Attitude Switch: unraveling breast cancer cell-intrinsic mechanisms that manipulate the immune system

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Discussion

Metastatic disease is still the main cause of death for breast cancer patients[1], despite the scientific advances that have been made in developing treatments including chemotherapy, radiation, hormone therapy, targeted therapy and immunotherapy. It has proven very challenging to successfully treat metastatic breast cancer patients because of the interpatient heterogeneity in patient characteristics and tumor characteristics[2], causing patients with the same cancer type to have a different disease outcome or treatment response. Cancer metastasis is a multi-step process that involves cancer cell invasion and migration from the primary tumor, dissemination through the circulation and colonization in distant organs[3]. The fate of disseminating cancer cells is dictated by genetic drivers that activate a migratory behavior via processes like epithelial-to-mesenchymal transition (EMT)[4], but it also depends on environmental cues that influence the interplay between cancer cells and non-cancerous cell types, including fibroblasts, immune cells and endothelial cells, in the tumor microenvironment (TME)[5,6]. Among these non-cancerous cell populations, immune cells are major interactors of cancer cells via secreted factors and the expression of regulatory receptors. The crosstalk between cancer cells and immune cells contributes to the progression of cancer, as cancer cells can prevent their recognition and destruction by hijacking the immune system and immune cells facilitate inflammatory environments that promote the metastatic process[7]. The abundance of specific immune cell populations in cancer patients has been associated with prognosis and therapy response. For instance, the presence of neutrophils in solid tumors or in the circulation of cancer patients has been correlated with poor prognosis[8,9], while tumor-infiltrating lymphocytes are indicative of a favorable prognosis and a good response to immune checkpoint blockade for most cancer patients[9,10]. Current immunotherapies aim at cancer cell destruction by blocking inhibitory receptors on lymphocytes or by adoptive transfer of tumor-specific lymphocytes, but these approaches are not beneficial for all cancer patients. This illustrates the need for the development of novel immunomodulatory therapies for cancer patients. The broad variety of immune cell populations and their dual functions in cancer provide new targets for immune-based therapies, yet finding the modulators of the immune landscape in cancer is critical for better stratification of cancer patients and the implementation of precision medicine.

A better understanding of the cancer-immune crosstalk, the different functions of immune cells in cancer, and the factors that modulate the immune system in cancer will aid the design of novel immune-based therapies for cancer patients. There is a growing recognition of the relationship between cancer genotypes and immune phenotypes in cancer that contributes to the heterogeneity between patients[11-14]. This phenomenon, that has led to novel insights into the cancer-immune cell crosstalk, is highlighted in this thesis in the context of breast cancer. Here, we demonstrate that genetic drivers of breast cancer shape the composition of the immune landscape to influence metastatic disease and therapy response via both local and systemic inflammation. In **chapter 2**, we discuss different cancer cell features, including epigenetic alterations, genetic aberrations, intracellular signaling pathways and metabolic state, that influence the communication with immune cells, with a focus on

neutrophils. Genotype-immunophenotype relationships are facilitated by cancer-immune cell conversations that can be assessed using an *in vitro* method of administration of conditioned medium from cancer cells to immune cells, as illustrated in **chapter 3**. **Chapters 4-6** describe mechanistic studies demonstrating how breast cancer cell-intrinsic genetic aberrations impact local and systemic inflammation, the communication between cancer cells and immune cells, and response to immunotherapy. Distinct immune regulatory mechanisms are described for breast cancer cell-intrinsic loss of *Trp53* (**chapter 4**), *Trp53* mutations (**chapter 5**) and *MET* amplification (**chapter 6**), together illustrating the importance of understanding cancer-induced immune modulation to enable the design of personalized immune-based strategies. In this final chapter, I will delineate the challenges and implications of the work described in this thesis. The interplay between cancer genetics and the immune system that can occur at a multi-organ level makes cancer a systemic inflammatory disease that requires systemic intervention strategies. Such therapeutic opportunities will be touched upon in this chapter, mainly focusing on neutrophil targeting and combining targeted therapies with immune-based therapeutic approaches. Moreover, I will describe how systemic neutrophilic inflammation is connected to distinct genetic backgrounds of breast cancer. The utility of model systems that have been used in the studies described in this thesis, together with an outlook on additional technologies that could improve future investigation of genotype-immunophenotype relationships in cancer will also be discussed. Finally, implications for personalized immune intervention strategies based on the genetic make-up of breast cancer cells are given.

CANCER TRAITS INFLUENCING THE IMMUNE SYSTEM

A major part of the heterogeneity in immune phenotypes that is observed between breast cancer patients is due to tumor-induced remodeling of the tumor microenvironment and alterations in cancer-immune cell and immune-immune cell communication. In this thesis, a profound role for the genetic make-up of breast cancer cells is described in shaping the immune landscape, as proposed in **chapters 4-6** and reviewed in **chapter 2**. However, not solely the genetic make-up of tumors is underlying the heterogeneity in immune composition and function in cancer. Additional cancer cell-intrinsic features that mediate the interaction with components of the tumor microenvironment exist, including epigenetic alterations, intracellular signaling molecules and metabolic pathways[12-14]. Most of these parameters are intertwined and can often be linked to genomic aberrations in cancer cells, but each of these processes can also form exclusive mechanisms of cancer-immune cell crosstalk. Indeed, our findings in **chapters 4-6** illustrate that alterations in cancer cell state, gene expression, or protein secretion can be led back to distinct genetic aberrations. All of these cancer cell-intrinsic processes affect the communication with neighboring cells in the local environment and at distant sites throughout the body. The communication between cancer and immune

cells is facilitated by the secretome that consists of cell-derived molecules, such as cytokines, chemokines, growth factors, extracellular vesicles and cell surface molecules. Here, we demonstrate that the genetic make-up of breast cancer dictates immune modulation and evasion facilitating cancer progression and resistance to immunotherapy. Specifically, the impact of cancer cell-intrinsic alterations in *TP53* and *MET* on immune modulation will be discussed in more detail.

TP53: an immune suppressor?

