrating lymphocytes (TIL) grown in vitro from a patient's own metastasis using IL-2 and anti-CD3 antibody, and re-administered following lymphodepletion of the recipient. Such melanoma TIL can be shown to recognize their autologous tumor in over 75% of patients. Preparation of a culture of TIL for administration requires initial surgery, 4–5 weeks of in vitro culture and preparative lymphodepletion using agents such as cyclophosphamide, fludarabine and in some protocols, total body irradiation. The adoptive transfer of expanded T-cells is typically administered only once and complete and partial responses can endure for years. The largest published experience with significant follow up showed an overall objective response rate of 56% in 93 patients (given a variety of lymphodepleting regimens) with ongoing complete responses in 19 patients (21%) with 5–9 year follow-up (6). Other investigators have published their experience with TIL adoptive transfer and report overall initial response rates of approximately 50%, again with complete responses (7, 8, 9).

The development of active oncogene-targeted therapy for metastatic melanoma was sparked by the discovery that approximately half of human melanomas contain an activating mutation in codon 600 of the BRAF gene (normally encoding valine) and the majority resulted in a substitution of glutamic acid. Vemurafenib is a small molecule that blocks V600E mutant BRAF kinase function. As reviewed more thoroughly elsewhere in this volume, the oral administration of vemurafenib twice daily to patients with BRAF mutant tumors has been highly effective in inducing tumor regression and delaying tumor progression. Chapman, et al. reported a Phase III clinical trial with vemurafenib versus dacarbazine in 675 patients with metastatic melanoma and the BRAF V600E mutation. They demonstrated significantly improved rates of overall and progression-free survival in patients treated with vemurafenib. Vemurafenib increased median progression free survival to 5.3 months compared to 1.6 months in the dacarbazine arm ($p < 0.001$). At six months, overall survival was 84% in the vemurafenib group and 64% in the dacarbazine group (10). However, only 8 of 132 (6%) of evaluable patients treated with vemurafenib achieved a complete response, and the majority of responding patients progressed within a year of therapy (11). Notwithstanding the frequent and rapid onset of tumor regressions mediated by vemurafenib monotherapy, in patients with BRAF-mutated melanomas, this targeted therapy does not appear to be capable of mediating curative outcomes. Similar results were obtained with dabrafenib, another highly-specific BRAF-inhibitor (12).

More recently, combining dabrafenib with trametinib, a MEK inhibitor, achieved even higher initial response rates than dabrafenib monotherapy with a strong indication of improved progression free survival and decreased toxicity. Flaherty, et al. conducted a phase I and II trial with dabrafenib, a selective BRAF inhibitor, and trametinib, a selective MAPK kinase (MEK) inhibitor, in combination. One hundred sixty two patients were randomized in 1:1:1 ratio to receive dabrafenib 150mg twice a day alone or with trametinib either at 1mg or

2 mg once a day. With a median follow-up of 14.1 months (range 10.8–17.6 months), median progression-free survival for patients in the 150/2 combination group was 9.4 months, and 5.8 months for the dabrafenib monotherapy group ($p<0.001$). At one year, 41% of the 150/2 combination group was alive and progression free versus 9% of the monotherapy group ($p<0.001$). Median overall survival was not significantly different although 80% of the monotherapy group had crossed over to the 150/2 regimen (13). Median overall survival with MEK inhibition may be lower than combination based on trials of MEK inhibition alone (14, 15). This clearly illustrates the therapeutic potential of targeting the MAPK pathway in patients with melanoma, but even these results do not determine if curative outcomes can be achieved.

