Breaking barriers: unraveling response mechanisms to immunotherapy in breast cancer

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English summary

Tumors are complex ecosystems consisting not only of malignant carcinoma cells but also a diverse range of endothelial, stromal, and immune cells that together form the tumor microenvironment. Throughout the process of tumor initiation, progression and metastasis, cancer cells are in constant communication with these non-cancerous cells. Of particular significance is the immune system, which plays a dual role in cancer. While certain immune populations such as cytotoxic T cells and NK cells can inhibit or eliminate cancer cells when properly activated, other immune cells are manipulated by the cancer to facilitate immune evasion, tumor growth, and metastasis. Cancer cells induce chronic inflammation via the release of cytokines that provoke the systemic expansion, recruitment, and pro-tumor polarization of immunosuppressive immune cells including macrophages, neutrophils, and regulatory T cells (T_{regs}), which impedes the development and effectiveness of the anti-tumor immune response. The fate of the tumor is ultimately determined by the balance between anti-tumor immunity and tumor-promoting inflammation and immunosuppression.

The objective of immunotherapy is to tilt this balance in favor of anti-tumor immunity. Immune checkpoint blockade (ICB), such as the inhibition of the immune checkpoints PD-1 and CTLA-4 used predominantly in this thesis, aims to enhance the activation, expansion, and effector functions of tumor-specific T cells to mount an effective anti-tumor immune response. Since its introduction in 2011, ICB has caused a revolution in the treatment landscape for patients with certain metastatic cancers such as melanoma and lung cancer. However, ICB efficacy is much lower against less immunogenic cancer types such as breast cancer. Durable responses are only seen in 5% of patients with metastatic breast cancer and mainly limited to the triple-negative breast cancer subtype. While response rates can be improved to around 20% by selecting patients on certain biomarkers such as tumor PD-L1 expression and high lymphocyte infiltration, or by combining immunotherapy with chemotherapy, our mechanistic understanding of the underlying biology of immunotherapy response remains limited. A deeper understanding of the mechanisms governing immunotherapy response is needed to rationally design more effective immunotherapeutic strategies.

Previous research on the mechanisms of ICB response has predominantly focused on T cells. However, it is important to recognize that effective immune responses rely on coordinated interactions between innate and adaptive immune cells. Furthermore, mounting an effective anti-tumor immune response requires coordinated crosstalk across various tissues including the lymph nodes, particularly in the context of metastatic disease. It's becoming increasingly clear that tumor-induced inflammation and immunosuppression play a significant role in hindering the success of cancer immunotherapy. In this thesis, I aimed to study the cellular determinants for response and resistance to immunotherapy in primary and metastatic breast cancer and to investigate how we can rationally exploit these mechanisms to improve immunotherapy for breast cancer. I argue that to improve our mechanistic understanding of the biology behind immunotherapy response and resistance in breast cancer, it will be critical to take a holistic approach, studying the crosstalk between innate – adaptive immune cells in the tumor micro- and macro-environment.

Chapter 2 delves into the intricate multistep process of metastasis formation and examines the dual role of the immune system herein. It focusses on the interactions between cancer cells and immune cells as well as the reciprocal communication among different immune cell populations that determine the tumor's fate. By gaining mechanistic insights into immune regulation of metastasis, we shed light on how these interactions can be therapeutically exploited to combat metastasis.

In **chapter 3** we investigate the mechanisms underlying response to ICB in breast cancer by combining unbiased immunophenotyping in breast cancer patients with mechanistic studies in clinically relevant breast cancer mouse models. We discovered a crucial role for a previously unrecognized player in anti-tumor immunity: the eosinophil. Our work reveals the importance of crosstalk between different innate and adaptive immune cell populations in the tumor microenvironment as well as systemically that underlies effective immunotherapy responses. We propose that therapeutic engagement of eosinophils could be a future avenue for improving immunotherapy responses in breast cancer.

In **chapter 4** and **5** we investigated whether simultaneous boosting of anti-tumor immunity and dampening of tumor-promoting inflammation and immunosuppression may improve response to immunotherapy. We focus on two key immunosuppressive mediators: regulatory T cells and neutrophils. **Chapter 4** describes how regulatory T cells (T_{regs}) are unintended targets for ICB therapy. Immunotherapy led to increased T_{reg} proliferation and activation, which limits the efficacy of the treatment. Depletion of T_{regs} during neoadjuvant ICB provoked a tumor microenvironment favorable for ICB response and induced sustained activation of systemic $CD8^+$ T cells, resulting in enhanced metastasis-related survival after primary tumor resection. Our findings highlight the potential of neoadjuvant ICB in combination with T_{reg}-targeting to improve metastasis-related survival, independent of primary tumor response.

In **chapter 5** we study the expansion and pro-tumor polarization of immunosuppressive neutrophils during tumor progression and metastatic spread and its consequences for immunotherapy response. We demonstrate that short-term neutrophil depletion during ICB induces a modest but significant improvement in survival against primary tumors by promoting $CD8^+$ T cell activation in the tumor. Our data underscore the dominant immunosuppressive role of neutrophils in the tumor microenvironment and their impact as a barrier to ICB response. Our findings suggest that strategies targeting neutrophils may enhance the effectiveness of immunotherapeutic approaches in breast cancer.

In **chapter 6** we explored the potential suitability of PD1-IL2v, a new immunomodulatory agent that has shown promise in pancreatic cancer models, for breast cancer treatment. We discovered that combining PD1-IL2v with the chemotherapeutic cisplatin activates both systemic and intratumoral adaptive and innate immunity, leading to effective immunotherapy responses.

Finally, **chapter 7** discusses the findings described in this thesis. I put our findings in the context of existing literature, explore future research directions and propose potential novel therapeutic strategies for breast cancer. Altogether, this thesis sheds light on some of the key mechanisms of response and resistance to immunotherapy in breast cancer, highlighting the significance of the interplay between innate and adaptive immune cells in the tumor microenvironment as well as systemic immunity.