With *TP53* being one of the most well-known tumor suppressor genes affected in the majority of solid cancers, detailed understanding of its role in immune modulation is of high relevance to develop novel cancer therapies. Aberrant *TP53* gene expression induces cancer cell rewiring that manifests in alterations in cellular processes like proliferation and survival, that ultimately lead to cancer progression, but *TP53* aberrations have also non-cell-autonomous consequences[15,16]. As the guardian of the cancer genome, p53 is a central player in tumorigenesis and cancer progression by facilitating a progressive tumor microenvironment that promotes immune evasion and metastatic disease[15,16]. As of now, limited studies have demonstrated how cancer cell-intrinsic p53 status relates to immune regulatory programs in cancer[17-24]. In **chapter 4**, we describe how loss of p53 in breast cancer cells activates a pro-metastatic inflammatory environment involving multiple immune cell populations. When tumor suppressor gene p53 is lost in breast cancer cells, miR-34a is no longer activated, which alleviates the inhibition of Wnt ligand secretion that subsequently activates IL-1β production by macrophages in the TME. In turn, IL-1β induces a systemic inflammatory response involving an interplay between γδ T cells, neutrophils and $CD8^+$ T cells to promote metastatic disease. Additional reports demonstrate that cancer cell-intrinsic loss of p53 induces distinct immune phenotypes in different mouse models of solid cancer types other than breast cancer. In $p53^{null}$ pancreatic and lung cancer models, cancer cell-intrinsic loss of p53 promotes macrophage recruitment into the TME and their polarization towards T cell suppressive functions[17]. Other studies confirm that cancer cell-intrinsic loss of p53 activity induces an immunosuppressive tumor niche that hampers anti-tumor immunity[18-20]. In PTEN-deficient prostate cancer, recruitment of macrophages and regulatory T cells is mediated via secretion of CXCL17 as a result of *Trp53* loss in cancer cells[18], while in KRAS-mutated pancreatic cancer cells that have lost the *Trp53* gene both JAK2/STAT3 signaling[17] and CXCL10/M-CSF secretion[19] were found to promote an immunosuppressive environment. In a GEMM-based lung cancer cell line inoculation model, loss of *Trp53* reduced intratumoral $CD8^+$ T cell infiltration and MHC-I expression on cancer cells leaving lung tumors irresponsive to immunotherapy, while doxycycline-mediated induction of p53 activity in cancer cells enhanced MHC-I expression and immunotherapy response[20]. Together, these studies indicate that loss of tumor suppressor gene p53 induces distinct immunoregulatory mechanisms, depending on the cancer type and additional genetic aberrations or signaling molecules present in cancer cells, that could be exploited for immune-based therapies.

In the studies described above, a comparison is made between the presence or absence of the p53 gene in tumors, while mutations in p53 also frequently occur across cancer types[25]. Some mutations lead to loss of p53 gene activity, thus recapitulating the $p53^{null}$ situation, and are considered loss-of-function (LOF) mutations, whereas other mutations can have so-called gain-of-function (GOF) consequences for p53 function[26]. **Chapter 5** details how single nucleotide mutations in the p53 gene alter the immune composition of breast tumors. We demonstrate that $p53^{null}$ tumors greatly differ from most p53 mutant tumors in terms of local immune phenotype, while systemic neutrophilic inflammation (as introduced in **chapter 4**) is similar between $p53^{null}$ and p53 mutant mammary tumor-bearing mice. A few p53 mutations lead to an immune-enriched phenotype that is comparable to the observed immune phenotype in $p53^{null}$ tumors, together representing the immunologically enriched mutants. However, the majority of p53 mutant breast tumors have an immune-deprived microenvironment that differs from the $p53^{null}$ situation, indicating a GOF for these p53 mutations. Several potential leads of immune regulation by p53 mutations are described in **chapter 5**, including autophagy, secreted factors and extracellular matrix (ECM) remodeling, but none of those stand out as the single factor that distinguishes immune-enriched from immune-depleted phenotypes, so there may be multiple mechanisms at play that influence the ability of immune cells to infiltrate p53 mutant breast tumors. Taking a closer look at all p53 hotspot mutations separately uncovers a distinct immune pattern for each single mutant (**chapter 5**), indicating that each individual p53 mutant may trigger unique mechanisms of immune regulation.

Nonetheless, our findings described in **chapter 5** show that T cell-inflamed immune phenotypes are related to a distinct group of p53 mutations in multiple murine breast cancer models. Strikingly, the mutant p53-induced immune phenotypes correlated with immunotherapy response, as we demonstrated that the group of p53 mutant breast tumors that show a T cell-inflamed immune phenotype responded better to anti-PD-1 treatment compared to T cell-depleted p53 mutant breast tumors (**chapter 5**). A similar trend of T cell infiltration, although non-significant probably due to a small sample size, was observed in human p53 mutant breast tumors of the TONIC dataset (**chapter 5**), yet additional validation of mutant p53-induced immune phenotypes in samples of human breast cancer patients and its correlation with immunotherapy response will aid the stratification of breast cancer patients that may respond to immune checkpoint blockade based on the p53 status of the tumor. However, a major challenge lies in treating the group of breast cancer patients bearing immunologically deprived p53 mutant tumors that may fail to respond to immunotherapy. For this group of patients, mechanistic understanding of mutant p53-induced immune phenotypes is essential to enable TME remodeling towards an immune-enriched milieu that alleviates immunotherapy resistance. Turning immune-deprived breast tumors into immune-enriched breast tumors is challenging and unfortunately, our attempt of genetically activating ATG2B or pharmacologically activating the autophagy process to promote an anti-tumor immune response via anti-PD-1 treatment in immune-depleted breast tumors was

unsuccessful, since intratumoral autophagy activation was not observed *in vivo* (**chapter 5**). Other studies focusing on single p53 mutations in cancer provided mechanistic insights into mutant p53-induced immune environments and showed that targeting of these processes led to remodeling of the tumor immune landscape. An immune-deprived phenotype was found in murine models of $p53^{R249S}$ breast cancer, $p53^{R172H}$ pancreatic cancer, and carcinogen-induced p53 mutant oral squamous cell carcinoma, in which reactivation of the STING signaling pathway improved cytotoxic immune cell infiltration[22,23] and response to immunotherapy[22] by unleashing type I IFN. Interestingly, in another study that also described a T cell-deprived phenotype in $p53^{R172H}$ pancreatic tumors, blocking of neutrophil recruitment improved immunotherapy response[24]. These studies show that the $p53^{R172H}$ mutation induces a T cell-deprived phenotype, like we described in **chapter 5**, that is regulated by multiple processes, which may depend on other acquired cancer cell-intrinsic features influencing the immune system. These data indicate that the search for a common immune regulator for a group of p53 mutant tumors with a similar immune phenotype may be challenging. However, these findings also illustrate the potential of targeting p53 mutant-induced processes to reshape the immune landscape and promote immunotherapy response in p53 mutant cancer.