The clinical benefits of immunotherapy and agents targeting the MAPK pathway appear to be complementary and for most patients with BRAF mutated melanomas, they will see both modalities sequentially during the treatment of their disease. This has led to a strong interest in rationally combining these modalities in an attempt to achieve synergy. On initial scrutiny, there was little to suggest such an outcome other than a fervent desire to achieve a high frequency of durable complete responses. This review will examine the data that has since been generated which suggests that such a synergistic result could be seen. These data lead to several hypotheses by which combinations of targeted agents and adoptive cellular therapy could be combined. First, mouse models show that ACT can be enhanced by vaccination and rapid tumor destruction by vemurafenib may provide a vaccine-like stimulus to adoptively transferred T-cells. Second, vemurafenib appears to increase T-cell infiltrates in tumors in mice and in early clinical data. Third, vemurafenib has been shown to upregulate the expression of some melanoma antigens and therefore may enhance T-cell recognition of vemurafenib treated melanomas. Fourth, vemurafenib may sensitize tumor cells to immune destruction. Lastly, some investigators have found that an optimal result from vemurafenib is dependent on an intact host immune response. Since some studies suggest that MEK inhibitors may actually inhibit T cell proliferation (13, 16), it may be counterproductive to add MEK inhibition to a combination of BRAF and ACT. Currently, the Surgery Branch of the National Cancer Institute has initiated a phase II trial combining vemurafenib with ACT using TIL in patients with BRAF mutant tumors to investigate the safety and efficacy of this combination.

1. AN ENDOGENOUS VACCINE-LIKE EFFECT FROM BRAF INHIBITION

Preclinical evidence from murine models indicates that the effectiveness of ACT can be improved by concurrent therapeutic vaccination against the nominal antigen recognized by the transferred T-cells. Overwijk, et al. found that when the B16 murine melanoma is treated with T-cells transgenic for a T-cell receptor against the gp100 antigen, the addition of a recombinant pox virus vaccine enhanced the in vivo proliferation potential of the transferred T-cells and greatly increased the anti-tumor efficacy (17). When one transfers autologous tumor derived TIL clinically, the repertoire of antigens they recognize from the autologous tumor is broad and largely unknown (including antigenic tumor specific mutations). Consequently, complementing the full antigen specificity of adoptively transferred TIL with an “off-the-shelf” standardized vaccine would not be feasible. Alternatively, using the tumor antigens released from in vivo killed autologous melanoma cells during the cytoreductive phase of MAPK-targeted therapy as a source of “endogenous” vaccine is an attractive concept with the potential for stimulating the patient’s immune system against the full range of autologous tumor specific antigens.

The proliferation and activation status of anti-tumor effector immune cells are controlled by interaction with “professional” antigen presenting cells (APC; e.g. dendritic cells). After picking up fragments of dead tumor cells, APC will present tumor specific antigens to T-cells both in a HLA Class II restricted manner (through the exogenous pathway) to CD4+ T-

cells as well as Class I restricted manner (by cross-presentation) to cytolytic CD8+ T-cells. When effective, cytotoxic therapies will cause cancer cell death that is associated with an increased uptake and presentation of tumor antigens to the effector immune cells by APC. Heterogeneity is observed with respect to the level of immune stimulation according to the nature of the cytotoxic therapy (18). The “intrinsic” immunogenicity of cancer cell death mediated by BRAF-inhibitors such as vemurafenib or dabrafenib is not yet well established. However, taking into account the rapid and often extensive cytoreductive effect of these agents, they mediate a massive shedding of tumor specific antigens. Alignment of the adoptive transfer of TIL with the window where presentation of antigens derived from BRAF-inhibitor killed melanoma cells is present is likely to be beneficial. Further benefit may be achieved by offering an immunostimulatory conditioning that activates the APC repertoire. Already treatment regimens involving ACT also involve co-administration of immunostimulatory cytokines, for instance interleukin-2 that could potentially increase the benefit of presenting captured tumor antigens via APC to the adoptively transferred T-cells. Yet a vaccine-like effect from vemurafenib with TIL is still only theoretical and there is no direct preclinical evidence that supports this concept.

