Improving immunotherapy for melanoma: models, biomarkers and regulatory T cells

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English summary

Therapeutic advances in the recent decade have revolutionized the treatment of advanced stage cutaneous melanoma, considered to be one of the deadliest forms of skin cancer. Currently approved immunotherapies include the use of monoclonal antibodies, which block the interaction of immune checkpoints like CTLA4 and PD1, expressed on immune cell subsets such as CD8 T cells, with their respective ligands expressed on antigen presenting cells or tumor cells. These immune checkpoint blockade (ICB) therapies have significantly prolonged the duration of response, thus achieving long-term relapse-free and overall survival rates in patients with melanoma. Nonetheless, a subset of patients do not benefit from currently available ICB therapies and require novel approaches.

Lack of response to ICB could arise from low infiltration and functionality of tumor-reactive CD8 T cells. Moreover, the presence of suppressive immune cells in the tumor microenvironment, such as regulatory T cells (Tregs) can also hinder the functionality of CD8 T cells resulting in low response to ICB. Strategies aiming to increase CD8 T cell infiltration, function, and reduce the suppression mediated by Tregs could be combined with existing ICB approaches to provide therapeutic benefit to the non-responding patients. Pre-clinical models that mimic the non-responding subgroup of melanoma patients are essential for research aiming to increase our understanding of resistance mechanisms that prevent durable responses to immunotherapy.

The work described in this thesis intended to bring about a shift in the balance towards pro-inflammatory environment in melanoma by the use of rational combination approaches and reduced suppression by targeting Tregs, all aiming to increase the response to ICB in patients with advanced cutaneous melanoma. Firstly, the need for syngeneic murine models is addressed by establishing novel melanoma models, described in chapter 2. Two cell lines, MeVa2.1 and MeVa2.2, were derived from a single primary tumor induced on $Braf^{V600E}/Pten^{-/-}$ mice. They were then transduced *in vitro* to express the foreign antigen ovalbumin (OVA), obtaining their immunogenic derivatives MeVa2.1.dOVA and MeVa2.2.dOVA, respectively. Despite being derived from the same primary tumor, the cell lines displayed differences in immune-mediated growth control. Taken together, the four novel cell lines recapitulate commonly occurring resistance mechanisms in patients.

Thereafter, in chapters 3 and 4 of this thesis the possibility of combining existing drug classes with ICB has been explored. Histone deacetylase (HDAC) inhibitors are known to modulate immune responses, controlling tumor growth. Chapter 3 of this thesis shows that the class I specific HDAC inhibitor, domatinostat, could be a rational addition to anti-PD1 + anti-CTLA4 in melanoma. Domatinostat addition to anti-PD1 + anti-CTLA4 resulted in immune-modulation leading to lower tumor volume and prolonged survival

of tumor-bearing mice. Chapter 3 of this thesis also describes the DONIMI clinical trial that tested the combination in a neoadjuvant setting in patients with resectable stage III melanoma. In this trial, a baseline IFNγ response score, calculated using the tumor biopsies obtained prior to treatment, was used to allocate patients to IFNγ response high and low arms, respectively. In contrast to the results of the pre-clinical study, however, addition of domatinostat to ICB did not result in immune modulation or increased responses to treatment in patients with melanoma.

In chapter 4, another class IIA specific HDACi, LMK235 was evaluated for its effect on reducing frequency of Tregs. Despite promising *in vitro* results, LMK235 failed to modulate Treg populations *in vivo* providing sufficient evidence to stop pursuing this compound for its therapeutic benefit in melanoma.

The focus of the later chapters of this thesis shifts towards reducing the suppressive function of Tregs in the tumor. Arguing that an increased understanding of the basic biology of Tregs is essential to find relevant therapeutic targets, the metabolic pathways that govern Treg survival and functionality in conditions of tumor microenvironment (TME) have been described in chapter 5.

Delving further into the metabolic advantage of Tregs, chapter 6 of this thesis shows the impact of extracellular lactic acid, often present at high levels in the TME, on the induction of Tregs. The presence of lactic acid resulted in increased induction of Tregs, irrespective of glucose availability. Moreover, acidity rather than the presence of lactate resulted in the observed increase in Treg induction *in vitro* and modulation of pH in the tumor by administration of sodium bicarbonate reduced Treg frequencies *in vivo*.

Finally, in chapter 7, I have discussed the implications of the work of this thesis for melanoma treatment and elucidated the ongoing clinical trials that utilize similar approaches as described in the thesis. I also highlight the need for utilizing biomarker-driven patient stratification approaches in future trials evaluating novel combinations to immunotherapy. In summary, the novel melanoma models described in this thesis provide a platform for pre-clinical research aiming to enhance response to immuno-therapy. The results of this thesis shed light on the limited efficacy of specific HDAC inhibitors in melanoma. In addition, the findings in the later part of this thesis increase our understanding of the metabolic advantage of Tregs in conditions of the TME, which could be harnessed for potential therapeutic targeting in melanoma.