Although p53-induced immune regulatory processes comprise promising molecular targets for immune modulatory strategies in cancer patients, other studies have proposed direct targeting of p53 as a therapeutic option for p53-affected cancer patients. Loss of DNA binding capacities of mutant p53 as a consequence of alterations in its protein structure were restored by the use of small molecule treatment[27-29]. Specifically small molecule APR-246 has been shown to restore p53 wild type transcriptional activity by binding distinct cysteine residues in the p53 core domain[29,30] and has undergone clinical trials in several cancer types[31-35]. Notably, some studies demonstrated anti-cancer effects of APR-246 independent of the p53 status of cancer cells, indicating off-target effects of APR-246 that may be applicable beyond p53-mutated cancers[36-38]. Interestingly, one of these studies demonstrated that administration of APR-246 enhances immunotherapy efficacy in a wild type p53 melanoma model via increasing p53 expression in macrophages in the TME[38]. There is very limited data available on APR-246 as a therapeutic agent for mutant p53-induced immune regulatory programs, but one study demonstrated that APR-246 reduced the expression of inhibitory immune checkpoint molecules and elevated expression levels of type I IFN-related genes in p53 mutant cancer cells[39], indicating the potential of APR-246 in impacting immunoregulatory programs and anti-tumor immunity in p53 mutant cancer. Still, restoring wild type function of p53 may not be suitable as an anti-cancer therapeutic for pan-cancer application, since loss of p53 function endows cancer cells with a growth and survival advantage over wild type p53 expression.

A major question that remains is why some p53 mutant breast tumors respond to immunotherapy, while others are resistant, and how immunotherapy resistance in these p53 mutant tumors can be overcome. A clear difference in the number of lymphocytes, but a similar expression profile of activation markers, was observed between p53 mutant breast

tumors (**chapter 5**), suggesting that the difference in efficacy of immunotherapy in different p53 mutant breast cancers depends on the number of activated lymphocytes infiltrating the local TME. Besides lymphocyte infiltration and activation, additional aspects are required for effective anti-tumor immunity, including cytotoxic lymphocyte priming, immune recognition of cancer cells and clearing of cancer cells[40]. As such, the efficacy of anti-tumor immunity in p53 mutant breast cancer may also rely on differential regulation of the antigen-presentation machinery by different p53 mutations. Whether the immunogenicity of distinct p53 mutations is dependent on differences in antigen presentation can be addressed by immunopeptidome analysis that provides antigen expression profiles, including quantity and identity of antigens, of cancer cells expressing individual p53 mutations. Indeed, introduction of the $p53^{R175H}$ and $p53^{R273H}$ mutations in human osteosarcoma cells induced distinct antigen repertoires compared to $p53^{null}$ cancer cells that led to the identification of shared peptides as potential novel molecular targets for immunotherapy in p53 mutant cancers, but it is still unclear how mutant p53 induces unique antigen profiles[41]. Interestingly, the identification of neoantigens derived from mutant p53 shows promise for novel therapeutic options to target mutant p53 cancers[42-45]. Multiple tumor-specific T cell receptors (TCRs) and TCR-mimic antibodies have been demonstrated to recognize these mutant p53-derived peptides[46-48]. Altogether, these findings implicate that mutant p53 targeting in cancer is a promising (immuno)therapeutic strategy that warrants further investigation.

MET: oncogenic regulator of the immune system

While the *MET* oncogene is well-known for its key role in regulating EMT and the migratory behavior of cancer cells that both support metastatic disease, only limited studies delved into the immunoregulatory function of the c-MET receptor in cancer[49]. There are some indications that the progressive nature of *MET*-expressing cancers is due to immune evading strategies, with the majority of evidence pointing at MET-associated increased expression of immune checkpoint molecules in lung cancer[50-53]. In patients with lung cancer, *MET* amplification was associated with reduced circulating $CD8^+$ T cell levels, together with poor survival and resistance to immunotherapy[54]. These studies illustrate that *MET* expression in lung cancer is associated with distinct immune phenotypes that correlate with prognosis and immunotherapy response. Similarly, our results in **chapter 6** demonstrate that *MET* expression in breast cancer induces a distinct systemic inflammatory milieu characterized by neutrophil accumulation and reduced lymphocyte levels in the circulation of multiple *MET*-amplified breast tumor models. Interestingly, targeting of MET using c-MET-inhibitor Savolitinib reduced the neutrophil-to-lymphocyte ratio, suggesting that the inflammatory milieu can be targeted by blocking c-MET (**chapter 6**). Furthermore, $CD8^+$ T cells in *MET*-amplified breast tumors presented with increased levels of PD-1 (**chapter 6**), implying that a combination of c-MET inhibition and immune checkpoint inhibition may be a beneficial therapeutic strategy. Indeed, previous studies have demonstrated enhanced immunotherapy efficacy when immune checkpoint inhibitors are combined with c-MET inhibitors in multiple

murine cancer cell line inoculation models[54-57]. Additional reports describe the successful use of bispecific antibodies that target c-MET and PD-1 simultaneously showing improved tumor control over single treatments that target c-MET or PD-1 alone in a human gastric cancer cell line-based xenograft model[58,59]. Together, these findings provide a rationale to assess the combination of c-MET inhibitors with immune checkpoint inhibitors in pre-clinical models of *MET*-amplified breast cancer to abrogate MET-induced immune suppression.

The application of combination strategies aiming at cancer cell inhibition and immunoregulation may prove beneficial for *MET*-expressing breast cancer patients. Mechanistic insights regarding MET-induced immune regulatory programs and phenotypes aid the development of novel immune-based therapeutic strategies to strengthen treatment efficacy of c-MET inhibitors in immunoregulation. The cancer cell secretome might play a role in modulating immune cell recruitment and activation in *MET*-expressing cancer, as delineated in **chapter 6** where c-MET signaling induced expression of immune modulators that potentially regulate observed immune phenotypes. For instance, cancer cell-intrinsic and systemic upregulation of G-CSF could be linked to systemic neutrophil expansion, although a causal relationship has not been tested yet in *MET*-expressing breast cancer (**chapter 6**). It remains unclear how *MET* amplification leads to distinct secretome profiles of cancer cells. Cancer cell-intrinsic signaling by mTOR and NFκB have been shown to regulate G-CSF expression[60,61]. These signaling molecules are known to act downstream of the c-MET receptor[62,63], potentially explaining MET-induced cytokine release. Other reports demonstrate a role for STAT-3 and STING signaling pathways in *MET*-expressing cancer, of which the latter pathway induces IFN responses that are important for anti-tumor immunity[54,55]. These findings provide some indications on the signaling molecules involved in MET-induced immune regulation by cancer cells, but the exact mechanisms underlying MET-induced immune phenotypes need to be elucidated in future studies. Mechanistic insights into immune regulation by other tyrosine kinase receptors in cancer, such as EGFR, HER2 and AXL[64-67], might provide additional clues that could aid unraveling novel targets for immune-based therapies in context of *MET*-amplified cancers.