2. ENHANCED INFILTRATION OF MELANOMA METASTASES BY T-CELLS

Several groups have described increased rapid infiltration of tumors by T-cells after vemurafenib treatment (19, 20, 21). Wilmott, et al. observed an increase in the density of tumor-infiltrating lymphocytes within and around the metastases in biopsies taken early after commencement of treatment. They enrolled patients with metastatic melanoma who had mutated BRAF and treated them with either dabrafenib or vemurafenib. Biopsied tumor specimens were collected prior to BRAF inhibitor treatment and 3–15 days after initiation of treatment and at disease progression. They found that CD8+ lymphocytes increased while the size and metabolic activity of tumors decreased. The density of lymphocytes then decreased at disease progression. The results were less compelling for CD4+ cells (21). The mechanisms by which this occurs are not known. Liu, et al. found similar results in mice (20); however, to the contrary, Hooijkaas, et al. found PLX4720 treated mice had decreased frequency of tumor-resident T cells (22). These observations were also not replicated in a murine tumor model expressing mutated BRAF, but that tumor model showed very limited sensitivity to vemurafenib (23). Our initial experience in a protocol combining vemurafenib with TIL adoptive cell therapy is in line with the observation of Wilmott that increased T-cell infiltrates are seen within 2 weeks of initiating therapy. This raises the possibility of several variations on the sequencing of treatments to garner different benefits and this will be discussed below.

3. INCREASED ANTIGENICITY OF MELANOMA CELLS

Early studies on inhibition of MAPK signaling testing a variety of small molecule inhibitors on melanoma lines showed that these could enhance the expression of the melanoma/melanocyte differentiation antigens (MDA), MART-1, gp100 and tyrosinase (24). More recent in vitro studies by Boni et al. suggest that PLX 4720 (a vemurafenib-like inhibitor of V600 mutant BRAF) would enhance T-cell recognition of melanoma without affecting lymphocyte function, supporting the rationale of combining vemurafenib with T-cell therapies. They used melanoma cell lines with or without a BRAF mutation, and compared PLX4720 to inhibitors of wild-type MEK (acting within the same pathway). When melanoma cell lines were treated with the wild type MEK inhibitor, all lines showed increases in expression of MDA (while targeting wild type cells), while treatment with the selective BRAF inhibitor only increased antigen expression in the BRAF-mutant lines (which had higher avidity). This was also done using fresh tumor digests and showed similar results. (Figure 1). They also showed that in vitro immune recognition by MDA-specific CTL was enhanced. (Figure 2). Finally, they demonstrated that BRAF inhibition at clinically

relevant dose levels did not intrinsically affect viability, proliferation or cytokine secretion of T-cells (16). Recent clinical data suggest that the combination of BRAF and MEK inhibitors are more active in antitumor responses although MEK inhibitors are not tumor specific and may be immunosuppressive (13, 16). Others have suggested that inhibiting mutant BRAF can also upregulate HLA molecules, facilitating antigen recognition by T-cells (25).

4. IMMUNOSENSITIZATION

A fourth concept supporting the combination of BRAF inhibition and ACT revolves around the concept of immunosensitization, where a pharmaceutical agent that inhibits an oncogenic driver function in cancer cells could also sensitize these same cancer cells to immune destruction. Vemurafenib may make residual melanoma cells more sensitive to immune damage. Not only does it increase cell-surface ligands for immune effector cells, it may trigger an intracellular pro-apoptotic process that would enhance immune effector cell destruction of cancer cells. In some systems, proapoptotic modulation of the bcl-2 pathway can predispose tumors to T-cell killing, and although not clearly documented, that could also apply to MAPK inhibition (26, 27). However, Hooijkaas, et al. did not see increased apoptosis in BRAF treated melanomas in a mouse model (22).

