The repercussions of recognition: imprints of T cells on the tumor microenvironment

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APPENDIX A

Summary

This thesis delves into the ways that T cells interact with the tumor microenvironment, the types of tumors that they can be expected to see as immunologically foreign, whether such antigen-recognition detectably leads to the specific destruction of antigen-carrying cells and how to enhance T cell activity to treat cancer patients, specifically those with triple negative breast cancer.

The span of the questions that are addressed in this thesis is perhaps rather broad, considering that present-day PhD theses are supposed to report deeply on an increasingly more narrow topic. What ties the individual projects together, beyond the theme of T cells in the cancer microenvironment, is the need for computational approaches to answer them - my primary area of interest. During my time at the Netherlands Cancer Institute, I have worked on projects that had direct *translational* potential, questions that could directly inform care for patients with cancer. I have also been interested in improving our fundamental understanding of T cell biology, as such insights can eventually, but not necessarily within the span of a PhD, have an impact on clinical practice. The history of science demonstrates that the greatest technological achievements could not have originated in absence of a prior fundamental understanding to fuel them. On a more personal note, exploring nature for the sake of it is in my view oftentimes also more intellectually stimulating.

Taking a step back on cancer, we see a large diversity in the cells that make up a tumor. Cancer cells arise by the accumulation of DNA damage in our own, healthy cells. But the tumors that such cells form are not solely composed of mutated, cancer cells. Many unmutated host cells will also be recruited to contribute to the growing tumor mass. These cells may - for example - provide structural support, or facilitate the perfusion of blood flow through the tumor, in turn important for the dis-

tribution of nutrients and oxygen. Various cell types of the *immune system* will also migrate to the nascent tumor mass. The immune system has classically been understood to clear pathogens, such as bacteria and viruses, but a clear role in the control of cancer, and oftentimes also its facilitation, is now a part of our immunological understanding as well. Cytotoxic T cells, those positive for the cell surface marker $CD8^+$ and capable of killing cells that they can form interactions with, can respond to antigens that directly results from DNA damage. These *neoantigens* are especially attractive from a therapeutic perspective as their expression is restricted to tumor cells. Hence, boosting neoantigen-reactive T cells should result in high *on target* activity, and be free of the *off-target* activity that more traditional treatment modalities (surgical, chemotherapeutic, and radiation therapies) are limited by.

The first part of this thesis is translational, focusing on questions that have the potential to directly affect the clinical care for patients with cancer. We now know that neoantigen-reactive T cells are frequently integral to the clinical efficacy of T cell boosting cancer immunotherapies. Taking a helicopter view in **Chapter 2**, we mapped the number of neoantigens and its diversity across human malignancies. Encouragingly, we found ~50% of assessed cancer samples to be sufficiently rich in predicted (neo)antigens for at least minimal T cell recognition, suggesting widespread applicability of T cell engaging therapies in cancer treatment. This means that a first critical requirement for T cell-mediated tumor control (i.e., 'immunological foreignness') is met. However, it does not imply that all these cancers respond to treatment. Specifically, tumors can throw up ingenious barricades and subvert the immune system in order to preserve themselves during immune attack. Hence, successful immune-mediated tumor clearance will require all environmental conditions to be conducive to tumor clearance, besides the primary requirement for a 'recognition point' (i.e., antigen) on the tumor cell surface.

In **Chapter 3**, we study the application of T cell checkpoint blockade in a triple negative breast cancer. Breast cancer as a whole may not immediately have been expected to frequently respond to immunotherapy, as it is generally lowly infiltrated by immune cells. However, *triple negative* breast cancer is rather highly mutated and so should at least have sufficient antigenic diversity to facilitate T cell recognition. The therapeutic class of T cell checkpoint inhibitors aims to stop a blockade that (cancer) cells can throw up to inhibit T cells and tone down their activity. Importantly, this can only be of use if the tumor is infiltratable by T cells in the first place. In order to increase immune infiltration before administration of T cell checkpoint inhibitors, we assessed different *induction* treatments. Using extensive molecular characterization, we could trace how these induction treatments affected the tumor microenvironment. We learned what clinical responses look like on a molecular level - i.e., massive immune cell activity - and, using longitudinally collected samples, could compare induction therapies in terms of their capacity to yield this immunoactive profile. Although we did see differences between induction therapies, the strongest T cell activating effects could be ascribed - not entirely unexpectedly - to the T cell checkpoint inhibition itself. This latter conclusion has since been replicated in many other studies.

The second part of this thesis delves into fundamental T cell biology in the context of cancer. T cells

are known to release a range of different signalling molecules (cytokines) upon their activation by antigen-positive ('visible') cells. In **Chapter 5**, we investigate the effects of T cells on tumor cells they could not directly recognize, in order to measure the range of cytokine spreading. Of these, based on its mode of secretion, TNF-α has been thought to diffuse far beyond the antigen-positive cells, whereas IFN-γ is thought to act in a more localized fashion, i.e., restricted to the target cell that is being engaged. Given the known effects of IFN-γ (upregulation of MHC molecules that are required for T cell recognition, increased expression of T cell inhibitory receptors), this localized action appeared illogical. Perhaps akin to sounding a battle horn, IFN-γś stimulation of antigen presentation machinery, but also induction of possible feedback mechanisms in case of profound T cell activity, would appear most beneficial to ready tissues for T cell screening, and not as much to modify the transcriptome of (cancer) cells that are already being screened by T cells. In line with this theoretical argument, we found IFN-γ to have large 'field' effects, whereas TNF-α's scope is more focal. Additionally, IFN-γ-experienced cells showed reduced TGF-β signaling, in a manner that appeared orthogonal to IFN-γ's direct effect on TGF-β-responsive genes, indicating a potential shift in (immune) cell activity around IFN-γ-experienced cells.

In **Chapter 4**, we revisit the neoantigen predictions of **Chapter 2** to explore the selective pressure exerted by T cells on developing cancers. Despite robust methodology, this study did not find evidence of neoantigen depletion in treatment-naïve tumors. Several factors could explain this, including limitations of current neoantigen identification capabilities and the possibility that tumors primarily use other mechanisms besides the (genetic) loss of neoantigens to evade the T cell based immune system.

This thesis concludes by discussing future directions for expanding the methodology developed in **Chapter 5**. With further algorithmic innovation, RNA-based signal inference could extend beyond the relatively small range of cytokines we studied in **Chapter 5**. Such methodology could further enhance our understanding of immune interactions in cancer and other immune-related diseases, and thereby inform the development of more effective immunotherapies.