A SYSTEMIC INFLAMMATORY DISEASE DEMANDS SYSTEMIC INTERVENTION

Secreted factors are key modulators of cancer-immune cell crosstalk in the tumor niche, and local cancer-induced inflammation can quickly expand to a systemic level via the secretome[6,7]. The phenomenon of breast cancer being a systemic inflammatory disease is highlighted in multiple chapters of this thesis. The systemic inflammatory environment of breast cancer is complex in the sense that it involves multiple immune cell populations and immune modulators. The complexity of these multi-component inflammatory cascades challenges finding suitable treatment strategies to reduce progressive disease, but

mechanistic understanding of the crosstalk between cancer cells and the immune system aids the discovery of novel therapeutic targets to interfere with cancer-induced inflammation. In **chapters 4 and 6**, we described that the systemic inflammatory milieu in breast cancer is characterized by the accumulation of neutrophils. Clinical studies have demonstrated the prognostic value of the neutrophil-to-lymphocyte ratio (NLR) in clinical practice[8,68,69] and cancer-induced neutrophils have been causally linked to contribute to cancer progression in pre-clinical mouse models of solid cancers[70-74]. In **chapter 2**, we discussed how cancer cell-intrinsic traits impact neutrophil behavior in both local and systemic immune environments and how the understanding of cancer-induced neutrophil modulation guides the design of novel therapeutic agents targeting cancer-neutrophil crosstalk. Here, we will discuss the opportunities of targeting systemic neutrophilic inflammation to interfere with breast cancer progression and the neutrophil-specific aspects to take into consideration when aiming for neutrophil targeting.

Connecting the dots between p53^{null}- and MET-induced neutrophilia

The discovery of immune regulatory programs of cancer cells that underlie systemic neutrophilic inflammation in the context of breast cancer has provided implications for novel immune-based therapeutic strategies. These therapeutic strategies aim at interfering with cancer cell-intrinsic mechanisms that regulate neutrophil recruitment, expansion or polarization, rather than depletion of the entire immune cell population, thus avoiding adverse effects on homeostatic neutrophil levels and function. It is important to unravel the cancer cell features that are associated with immune modulation for the application of effective therapies to progressive breast cancer patients. In this thesis, we describe that different genetic aberrations in breast cancer cells can induce systemic neutrophilia that may be facilitated by distinct immune regulatory programs. We demonstrate that loss of the tumor suppressor gene *Trp53* in breast cancer leads to systemic neutrophil expansion that promotes metastasis formation via a multi-component inflammatory cascade (**chapter 4**) and that systemic neutrophil levels are enhanced when the *MET* oncogene is amplified on top of *Trp53* loss in breast tumors (**chapter 6**). The common immune modulator that was found at elevated levels in p53^{null} and *MET*-amplified breast tumor-bearing mice was the neutrophil growth factor G-CSF (**chapter 4 and 6**). The production of G-CSF is an indirect result of cancer-immune cell crosstalk in the local microenvironment of p53^{null} breast tumors, while breast cancer cells seem to be the source of G-CSF in a *MET*-amplified breast cancer setting. These findings suggest that G-CSF plays an important role in both p53^{null} and *MET*-amplified breast cancer models, yet the different sources of G-CSF imply distinct regulatory mechanisms of cytokine production and cancer-immune cell crosstalk by the distinct genetic aberrations in breast tumors. Although multiple underlying mechanisms are at play here, the role for G-CSF in mediating neutrophilic inflammation gives a rationale to target G-CSF in both p53^{null} and *MET*-amplified breast cancer. In other pre-clinical models for breast cancer, targeting of G-CSF or the G-CSF receptor has been shown to successfully reduce neutrophil-

mediated inflammation and progressive disease[75,76], underscoring the potential of targeting G-CSF in p53^{null} and *MET*-amplified breast cancer. Unfortunately, anti-G-CSF is not a feasible agent to be used for long-term treatment in breast cancer patients, since it will affect the entire neutrophil population that includes both steady-state and cancer-induced neutrophils, and may impair innate immune responses. Ideally, neutrophil levels should be normalized to baseline levels instead of depletion of the entire neutrophil population that occurs when G-CSF is being targeted. Therapeutic strategies that interfere with the crosstalk between cancer cells and neutrophils may form a better approach in a clinical setting, and more insights into the cancer cell-intrinsic mechanisms that promote systemic neutrophilia in breast cancer will provide strategies for personalized immunomodulation based on the tumor genetic profile.

The cancer cell secretome plays a major role in breast cancer-induced inflammation and can be utilized for therapeutic targeting, but it is still unclear whether the systemic accumulation of neutrophils that is observed in context of p53^{null} breast cancer and p53^{null}-MET^{high} breast cancer is regulated via identical or independent processes. Mechanistically, breast cancer cell-intrinsic loss of the *Trp53* gene prompts the production of Wnt ligands by breast cancer cells that activate tumor-associated macrophages to produce IL-1β, the kick starter of systemic pro-metastatic inflammation. Targeting of Wnt ligand production by breast cancer cells via either genetic disruption of porcupine or systemic treatment with the porcupine-inhibitor LGK974 strongly diminished metastasis formation in the lungs of p53^{null} tumor-bearing mice. Based on these data, we speculate that inhibition of Wnt ligand secretion may be an effective therapy for metastatic p53^{null} breast cancer patients. In a clinical trial involving cancer patients of multiple solid tumor types, treatment with LGK974 was well-tolerated and reduced Wnt pathway activity in the majority of tumor samples[77]. Correlative analysis on a small group of tumor samples from this clinical trial showed an inversed association between Wnt pathway activity and T cell-recruiting chemokines[77], indicating a potential role for porcupine inhibitors in modulating the immune system towards anti-tumor immunity. Indeed, additional findings from pre-clinical transplantation-based cancer models treated with a different porcupine inhibitor (RXC004) demonstrated that the treatment-induced reduction in primary tumor growth was dependent on the immune system[78]. Although additional testing of porcupine inhibitors in pre-clinical breast cancer models is needed, these findings indicate that targeting of Wnt ligand secretion alters the immune landscape and provide a rationale for porcupine inhibition in Wnt-dependent p53^{null} breast cancer patients. Given that both p53^{null} and p53^{null}-MET^{high} breast cancer models show systemic neutrophil accumulation, one could speculate that Wnt ligand inhibitors in combination with MET inhibitors may be beneficial to target systemic neutrophil accumulation in p53^{null}-MET^{high} breast cancer. Indeed, the MET inhibitor Savolitinib was successful in reducing pulmonary neutrophil levels in *MET*-amplified breast cancer (**chapter 6**), but the enhanced neutrophilic inflammatory phenotype in the *MET*-amplified breast cancer setting cannot be explained by differential presence of Wnt ligands or macrophages in the tumor microenvironment compared to p53^{null}-MET^{low} breast cancer setting. Protein analysis of *MET*-amplified breast tumors illustrates similar levels of

