The kinase inhibitors dabrafenib and trametinib affect isolated immune cell populations

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Metastatic melanoma is frequently fatal. Optimal treatment regimens require both rapid and durable disease control, likely best achieved by combining targeted agents with immunotherapeutics. In order to accomplish this, a detailed understanding of the immune consequences of the kinase inhibitors used to treat melanoma is required.

Following the identification of oncogenic driver mutations in the BRAF kinase in cutaneous melanoma, BRAF inhibitors (BRAFi), such as vemurafenib and dabrafenib, are now standard-of-care treatments for patients with metastatic melanoma bearing V600 BRAF mutations. The durability of clinical benefit is often short-lived, however, with BRAFi resistance developing within a median of 6 to 7 months after treatment initiation (reviewed in) often due to MAPK pathway reactivation or P13K pathway activation. Recent efforts to extend the durability of BRAFi and to reduce side-effects due to paradoxical pathway activation in cells harbouring RAS mutations have focused on combining BRAFi with MEK inhibitors (MEKi), such as trametinib.

Several reports have demonstrated that BRAF inhibition can be pro-immunogenic, with increasing expression of melanoma differentiation antigens and some Cancer Testis antigens in BRAF V600-mutated melanoma cells in vitro and in vivo following BRAFi exposure. Furthermore, treatment of patients with BRAFi is associated with infiltration of the tumor by T lymphocytes, increased markers of T-lymphocyte cellular cytotoxicity, and decreased expression of immunosuppressive cytokines, correlating with response to therapy (reviewed in). Together with the recent clinical successes using antibodies that target the molecular immune checkpoints CTLA4 and PD-1, the combination of kinase inhibition with immunotherapy is now being pursued for the treatment of melanoma (reviewed in).

However, the degree to which kinase inhibition may directly affect immune function remains poorly defined.

In our recent study we examined the effect of BRAFi (dabrafenib), alone or in combination with a MEKi (trametinib), on isolated immune cell populations including lymphocytes and monocyte derived dendritic cells (moDC) in vitro. We found that dabrafenib had no detectable impact on isolated CD4+ or CD8+ T-lymphocyte function and phenotype, or on moDCs, while trametinib, alone or in combination with dabrafenib, modulated immune cell function.

While our results with dabrafenib were similar to the reported effects of vemurafenib on T lymphocytes, the BRAFi BMS908662, has been shown to enhance T-cell proliferation (and reviewed in). Furthermore, a recent retrospective analysis of peripheral lymphocytes reported that vemurafenib, but not dabrafenib, treated patients had decreased peripheral lymphocyte counts and altered CD4+ T-lymphocyte phenotype and function, as compared with baseline samples. Taken together, these findings highlight the need for further comparative studies to decide which BRAFi should best be combined with immunotherapy.

Unlike BRAFi, MEK1/2 inhibitors, including U0126 and PD0325901 block ERK phosphorylation regardless of BRAF mutational status and have previously been shown to reduce T-lymphocyte viability, proliferation, IFNγ production and cytokine secretion in vitro (Fig. 1). We therefore sought to determine the effect of trametinib on isolated T-lymphocytes and moDCs. While dabrafenib did not impair healthy T-lymphocyte function, trametinib, alone or in combination with dabrafenib, reduced viability, proliferation, IFNγ production and cytokine secretion in our in vitro experiments on isolated cells. In addition, the activation of antigen-specific CD8+ T-lymphocytes was inhibited.

ERK signaling helps maintain dendritic cells (DCs) in an immature state, and MEK inhibition has previously been shown to promote maturation of moDCs induced by agents such as lipopolysaccharide (LPS) or tumor necrosis α (TNFα). Similarly, we found that inhibition of ERK phosphorylation with trametinib promoted the maturation of moDCs in the presence of LPS. If matured in the
presence of trametinib, alone or in combination with dabrafenib, moDCs lost their ability to cross-present the tumor antigen NY-ESO-1 in vitro.5

Taken together, our findings showed that the combination of trametinib with dabrafenib can reduce the proliferation and function of isolated human T lymphocytes and modulate moDC cross-presentation (Fig. 1), indicating that the immune enhancing effects of BRAF inhibition can be countered by MEK inhibition in vitro. Despite this, there was no difference in the frequency of CD8+ tumor-infiltrating lymphocytes (TILs) between patients receiving BRAFi alone or BRAFi plus MEKi, although TIL functionality was not assessed.8 It has been suggested that MEKi may affect naïve T lymphocytes more than memory T cells.8 Moreover, in an in vitro study by Jiang et al, MEK inhibition suppressed PD-L1 expression making melanoma cells more susceptible to immune destruction.9 Thus, combining MEKi with blockade of the PD-1 pathway may be clinically useful. Clearly the immune consequences of MEKi, both in isolation and in combination, requires further study in vivo where complex interactions between multiple cell populations occur.

The era of molecularly targeted cancer therapy has arrived, yet many of the agents now in clinical use have multi-faceted, and, so far, poorly characterized effects on the immune and stromal components of the tumor. With recent successes for both kinase inhibition and immunotherapy there is increasing interest in combining these treatment approaches. To optimize such combinations a detailed understanding of the immune consequences of administering such pharmacological agents is needed. This will require studies evaluating the effects of drugs, individually and in combination, on isolated cell populations in both pre-clinical models and in clinical trials designed to prospectively evaluate immune cell function and validate biomarkers alongside clinical endpoints (for a review of completed clinical trials see).10 Such studies will provide the scientific rationale for the selection, timing and sequencing of kinase inhibitors and immunotherapeutics in order to maximise the rate, depth and duration of disease control. Finally, these evaluations should permit
further understanding of the potential for each agent to amplify the anticancer toxicities of the partnered drug as well.

Disclosure of Potential Conflicts of Interest

JSC has honoraria from the speaker bureau of GSK and has served as a consultant/advisory for GSK, BMS and Merck.

MCA has received an honorarium in a scientific advisory role for GSK.

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