Synergistic effects of MAPK and immune checkpoint inhibitors in melanoma: what is the best combination strategy?

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Recent advances in the treatment of metastatic melanoma

The use of targeted kinase and immune checkpoint inhibitors have significantly improved the outcome of patients with metastatic melanoma.

MAPK inhibitors & metastatic melanoma

Selective BRAF inhibitors (BRAFi) are highly active in patients whose tumor harbor an activating V600 mutation in the *BRAF* gene (approximately 40–50% of cutaneous melanomas [1,2]). Clinical trials of vemurafenib and dabrafenib achieved response rates of 50–60%; however, disease progression occurred in the majority of patients within 5–7 months [3,4]. The combination of a BRAFi with an inhibitor of the downstream signaling protein MEK (MEKi) enhances response rates (61–80%) and extends progression-free survival to 9–12 months [5–7].

Immune checkpoint inhibitors & metastatic melanoma

Furthermore, immune checkpoint inhibitors have also proven to be extremely effective in the

treatment of metastatic melanoma. These include antibodies specific to PD1 and CTLA-4 which block co-inhibitory binding between T-cell receptors and their corresponding ligand expressed on tumor and antigen presenting cells. Phase I clinical trials utilizing the anti-CTLA-4 antibody ipilimumab, in combination with the anti-PD1 antibody nivolumab, have attained rates of tumor reduction of greater than 80% in approximately 41% of patients with 75% of patients alive at 2 years [8]. The aforementioned clinical trials were recently extensively reviewed elsewhere [9].

Improved treatment strategies are still required for metastatic melanoma

Neither MAPK inhibitor targeted therapies nor immune checkpoint inhibitors achieve both complete responses (10–13% with combined BRAF and MEK inhibition, and 17% with combined nivolumab and ipilimumab [6,8]) and long-term remission in the majority of patients. As a consequence, much research and clinical trial's interest has now focused on identifying the most effective combination and timing of these treatments to

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improve response rates and duration of responses, whilst reducing toxicities. However, understanding the effect of MAPK inhibitors on the melanoma tumor and its microenvironment, including their effects on tumor immunity, may prove critical in determining the optimal combination and timing of different systemic therapies.

Synergistic effects of combining MAPK inhibitors & immune checkpoint inhibitors: preclinical evidence

Preclinical evidence supporting the efficacy of concurrent administration of MAPK and immune checkpoint inhibitors is based on studies that show BRAFi can induce the expression of melanoma-associated antigens [10], reduce MAPK-driven immunosuppression [11], enhance host immune responses [12,13] and stimulate the infiltration of cytotoxic T cells within a tumor [10,14–16]. We recently investigated this further by assessing changes in the levels of cytokines and chemokines in the serum of patients before and during treatment with MAPK inhibitors. This identified significant increases in cytokines that are indicative of a Th1 T-cell mediated response (IFN γ and TNF α) early during treatment [17]. This is of particular relevance because the presence of a Th1 cell mediated immune response has been shown to predict improved responses to anti-PD1 therapy. Powderly *et al.* presented data showing that a higher baseline expression of cytotoxic Th1 T-cell markers (CD8, IFN γ and granzyme-A) correlated with improved response to the anti-PD1 therapy in a Phase I clinical trial that included metastatic melanoma patients (n = 96, MPDL3280A, Phase 1a) [18]. Additionally, they reported that baseline and week 4 on treatment biopsies taken from anti-PD1 responding patients demonstrate an increase in Th1 T-cell marker expression (IFN γ , TNF α and others), while non-responders experienced a decrease in T-cell activation over the same time period [18]. Therefore, MAPK inhibitors could provide strong immunogenic sensitization that drives the immunophenotype of the patient's disease into the high Th1 T-cell expression profile and enhance the rate and duration of responses to both therapies (Figure 1).

Lack of synergistic benefit of sequential administration of MAPK inhibitors & immune checkpoint inhibitors: clinical evidence

Retrospective analyses of clinical trials in which patients received sequential treatment with

ipilimumab and vemurafenib have found that patients who received a BRAFi prior to ipilimumab had rapid disease progression with little or no response to the immunotherapy, although selection bias makes firm conclusions regarding sequencing difficult [19,20]. Other studies suggest that BRAFi treatment can induce early on treatment immune cell infiltration [10,14,21]. However, this infiltrate is transient and is followed by an increase in regulatory T cells (FOXP3), markers of T-cell exhaustion (TIM3 and PD1) and expression of immunosuppressive ligands, particularly PDL1 [10,21]. Therefore, the sequential use of BRAFi followed by immunotherapies may induce an initial BRAFi-related intratumoral immune reaction while the patient responds to therapy. However, at the time of disease progression on BRAFi and the commencement of immune checkpoint inhibitors, the immune response is diminished and the tumor has gained additional immunosuppressive mechanisms induced by the time BRAFi resistance supervenes which leads to the subsequent failure on immunotherapy (Figure 1A). Conversely, retrospective analyses of clinical trial data suggest that BRAFi are as effective even after progression on ipilimumab [22]. However, this sequencing of drugs means response rates of ipilimumab are unaltered and BRAFi resistance remains a problem (Figure 1C). Although these data are derived from a small numbers of patients and are retrospective or *post-hoc* analyses, they seem to suggest that the sequential use of immunotherapies and BRAFi have little effect on durable response rates and first-line BRAFi may even negate the effectiveness of immunomodulators.