Wnt ligand expression compared to other p53^{null} models with normal *MET* status (**chapter 4**), and not all *MET*-amplified breast cancer models showed an intratumoral enrichment of macrophages (**chapter 6**). Together, these data imply that, besides Wnt-dependent crosstalk, additional immunomodulatory mechanisms are at play when *MET* is amplified on top of *Trp53* loss in breast cancer that requires different targeting approaches than p53^{null} breast cancer.
Another key question that remains is whether *MET* amplification in p53-proficient breast cancer cells also promotes systemic neutrophil accumulation. *MET* amplification and *Trp53* loss have been reported to cooperate in inducing breast tumorigenesis[79-81], implying a clear advantage for cancer cells when *MET* and *Trp53* are both affected. Their cooperative nature in cancer progression implies that these two genes form a collaborative effort in cancer cell-intrinsic processes that may include immune-regulatory programs. A variety of functions is known for the *Trp53* tumor suppressor gene in terms of immune regulation[82], while the *MET* oncogene is relatively understudied in that context. Uncovering interdependence of these cancer-related genes is an important aspect to investigate in light of genotype-immunophenotype networks in order to improve breast cancer patient stratification based on the *Trp53* and *MET* status of breast cancer.

Neutrophil reprogramming: exploiting the anti-tumor function of neutrophils

Neutrophils form an heterogeneous population that have a core function in the first line of immune defense during infection and inflammation, thus exploiting the effector functions of neutrophils could be beneficial as anti-cancer strategy. In situations where neutrophils contribute to cancer progression, hampering their expansion, their mobilization from the bone marrow, or their recruitment, are strategies to tackle the pro-tumoral outcome of neutrophil presence (as demonstrated in **chapter 4**). **Chapter 4** describes how blocking of neutrophil accumulation via inhibition of Wnt-ligand secretion by breast cancer cells leads to reduced metastasis formation in breast tumor-bearing mice. Notably, metastatic disease is not fully eradicated by LGK974 treatment, which could be explained by the fact that LGK974 treatment did not reduce the level of neutrophils to wild type levels. Another explanation may be that besides the increased number of circulating neutrophils, this population has distinct functional properties that warrants specific targeting.

Therapeutic reprogramming of neutrophils from a pro-tumoral state towards an anti-tumoral state involves altering the phenotype of existing neutrophils or neutrophil progenitors in the bone marrow rather than blocking neutrophil production or recruitment. In lung cancer patients, multiple subsets of circulating neutrophils were identified based on granular density and cell surface marker expression in different disease stages and showed distinct immune signatures between neutrophil subsets[83]. These findings highlight the heterogeneity of neutrophils in cancer patients that potentially influences the efficacy of neutrophil-targeting therapies. Additional recent evidence points at distinct polarization states of neutrophils that support immunotherapy efficacy in multiple murine cancer models, including melanoma, lung cancer and breast cancer[84-87]. Administration of immunotherapy in these settings led

to the recruitment and activation of neutrophils that enhanced immunotherapy response via multiple mechanisms, including IRF1-mediated interferon response[84], production of iNOS and ROS[85,86], and microbial-induced chemokine production[87]. These studies illustrate immunotherapy-associated neutrophil activation towards anti-tumorigenic states that support anti-tumor immunity. Since neutrophil state can be affected by a variety of cancer features[13], the functional characterization of neutrophils in different cancer settings will aid the development of treatment strategies that promote the anti-tumor function of neutrophils. Activation of neutrophil effector functions may be achieved by targeting cancer cell-induced immune regulatory programs involved in neutrophilic inflammation, or by activating or administering immune mediators that induce the anti-tumorigenic function of neutrophils. A major challenge in finding soluble mediators that polarize neutrophils towards an anti-tumorigenic state is that several cytotoxic mediators involved in neutrophil-mediated cancer cell elimination have also been reported to exert pro-tumoral effects in certain conditions. For instance, ROS production by neutrophils mediates cancer cell killing[88,89], while ROS also promotes cancer progression via stimulating tumorigenesis or suppressing the anti-tumor immune response[90,91]. In addition, signaling in neutrophils via the HGF/MET axis has been demonstrated to both promote and counteract cancer progression in different murine cancer models[56,92]. These studies highlight the importance of uncovering the underlying factors that drive such specific functions of these immune mediators. In addition, as these findings also illustrate that a single mediator can polarize neutrophils into multiple functional states, including both anti- and pro-tumoral phenotypes, depending on a variety of tumor characteristics, the application of these targeting approaches for neutrophil reprogramming is limited to distinct cancer conditions.

Current immunotherapy strategies aim at blocking inhibitory immune checkpoints on lymphocytes, yet antibody-based immune checkpoint inhibitors targeting neutrophils to promote their anti-tumorigenic functions have also been introduced in the cancer field[93]. For instance, the innate immune checkpoint SIRPα-CD47 that is expressed by macrophages and neutrophils forms a barrier of anti-tumor immunity and antibodies targeting either SIRPα or CD47 have demonstrated to effectively block this axis leading to destruction of cancer cells[94-96]. Engaging neutrophils as effector cells is most efficient when IgA monoclonal antibodies are used instead of the IgG monoclonal antibody isotype that is currently being used in lymphocyte-targeting immune checkpoint blockade[97]. A novel approach using a therapeutic bispecific antibody TrisomAb bearing the favorable features of both IgG and IgA antibodies successfully engaged NK cells, macrophages and neutrophils to eliminate cancer cells in a cell line-based mouse model for melanoma[98]. This study illustrates the use of a single antibody activating immune-immune cell crosstalk of multiple immune cell populations mediating cancer cell destruction. Further characterization of neutrophils in terms of immune checkpoint expression and cytotoxic potential in relation to distinct genetic aberrations in breast tumors may enable exploiting such neutrophil-engaging therapeutics to promote anti-tumor immunity in breast cancer.

