Improving response and reducing toxicity to immune checkpoint blockade therapy in melanoma

Hoefsmit, E.P.

Appendices

A

English summary

Unprecedented success has been made by the introduction of immune checkpoint blockade (ICB) therapy for the treatment of advanced melanoma, improving the prognosis for these patients. This therapy blocks the interaction between tumor cells and immune cells by the use of monoclonal antibodies, thereby preventing immune inhibition, and releasing an anti-tumor immune response. The molecules cytotoxic T-lymphocyte antigen-4 (CTLA-4) and programmed cell death 1 (PD-1) are currently the clinically most studied immune checkpoints, having non-redundant roles in regulating the immune response. These therapies, targeting only one of these checkpoints, have shown efficacy in a proportion of patients. However, combination ICB therapy showed to be more effective, resulting in a response rate of 58% of patients, with a 5-year landmark overall-survival (OS) of 52% (1). Despite this great achievement for the treatment of a once considered deadly disease, not all patients achieve benefit from these therapies. In addition, as these checkpoint molecules are involved in self-tolerance and limiting autoimmunity, blocking their interactions causes immune related adverse events (irAEs) in a substantial group of patients. Therefore, there is a need to improve the outcome for melanoma patients treated with ICB therapy. Therefore, in this thesis, I provide novel insight into response efficacy and resistance mechanisms. Additionally, I focus on mechanisms that could explain the development of irAEs.

Although ICB adjuvant treatment improves the prognosis for patients with surgically resectable melanoma, this might be the suboptimal scheduling of ICB treatment. In **chapter 2**, we describe the promise of neoadjuvant immunotherapy for cancer treatment. In this setting, immunotherapy is given before surgery, allowing to determine treatment response, reducing tumor burden and giving the opportunity to use pathologic response data as surrogate biomarker for recurrence-free survival (RFS) and OS. Moreover, as the major tumor mass, including infiltrated T cells, is still present, neoadjuvant ICB could enhance T cell activation while they are still exposed to tumor (neo)antigens. Pre-clinical studies showed that neoadjuvant ICB treatment might be the optimal setting compared to adjuvant treatment. Encouraging findings from early-phase clinical trials in melanoma support the concept of neoadjuvant therapy. As outlined in **chapter 3**, we observe durable responses upon neoadjuvant ipilimumab (anti-CTLA-4 therapy) plus nivolumab (anti-PD-1 therapy) in patients with stage III melanoma, with a 2-year event-free survival (EFS) of 84%. Especially patients achieving a pathologic response had a good prognosis, showing a RFS of 97%, which was only 36% for patients without a response.

To improve outcome for patients treated with ICB therapy, it is important to identify those, preferably upfront, who are (un)likely to respond to the treatment. In **chapter 3**,

we observe that patients with a tumor baseline high interferon-gamma-related gene expression signature score (IFN-γ score), a signature indicative for tumor inflammation, have a better prognosis. In addition, patients with a high tumor baseline expression of the Batf3 dendritic cell (DC)- associated RNA gene signature (Batf3-DC score), a DC subtype that excels in (tumor) antigen cross-presentation, proved to have a better prognosis, as outlined in **chapter 4**. When either of these scores is combined with tumor mutational burden (TMB), an even stronger association with response upon neoadjuvant ICB is found. Patients with low expression of these markers have a poor prognosis, and this subgroup may benefit from additional therapies. Based on this idea, patients with a low infiltration of Batf3 DCs may profit from therapies that enhance cross-presentation of tumor antigens. This motivated us to conduct a repurposing compound screen (**chapter 4**), to identify molecules that could enhance cross-presentation of tumor antigens by DCs. We discovered 145 compounds that significantly enhanced T cell proliferation after cross-presentation of tumor antigens by DCs. One of these hits is AZD5582, an antagonist of inhibitor of apoptosis proteins (IAPs) cIAP1, cIAP2 and XIAP, which induces DC activation, enhances antigen import from endolysosomes into the cytosol and increases expression of genes involved in cross-presentation. We also observe that AZD5582 has an additive effect to anti-PD-1 treatment in vivo, reducing tumor outgrowth.