Concurrent combination MAPK & immune checkpoint inhibition is most likely to achieve durable responses in metastatic melanoma

On the basis of the above-mentioned preclinical and clinical data, it is appropriate to suggest that concurrent treatment with MAPK and immune checkpoint inhibitors would have the greatest likelihood of achieving more durable and complete responses. Cooper *et al.* [21] reported that addition of anti-CTLA4 whilst on BRAFi therapy sustained an increase in the ratio of cytotoxic T cells to regulatory T cells (FOXP3) in the patient's tumor suggesting that the addition of an immune checkpoint inhibitor may halt the increase in immunosuppressive factors when given early during BRAFi treatment

Figure 1. Sequential and concurrent use of MAPK and immune checkpoint inhibitors for the treatment of metastatic melanoma. (A) Treatment of metastatic melanoma with upfront BRAFi ($\pm$ MEKi) results in cell death and subsequent release of antigens, inflammatory molecules and shifts the tumor-cytokine microenvironment to an immunoresponsive phenotype. However, this response is transient and when MAPK signaling is reactivated the residual tumor has now been sensitized to the host immune response which may render the tumor insensitive to subsequent immune checkpoint inhibitor therapy. Conversely, upfront anti-CTLA-4 and/or anti-PD1 (B) will stimulate a host response in a proportion of patients, many of whom will progress on therapy, and will be given a BRAFi as second-line therapy if their tumor is *BRAF* mutant. The response of the patient to BRAFi may be comparable to front-line BRAFi treatment but neither the immune checkpoint inhibitor therapy nor the BRAFi will have the additive effect and the disease will usually progress through each treatment modality. (C) Concurrent use of an immune checkpoint inhibitor and an appropriate kinase inhibitor will ensure the additive immunosensitizing effect of the BRAFi and the immunostimulatory effects of the immune checkpoint inhibitor therapy are achieved. The BRAFi will likely reduce the tumor burden and drive the tumor microenvironment into an immunotherapy responding phenotype with the release of immunostimulatory molecules and infiltration of cytotoxic T cells. The concurrent immune checkpoint inhibitor will ensure the immune response is maintained and potentially converted to a long-term immune response and tumor eradication.
BRAFi: BRAF inhibitor.

(weeks 2–4). This conclusion is supported by studies in murine models that found the combination of BRAFi and anti-PD1 or anti-PDL1 increased the production of IFN γ , TNF α and the cytotoxic T-cell activation marker granzyme B [21]. This contrasted with the mice treated with BRAFi alone that had an influx of CD8 $^{+}$ T cells that produced IFN γ and TNF α and an increase in PDL1 but no increase in T-cell activation (granzyme B), suggesting functional inhibition

in the T cells in the BRAFi-only treated mice. Several human clinical trials are currently investigating the combination of BRAFi with immune checkpoint inhibitors; however, an initial trial that combined vemurafenib and ipilimumab was discontinued due to hepatotoxicity during the dose escalation phase [23]. In contrast, the combination of dabrafenib and ipilimumab was well tolerated [24], although the addition of the MEK inhibitor trametinib resulted in bowel

perforation in several patients. Combining MAPK inhibitors with anti-PD1 antibodies (which are less frequently associated with grade 3 or 4 toxicities than ipilimumab) may be better tolerated and show improved patient outcomes.

Nevertheless, these findings highlight the urgent need to improve preclinical understanding of the mechanisms of immune action of both therapies, to better anticipate potential toxicities, to maximize opportunities for synergy and to enhance prediction of patient response. Ultimately, it is this knowledge that will inform rational decisions regarding optimizing the best combination and timing of therapies and lead to further improvements in disease outcomes of metastatic melanoma patients.

Future perspective

Preclinical and clinical evidence suggest that combining MAPK inhibitors with concurrent administration of immune checkpoint inhibitors is the treatment strategy that is most likely to provide durable responses in patients with

BRAF-mutant metastatic melanoma. However, the efficacy and safety of such treatment combinations will need to be established in clinical trials before this can be recommended as standard of care. In the future, greater understanding of the regulation of tumor immunity in the tumor microenvironment as well as molecular modifiers of treatment responses will likely provide novel and more effective treatment strategies and may ultimately result in metastatic melanoma becoming a manageable chronic disease in many patients.

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