Targeting systemic neutrophilia: aspects to consider

Neutrophils appear in multiple compartments of the systemic immune environment in the context of cancer, including the bone marrow and spleen for their development via (extramedullary) granulopoiesis, the circulation, and distant organs like the lungs and liver for pre-metastatic niche formation[7,99,100]. Neutrophil phenotype and behavior can differ between these different organs, as the transcriptional programs and polarization states of neutrophils have been demonstrated to depend on the time of the day and the tissue they reside in[101-104]. Although distinct neutrophil polarization states correlate with disease progression and outcome in cancer, and provide potential leads for future neutrophil-targeting treatments[75,105], the finding that neutrophil function depends on the time of the day, the tissue of residence and its transcriptional regulation may influence therapy response when targeting cancer-induced systemic neutrophilia that possibly covers multiple tissues. Together, these indications provide a rationale for the administration of neutrophil-targeting agents at specific times during the day to enhance treatment efficacy in cancer patients. In addition, the short lifespan of neutrophils demands a therapeutic approach that acts at an early step of neutrophil development or mobilization to ensure efficient targeting of this relatively fast aging immune cell population[106]. Using parabiosis experiments, it was demonstrated that there are different lifespans of neutrophils between tissues in both homeostatic conditions and cancer[103,104]. In a recent study, the iLy6G^{tdTomato} mouse model for tamoxifen-inducible neutrophil labeling was used to assess the longevity of neutrophils in a setting of breast cancer metastasis in the brain, showing that tumor-associated neutrophils lived longer than neutrophils in healthy brains[107]. These studies provide the first indications that healthy neutrophils show differential lifetimes compared to cancer-associated neutrophils, but the lifespan of neutrophils in different cancer settings still warrants better understanding. Adoptive transfer studies with labeled neutrophils into different disease settings may also contribute to the understanding of neutrophil longevity in cancer. The transgenic hMRP8-ATTAC mouse model that we have generated (**chapter 7**) contains a neutrophil-specific GFP label that allows such adoptive transfer studies to assess the neutrophil longevity in various cancer settings. Together, these findings indicate that it is worthwhile to incorporate time- and tissue-dependency in cancer research that is aiming at finding immunomodulatory strategies to be able to define optimal treatment conditions for cancer patients.

MODEL SYSTEMS AND TECHNOLOGIES TO STUDY GENOTYPE-IMMUNOPHENOTYPE RELATIONSHIPS

In this thesis, we describe how distinct genetic aberrations in breast cancer lead to specific immune phenotypes, immune cell polarization and response to (immuno)therapy. In order to assess cancer-immune cell crosstalk, we made use of pre-clinical mouse models of breast cancer and culture-based methods in combination with multiple omics approaches. Besides

the methodologies that are described in this thesis, other techniques have been applied to cancer samples to study cancer-immune cell crosstalk, such as approaches involving bioinformatics and *in silico* models on datasets of cancer patients. Several previous studies have indicated immune signatures or phenotypes that can be applied to considerable groups of cancer patients or even across solid tumor types[108-113]. Correlative analyses showed that oncogenic driver genes and pathways were significantly associated with distinct immune phenotypes, indicating the existence of genotype-immunophenotype relationships across cancers[112,113]. These approaches mostly give correlative indications of genotype-immunophenotype links based on the deconvolution of gene expression profiles that fit known immune phenotypes or pathways, and thus do not reveal the underlying mechanisms of genotype-immunophenotype correlations. In addition, cancer type-specific studies allow for a more accurate assessment of cancer cell-intrinsic regulators of the immune system and estimation of response to immunotherapy over pan-cancer analyses, since the heterogeneity of the immune landscape and the response rates to immune-based therapies greatly differ between solid cancer types[113,114]. Still, these datasets of human cancer samples are valuable for explorative analyses linking cancer genetics to immune pathways or disease outcomes and for the clinical validation of mechanistic findings that have been revealed in pre-clinical models.

Functional methods and model systems that allow perturbations are necessary for the identification of causal relationships between cancer genetics and the immune landscape and for the mechanistic investigation of cancer-immune cell crosstalk. Ideally, a combination of screening methods, bioinformatics and pre-clinical mouse models is used to unravel the immunoregulatory properties of cancer cells. Since transgenic mouse models for breast cancer recapitulate the spontaneous course of tumorigenesis and cancer progression as observed in human breast cancer patients[115], they are very valuable to identify genotype-immunophenotype relationships. Additional model systems, such as somatic or cell line-based mouse models[115,116], and *in vitro* model settings that mimic the cancer-immune cell crosstalk[117,118], are beneficial to better understand the mechanisms of cancer cell-intrinsic genetic aberrations impacting the immune landscape. *In vitro* genetic targeting followed by orthotopic transplantation of matched cancer cell lines is a useful approach to dissect the role of specific genes in cancer-induced inflammation (**chapter 4-6**). Moreover, somatic modeling of genetic aberrations in pre-clinical animal models allows conditional manipulation of target genes in an *in vivo* setting, while maintaining the spontaneous development of breast cancer (**chapter 6**). These technologies enable the pre-clinical investigation of the role of candidate genes involved in cancer-induced inflammation in model systems that recapitulate human breast cancer and the cancer-immune cell crosstalk.

Additional sophisticated technologies that could support genotype-immunophenotype studies include single-cell RNA sequencing, intravital imaging, spatial transcriptomics and proteomics, and a few examples of each of these methods will be discussed here. The application of these techniques alone will not reveal the mechanistic processes that underlie genotype-immunophenotype relationships, but it will give insights into the immune

composition and immune-related expression profiles of cancer. For instance, single-cell transcriptomic analysis and antigen receptor profiling of B cells provided mapping of distinct B cell subtypes in the systemic versus local environment of breast cancer patients and based on the transcriptomic data, a correlation between naïve B cell and memory B cell signatures and improved survival in TNBC patients was found[119]. This study demonstrates the utility of single-cell technologies to profile immune cell heterogeneity in breast cancer that can be applied to both local and systemic inflammatory settings. Single-cell transcriptomic and proteomic analyses have also been used to determine interpatient immune cell heterogeneity in the breast by comparing breast tissue from healthy individuals and patients with different subtypes of breast cancer[120,121]. For instance, triple-negative and HER2$^+$ breast tumors contained proliferating CD8$^+$ T cells, while ERα$^+$ breast tumors primarily consisted of tumor-associated macrophages[120]. These single cell maps of breast cancer illustrate distinct immune phenotypes between different breast cancer subtypes and show a link between cancer cell characteristics and the immune landscape, together providing leads for mechanistic follow-up studies of cancer-immune cell crosstalk, such as hormone receptor-induced immune phenotypes.