Koya, et al. developed a BRAF V600E-driven transplantable murine model of melanoma, SM1, with low to moderate sensitivity to vemurafenib in vitro and in vivo. In two adoptive transfer models (one with genetically engineered T-cells targeting the artificial antigen, OVA introduced into SM1 and the other with transgenic T-cells targeting the endogenous antigen gp100), modest improved anti-tumor effects were seen with the addition of systemic vemurafenib. They found an increase in antigen-specific cytotoxicity in the splenocytes of mice receiving vemurafenib and ACT and increased IFN-gamma secretion on antigen restimulation in the lymphocytes infiltrating the tumors in both these mice. These experiments did not clearly determine if the effects of vemurafenib were on the T-cells directly or via effects on the tumor and its subsequent stimulation of T-cells (23). Khalili et al, looked at the effects of introducing mutant BRAF into melanocytes or wt BRAF tumor lines and saw the induction of IL-1 that could then be inhibited by vemurafenib. They then showed that IL-1 could induce expression of the ligands for PD-1 (a T-cell inhibitory receptor) on tumor stromal cells (28). Thus vemurafenib could potentially reduce the degree of TIL immunosuppression by this complicated mechanism. On the other hand, Frederick, et al. noted an increase in the inhibitory markers TIM3, PD1, and PDL1 on human TIL and tumors after BRAF inhibition in melanoma patients (19). Because markers such as PD-1 are induced on T-cells after they are activated, it is not clear if this finding was in response to increased T-cell activation or a direct effect of MAPK inhibition. Yet it points out the need for a concerted approach to immunotherapy to achieve optimal results.

5. INTACT IMMUNE SYSTEM NECESSARY

The final concept supporting a combination of BRAF inhibitors and cellular immunotherapy comes from evidence that optimal response to targeted therapy may require an intact immune system and a vigorous anti-tumor immune response. Knight, et al. demonstrated that immunodepleting mice during treatment of a BRAF-mutant murine melanoma with PLX4720 attenuated the efficacy of that treatment. Antibody depletion of CD8+ cells (or total T-cell deficiency in Rag KO mice) decreased efficacy of PLX4720 therapy that did not occur with CD4+ or NK cell depletion alone. They also showed that mice deficient in both IFN-g and perforin had lesser responses to PLX4720. They present circumstantial data to support the concept that a reduction in the tumor-produced T-cell chemoattractant CCL2 (caused by BRAF inhibition) reduces tumor-infiltrating T-regulatory cells, enhancing a native CD8-based immune response against the tumor (29). Although it is not clear if this is

a general finding or if the proposed mechanism is specific to this tumor model, it serves as an example of a principle with much wider implications. It is clear that the tumor-bearing host harbors many immunosuppressive mechanisms dependent on the presence of the tumor. These include not only T-regulatory cells and immunosuppressive myeloid cells, but a host of soluble factors and cell surface ligands associated with a vigorous tumor microenvironment that can inhibit T-cells and render tumor cells resistant to apoptotic destruction like TIM3, PD-1, and PD-L1 (19, 30). The sudden disturbance of this microenvironment associated with catastrophic tumor disruption from MAPK inhibition may open a window of opportunity for a more effective immune attack (via any of the mechanisms described above. Because this window may only be open for a brief time, the ability of adoptive T-cell transfer to deliver the immune attack quickly and in full force makes it more attractive than other approaches (such as vaccination or checkpoint blockade) which often take longer to reach their maximum efficacy in patients.

VEMURAFENIB/TIL PROTOCOL

At the Surgery Branch of the National Cancer Institute, a pilot trial has begun combining vemurafenib with TIL adoptive cell therapy in patients with BRAF-mutant melanoma. The objective is to determine the safety and immunological effects of administering vemurafenib before and concurrently with TIL and high dose aldesleukin after a non-myeloablative lymphodepleting preparative regimen. Secondary objectives are to gain information on how this combination of therapies mediates clinical tumor regression in patients with metastatic melanoma and to study the impact of vemurafenib administration on the lymphoid infiltrate in melanoma deposits by performing serial tumor biopsies. We have treated six evaluable patients with a target accrual of 25 patients.

Patients eligible for the protocol, and documented to have BRAF V600E mutant melanoma, undergo resection of a tumor in order to grow TIL. Once TIL cultures have grown to approximately 1×10^8 cells, they are cryopreserved. Patients then undergo staging that includes an FDG-PET scan and begin vemurafenib at 960mg twice a day. After a week, the TIL are thawed and begin a two week final expansion in vitro. After approximately 14 days on vemurafenib, patients are fully restaged and if possible, they undergo an additional tumor biopsy. While vemurafenib is continued, the patient begins the lymphodepleting preparative regimen consisting of 60mg/kg cyclophosphamide daily for two days followed by 25mg/m² of fludarabine daily for five days prior to cell infusion and aldesleukin administration. The TIL infusion is typically between 1×10^{10} to 1×10^{11} TIL cells followed by the administration of high-dose aldesleukin at 720,000 IU/kg every 8 hours as tolerated. Patients will be evaluated for response 4–6 weeks following completion of aldesleukin and monthly thereafter. Vemurafenib will be continued until the patient is taken off study or has disease progression. (Figure 3).