In the next part of the thesis, I focus on melanoma patient groups that have a worse prognosis. In **chapter 5**, we characterize liver metastases from patients with cutaneous melanoma (CM) and uveal melanoma (UM). Despite their shared origin (melanocytes), ICB response rates in UM are disappointingly low (ranging from 0% to 15%) (2-4). In this study, we observe a difference in TMB, while there is no difference in the extent of immune infiltration between CM and UM liver metastases. Only the ratio of exhausted CD8 T cells to cytotoxic T cells, to total CD8 T cells, and to Th1 cells, is significantly higher in UM metastases. Therapies that target exhaustion could be a future therapeutic direction for patients with UM.

The response rates and irAEs to neoadjuvant ICB in stage III melanoma are higher compared to stage IV melanoma. In **chapter 6**, we hypothesize that systemic immune suppression might be the underlying mechanism, and therefore, we analyzed plasma and serum for circulating proteins. In this unbiased approach, we show that patients with progressive disease have a higher expression of systemic leucine-rich alpha-2-glycoprotein 1 (LRG1). In addition, we observe that non-responder patients to neoadjuvant ICB with high LRG1 expression have a worse prognosis in two independent cohorts. Together, the findings in this study suggest that LRG1 can be used as a biomarker to identify patients with high risk for disease progression and recurrence. In addition, therapy that targets LRG1 could potentially be combined with neoadjuvant ICB in order to improve efficacy.

In the last part of this thesis, we investigate potential pre-disposition factors that contribute to development of irAEs. Based on the similarity of irAEs to autoimmune disorders, it can be hypothesized that irAEs may be linked to susceptible genetic loci related to various autoimmune disease. In **chapter 7**, we discuss susceptible loci associated with autoimmune disease that could be relevant for ICB-induced irAEs. In **chapter 8**, we focus on ICB-induced neurotoxicity, since these are usually severe and irreversible, and therefore, it is key to identify patients who are more likely to develop neurotoxicity upon ICB therapy. We investigate whether previous infection with neurotropic bacteria and viruses predispose patients to develop ICB-induced neurotoxicity. We show that there is no association between previous neurotropic infections and development of neurologic irAEs. More in depth studies into potential immunological cross-reactivity will give more insight whether this is (one of) the underlying cause of neurotoxicity after ICB treatment.

In the final chapter, I summarize the research presented in this thesis. In **chapter 9**, I discuss the implication of the work in this thesis for the immunotherapy field, contextualize the results and share my view on remaining challenges that need to be addressed to improve the efficacy of ICB therapy.

Together, the work in this thesis provides new insights to improve the response to ICB therapy in melanoma. We demonstrate the promise of neoadjuvant ICB therapy and analyze different cohorts of melanoma patients. This results in the identification of several markers that are associated with prognosis. These markers can potentially be targeted and might facilitate rational combination therapies that can boost the efficacy of ICB therapy. For this purpose, we perform a repurposing compound screen that targets antigen cross-presentation. Collectively, this work increases our understanding of factors that determine ICB therapy efficacy and toxicity, with the goal to identify novel strategies to improve outcome of melanoma patients in a rationale and personal manner.

References

1. Larkin J, Chiarion-Sileni V, Gonzalez R, Grob J-J, Rutkowski P, Lao CD, et al. Five-year survival with combined nivolumab and ipilimumab in advanced melanoma. New England Journal of Medicine. 2019;381(16):1535-46.
2. Luke JJ, Callahan MK, Postow MA, Romano E, Ramaiya N, Bluth M, et al. Clinical activity of ipilimumab for metastatic uveal melanoma: a retrospective review of the Dana-Farber Cancer Institute, Massachusetts General Hospital, Memorial Sloan-Kettering Cancer Center, and University Hospital of Lausanne experience. Cancer. 2013;119(20):3687-95.
3. Kelderman S, van der Kooij MK, van den Eertwegh AJ, Soetekouw PM, Jansen RL, van den Brom RR, et al. Ipilimumab in pretreated metastatic uveal melanoma patients. Results of the Dutch Working group on Immunotherapy of Oncology (WIN-O). Acta Oncologica. 2013;52(8):1786-8.
4. Maio M, Danielli R, Chiarion-Sileni V, Pigozzo J, Parmiani G, Ridolfi R, et al. Efficacy and safety of ipilimumab in patients with pre-treated, uveal melanoma. Annals of Oncology. 2013;24(11):2911-5.