By utilizing spatial transcriptomics, clusters of cell types and gene activity are determined in immunohistochemical tissue sections based on mRNA content. As such, in HER-2$^+$ breast cancer, tumor regions showing co-localization of myeloid and T cell subsets were found to be enriched for active type I IFN pathways[122], indicating how spatial transcriptomics provides information on both the distribution of specific cell types and genetic expression profiles of these cell subsets in tissue sections, although follow-up studies are essential to address whether co-localization of immune cell subsets and active immune signaling are causally related. Another technology to visualize the spatial organization of the tumor microenvironment is intravital imaging, a method that allows live fluorescent microscopic imaging via a window placed at the primary tumor site or a metastatic organ. Live *in vivo* microscopy enables visualization of the interaction between cancer cells, stromal cells and immune cells at a cellular and tissue level, and has been used to study cancer cell migration and metastatic spread, cancer-immune cell interactions, and therapy-induced immune responses[123-125]. Live tracking of immune cells in the tumor microenvironment during anti-PD-1 immune checkpoint blockade revealed that macrophage-T cell interactions mediate reduced immunotherapy efficacy in a cell line-based mouse model for colorectal cancer[125], indicating the relevance of spatially visualizing cell-cell interactions using intravital imaging under different experimental conditions. Likewise, intravital imaging enables investigation of cancer-immune cell interactions in the tumor microenvironment or metastatic niche comparing breast cancer models with distinct genetic origin. Together, these sophisticated tools give insights into the heterogeneity of cancer and cancer-immune cell crosstalk based on single-cell genetic transcripts or fluorescence-labeled cell imaging *in situ* and these technologies could also be applied to find genotype-immunophenotype relationships in cancer.

Although transcriptomic analyses can reveal candidate genes or gene signatures involved in genotype-immunophenotype relationships, assessment of the proteome of cancer

cells in combination with follow-up studies is a powerful strategy to better understand the communication between cancer and immune cells. Spatial quantification of protein expression in human triple-negative breast cancer (TNBC) showed enhanced epithelial MHC-II expression and stromal CD_4 expression in patients with long-term disease-free survival[126]. This study revealed distinct immune cell compositions in breast tumors that were associated with patient disease outcome based on proteomics[126], an approach that can also be applied to compare activation of signaling molecules and immune cell localization in breast tumors of distinct genetic backgrounds. In two other studies, the spatial composition of the tumor microenvironment was mapped using high dimensional imaging on single-cell level[127,128]. Combining proteomic data with genomic data resulted in connecting cancer cell genetic make-up and cellular phenotypes in the tumor microenvironment, and in correlating breast cancer subtypes with clinical outcomes[127,128]. Interestingly, among other genomic alterations, mutations in *TP53* were significantly associated with T cell infiltration[127], similar as our observations (**chapter 5**). Both studies illustrate the power of integrating multiple omics approaches to identify genotype-immunophenotype relationships in cancer using single-cell analytical strategies, but mechanistic studies are crucial to proof causality in genotype-immunophenotype relationships and to enable the design and implementation of novel therapeutic strategies. As described in this thesis, detection of immunomodulatory proteins in serum of pre-clinical breast cancer models with distinct cancer cell-intrinsic genetic aberrations gives insights into the systemic immune cell crosstalk induced by tumors with distinct genetic make-up (**chapter 4 and 6**). Also *in vitro* co-culture settings have proven to be useful model systems to find immunoregulatory mediators produced by cancer cells that harbor specific genetic aberrations (**chapter 3 and 4**). Proteome detection in fresh breast tumor lysates from pre-clinical mouse models or human patients could reveal soluble factors in the tumor microenvironment involved in shaping the immune landscape of breast cancer. Altogether, these single-cell proteomic technologies could provide leads of immunomodulation for mechanistic follow-up studies aiming at deciphering the cancer-immune cell crosstalk based on the genetic make-up of breast cancer cells.

FUTURE PERSPECTIVE: AIMING FOR PERSONALIZED IMMUNE INTERVENTION

The emerging insights on genotype-immunophenotype links and the underlying regulatory programs have extended the list of potential therapeutic targets that can interfere with cancer-induced immune modulation over recent years. These proof-of-concept studies provide important knowledge on the mechanisms of cancer-immune cell crosstalk as a result of distinct genetic aberrations and contribute to the design and implementation of personalized immune intervention strategies for cancer patients based on a tumor's genetic profile. The findings described in this thesis demonstrate that targeted therapy can

be used as a therapeutic strategy for immunomodulation in breast cancer (**chapter 4 and 6**). Although single treatment with targeted therapy was shown to be sufficient to collapse the inflammatory response and associated disease progression in these breast cancer settings (**chapter 4 and 6**), combining targeted therapies with immune-directed agents could improve treatment response even further. Immune checkpoint blockade has proven successful for a subset of breast cancer patients over recent years[129], but the majority of advanced breast cancer patients that fail to respond to immunotherapy may benefit from combination strategies that collectively modulate the immune system. In TNBC patients, three distinct immune phenotypes have been found to differentially correlate with anti-PD-1 treatment response[130]. One of the immune phenotypes that showed resistance to anti-PD-1 treatment was characterized by high infiltration of $CD163^+$ macrophages or activation of the Wnt pathway, suggesting that a combination of treatments targeting either macrophages or Wnt activity and immunotherapy would be more eligible than immunotherapy alone[130]. In our $p53^{null}$ breast cancer models, we found a similar intratumoral immune phenotype consisting of abundant macrophage infiltration and increased Wnt activity, and blockade of the Wnt pathway reduced – but did not fully eradicate – metastasis formation (**chapter 4**). In addition, **chapter 5** delineates that $p53^{null}$ breast tumors do contain relatively high levels T cells and responded modestly to anti-PD-1 treatment, but the effect on metastasis formation was not addressed here. Taken these studies together implicates that immune modulation in $p53^{null}$ breast cancer may be most beneficial when using a combination of Wnt blockade and anti-PD-1 immunotherapy to further eliminate metastases that remained upon Wnt inhibition alone, providing an example of personalized immune intervention based on mechanistic understanding of breast cancer-immune cell crosstalk.