As permitted, biopsies of cutaneous and subcutaneous tissue will be obtained prior to the start of vemurafenib, prior to the start of the preparatory regimen, and after TIL infusion. Biopsies will evaluate antigen expression by the tumor and the reactivity of lymphocytes grown from these biopsies. (Figure 4). It is not clear if the administration of a lymphodepleting regimen after initiating vemurafenib (but before infusing tumor-reactive TIL) might have a detrimental effect on vemurafenib efficacy. If BRAF inhibition is serving as an “auto-vaccination,” the optimal timing of such supportive vaccines is not known. Exploiting the upregulation of target antigens by vemurafenib would suggest the sequence we have chosen, but to take advantage of the increased T-cell infiltrates in melanomas treated with vemurafenib, one might consider initiating vemurafenib before tumor harvest for TIL. On the other hand, if any of the action of vemurafenib is mediated by resident immune cells as suggested by Knight et al., the lymphodepletion that accompanies ACT

could be detrimental to vemurafenib efficiency. Therefore, depending on long term outcomes and correlative immunological studies, the timing of TIL harvest, TIL therapy, and initiation of vemurafenib could all be reconsidered. Preliminary results indicate that combining vemurafenib with TIL ACT in this fashion is safe, associated with high initial response rates and there have been no unexpected new toxicities in the first six patients.

CONCLUSION

The availability of two highly effective new approaches for treating metastatic melanoma calls out for devising rational ways to combine or sequence them. Inhibition of the MAPK pathway in susceptible tumors is associated with excellent convenience, high initial response rates, but eventual resistance is a near universal problem. Adoptive cellular transfer is a more cumbersome and toxic approach, but consistently achieves a higher rate of durable complete responses. These complementary strengths and weaknesses make it intuitively attractive to combine them in an effort to garner the best of both approaches. Yet at this time, we are only beginning to generate the data to understand the mechanisms and to rationally combine them clinically. Much of the preclinical data is either conflicting or incomplete and there are innumerable permutations in the design of combination trials. Nevertheless, because of the potential gains, clinical efforts are ongoing, in parallel with laboratory investigation, to try to achieve this ideal marriage of modalities that would be a leap forward in the treatment of advanced melanoma.

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Figure 1. Increased antigenicity of melanomacells

Increased levels of RNA of melanoma antigen seen with selective BRAF inhibitor in BRAF mutated (A) cell lines and (B) fresh tumor digests versus BRAF wild type melanomas.

Adapted from Boni, et al. (16).

A

B

Figure 2. Enhanced in vitro immune recognition by MDA-specific CTL
(A) Artistic rendition of TCR recognizing MDA. BRAF inhibition would increase MDA expression. (B) IFN-gamma production measured by ELISA increased in PLX4720 treated UACC903 melanoma cells cultured with control lymphocytes or CTL specific for MART-1 or gp100. Panel B adapted from Boni, et al. (16).

Figure 3. The vemurafenib/TIL Protocol Schema

Schema summarizing vemurafenib/TIL protocol. TIL harvest obtained and culture initiated. Patient is started on vemurafenib 21 days prior to TIL infusion (and continued indefinitely). Cyclophosphamide and fludarabine given days -7 through -1. TIL infused on day zero along with systemic interleukin-2. Biopsies are obtained before and after vemurafenib initiation and after TIL infusion.

Figure 4. T cell infiltration from Tumor Biopsies from vemurafenib/TIL patients
Immunohistochemical staining for CD8+ cells from a patient treated on the vemurafenib/TIL protocol at NIH. (A) pre-treatment, (B) after 13 days of vemurafenib.