The Pig as a Large Animal Model for Studying Anti-Tumor Immune Responses

The immune system plays a crucial role in cancer development and progression. Cancer immunoediting encompasses three phases: elimination, equilibrium, and escape; together, describing the complex interplay between tumor and immune cells. Specifically, the immune system both protects against cancer but also generates a selective pressure, which may lead to selection of tumor cell variants with reduced immunogenicity; thereby, increasing the risk of tumor escape. Cancer immunotherapy includes treatment strategies aimed at activating anti-tumor immune responses or inhibiting suppressive and tumor-favorable immune mechanisms. One of the promising arms of cancer immunotherapy is peptide-based therapeutic vaccines; yet, no such vaccine has been approved for use in human oncology. For many years, mouse models have provided invaluable understanding of complex immunological pathways; however, the majority of preclinical results are lost in translation from mice to humans. In particular, the success rate when translating therapeutic cancer vaccines has been extremely low; thus leaving room for improvement.

The overall aim of this Ph.D. project was to investigate the potential for the pig as a large animal model for cancer immunology research and preclinical testing of cancer immunotherapies. We hypothesized that a physiologically relevant model with high degree of homology with humans can provide a crucial link between murine studies and human patients. This may increase the success rate when translating preclinical findings in the future.

As T cells are important mediators of anti-tumor immune responses, we first developed an immunization protocol allowing the induction of a cytotoxic T lymphocyte (CTL) response and evaluation of the effect of vaccine antigen dose. Göttingen minipigs received intraperitoneal (i.p.) injections with tetanus toxoid, an exogenous model antigen, formulated in CAF09 adjuvant. We demonstrate induction of a polyfunctional CTL response upon low antigen dose immunization, while a CAF09-formulated high antigen dose generates antigen-specific IgG antibodies.

Secondly, we investigated the effect of antigen dose, when immunizing Göttingen minipigs against Indoleamine 2,3-dioxygenase (IDO); an endogenous target relevant for cancer immunotherapeutic purposes. By repeated i.p. administration of CAF09-adjuvanted IDO-derived peptides, we show a vaccine-induced break in the peripheral tolerance towards IDO and the establishment of an antigen-specific cell-mediated immune (CMI) response. When comparing the different CAF09-formulated antigen doses, we demonstrate the induction of a CMI-dominant response upon exposure to a low endogenous peptide dose. In contrast, a mixed CMI and humoral immune response could be shown following repeated high peptide dose immunization. Together, our data underline the importance of correctly determining the first-in-human vaccine antigen dose, which may be more accurately predicted in a large animal like the pig.

Finally, we performed a T-cell focused immunological characterization of the novel transgenic Oncopig model. Following injection with an adenoviral vector Cre-recombinase (AdCre), these animals develop sarcomas at the injection site resulting from expression of two mutant transgenes: KRASG12D and TP53R167H. We demonstrate pronounced T-cell infiltration to the tumor site with a specific enrichment in both regulatory and cytotoxic subsets when compared to peripheral blood. Thus, Oncopig subcutaneous tumors can be classified as hot in accordance with the Immunoscore classification.

In an in vitro setup, we show immune-mediated specific lysis of autologous tumor cells, underlining the capacity of the Oncopig immune system to mount a cytotoxic anti-tumor response. Using the results from RNA-seq analysis, we propose a potential mechanism for in vivo inhibition of anti-tumor cytotoxicity based on elevated expression of the immunosuppressive genes IDO1, CTLA4, and PDL1 within Oncopig leiomyosarcomas. As a high rate of spontaneous regression of subcutaneous tumors occurs over time, we speculate that the anti-tumor immune responses become dominant at the later stages post AdCre injection; eventually leading to tumor elimination. Combined, our data support that the Oncopig provides a crucial platform for studying anti-tumor immune responses in a large in vivo system, although the model currently only allows preclinical testing of therapeutics against the early stages of cancer.