Besides *Trp53* loss, the mutation status of p53 underlies immunotherapy resistance in breast cancer, as we demonstrated in **chapter 5** that a specific group of p53 hotspot mutations lead to immune-deprived breast tumors that are unable to respond to anti-PD-1 treatment. Mechanistic understanding of the immunoregulatory programs that control immunotherapy resistance in p53 mutant breast cancer will provide novel targets that could be exploited as therapeutic strategy in combination with immune checkpoint blockade to shift the immune-deprived TME into an immune-enriched niche and to overcome immunotherapy resistance in breast cancer. The administration of targeted therapies on top of immune checkpoint blockade could improve response rates to immunotherapy for breast cancer patients with distinct genetic profiles. Of note, when applying therapies targeting cancer cell-intrinsic features as immunomodulation strategy, the potential detrimental effects on the immune system of such agents should also be considered, since multiple cancer-related signaling molecules, such as c-MET[56,92], CDK4/6[131], STAT-3[132,133] and PI3K[134,135], have been demonstrated to be involved in immune cell regulation in the context of cancer. Targeted therapies against these signaling molecules that may directly affect immune cells potentially limit therapy efficacy and response, while it may also be advantageous when pro-tumorigenic immune cells are inhibited or eliminated by targeted therapy. These indications highlight the importance

of clarifying the systemic effects of therapies targeting cancer cell-intrinsic features on the immune landscape. Together, work described in this thesis and by others emphasizes the relevance of mechanistic genotype-immunophenotype studies for the implementation of precision medicine and to guide patient stratification and clinical decision making for successful therapy response based on cancer cell features.

In light of the overarching goal of aiming for precision medicine for breast cancer patients based on the genetic make-up of cancer cells, one could argue what the ideal starting point of genotype-immunophenotype studies should be to obtain clinically relevant results. There is ample clinical data available linking genetic alterations, prognosis and therapy response of cancer patients to be used for validation of mechanistic findings covering genotype-induced immune phenotypes that have been discovered in pre-clinical mouse models and other *in vivo* and *in vitro* model systems. The initial causal relationships between the genetic make-up of cancer cells and immune phenotypes have been identified in animal models and the identified immune modulators or gene expression profiles were later validated in human datasets. The use of pre-clinical research as a starting point is illustrated in **chapter 4** that describes a detailed immune analysis of 16 different GEMMs for breast cancer that has led to the discovery of cancer cell-intrinsic loss of *Trp53* regulating systemic inflammation that is involved in metastasis formation. On the other hand, utilizing a human patient dataset as a starting point to unravel novel genotype-immunophenotype relationships will provide leads for focused mechanistic follow-up studies to gain a better understanding of the clinical observations. The latter is illustrated in a recent study that combines genomic and immunological profiling of human breast-to-brain metastatic lesions to show that an hypermutated tumor profile induces a distinct immune phenotype in human brain metastases[110]. The clinical relevance of these observations gives a rationale to perform mechanistic studies using pre-clinical animal model systems in order to unravel underlying processes of cancer-induced shaping of the immune landscape and to identify potential therapeutic targets tailored at distinct cancer cell features. Both perspectives confirm that the combination of pre-clinical and clinical research is key to achieve clinically relevant causal relationships for the implementation of precision medicine based on a tumor's genetic profile.

THE CLINICAL UTILITY OF A CANCER CELL'S GENETIC MAKE-UP

Based on the work described in this thesis and by others, genotype-immunophenotype relationships offer novel therapeutic strategies to alter the immune landscape by targeting the immunoregulatory function of cancer cells and enable the stratification of breast cancer patients for immune-based therapies. Several genetic aberrations are touched upon here, while many more (epi)genetic alterations that are involved in immunomodulation still go unnoticed. In an ideal situation, a common mechanism that is applicable to a large group of cancer patients or even across cancer types could provide novel treatment strategies that

substantially improve disease outcome for patients with cancer. With *TP53* – either lost or mutated – being affected in the majority of cancer cases and being a key player in impacting the immune landscape covering both systemic pro-metastatic inflammation and local immune phenotypes that are related to immunotherapy response, stratification for LGK974 treatment or immunotherapy based on *TP53* status might be applicable to a large group of patients. More challenging cases comprise patients bearing tumors with genetic alterations that are less abundant or with multiple (epi)genetic aberrations that warrant complex targeting strategies. The most challenging group includes metastatic cancer patients that harbor complex mutational profiles that differ per organ site and these patients may need multiple treatment strategies to obtain an effective response. Current focus of research directed to genotype-immunophenotype relationships is aiming for personalized immune intervention that is applicable to a group of cancer patients with comparable characteristics. The findings described in this thesis may contribute to the design of novel therapies targeting cancer-immune cell crosstalk, but there is still a substantial knowledge gap in this upcoming research field. Collaborative efforts of experts in pre-clinical mechanistic studies, multi-omics analyses, single cell technologies and immune profiling of patient samples will aid filling this knowledge gap and translating mechanistic findings to the clinic.

CONCLUDING REMARKS

The work described in this thesis delineates that the concepts of cancer being a genetic disease and cancer being accompanied by inflammation are connected via cancer-immune cell crosstalk. The complexity of this crosstalk is depicted by the continuous adaptation of tumors and the immune system as part of evolutionary selection and by the heterogeneity that exists between cancer patients. Understanding the details of this process is challenging, as the composition of tumors, associated communication networks and cellular functions depend on a variety of factors and each combination of factors differentially impacts disease outcome and therapy response. This dynamic nature and continuous adaptation of cancer demands sophisticated multi-target treatment strategies to preserve effective therapy responses for cancer patients. Ideally, the communication between cancer cells and immune cells that promotes disease progression is therapeutically exploited to reprogram immune cells with a pro-tumorigenic phenotype to an anti-tumor immune state – as an Attitude Switch – without affecting the steady-state abundance and homeostatic functions of these immune cells. With the work in this thesis a foundation is laid for the stratification of breast cancer patients based on the cancer cell's genetic make-up and associated immune-regulatory programs that supports the clinical utilization of personalized immune intervention strategies.

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