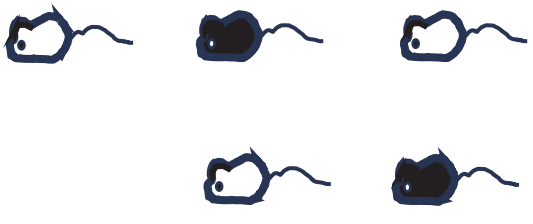
Dissecting the immune microenvironment of breast cancer

Ciampricotti, M.

Chapter 5



General discussion

Breast cancer is a very heterogeneous disease; distinct subtypes of breast cancer are dependent on different oncogenic pathways and are likely to be differentially regulated by the immune system. Chemotherapy denotes one of the main treatments that breast cancer patients receive, but response rates vary amongst patients. A better understanding of the adaptive and innate immune system in breast cancer initiation, progression, metastasis formation and chemotherapy response is essential for the development of new therapeutic approaches to improve survival rates. For instance, immunomodulatory agents targeting myeloid cells are currently being assessed in clinical trials. To maximize the success of these compounds, it is essential to understand the effects and mechanisms of these drugs. The overall goal of the research described in this thesis is to better understand the interaction between the immune system and breast cancer. I have studied the roles of the adaptive immune system during breast cancer tumorigenesis and chemotherapy response. In addition, I have studied the consequences and underlying mechanisms of targeting macrophages via CSF-1R blockade during breast cancer development and chemotherapy treatment. We focused on the following main research questions by using genetically engineered mouse models (GEMMs) for metastatic breast cancer:

1. Does the adaptive immune system play a role during HER2-positive breast cancer formation, progression and metastasis?
2. Is the adaptive immune system important for chemotherapy response of breast cancer?
3. What is the impact, optimal combination partner and mechanism of anti-CSF-1R antibody targeting during breast cancer development and chemotherapy treatment?

Impact of the adaptive immune system on HER2-positive breast cancer

A body of accumulating clinical data indicates that different molecular subtypes of tumors are characterized by distinct immune landscapes [1-3]**.** Depending on the tumor type, stage and treatment, different types of adaptive immune cells can play opposite functions, ranging from tumor-promoting, tumor preventing to no role [4-8]. In breast cancer, preclinical studies with a variety of mouse models have demonstrated that certain

tumor-associated adaptive immune cell subsets are important for metastasis formation [8-11]. For example, metastasis formation in the transgenic MMTV-PyMT mouse model for spontaneous breast adenocarcinomas was shown to be dependent on interleukin 4 (IL-4)-expressing $CD4^+$ T cells that stimulated EGF production from tumor-associated macrophages (TAMs) [9]. In **Chapter 2** the causal link between the adaptive immune system in HER2-positive breast cancer formation and metastatic spread was investigated. Using a mouse model for spontaneous HER2-driven mammary tumorigenesis *i.e., MMTV-NeuT* mice, our findings reveal that genetic elimination of the complete adaptive immune system did not affect premalignant progression, tumor latency, tumor growth, tumor multiplicity, and *de novo* pulmonary metastasis formation. These findings indicate that HER2+ breast tumors and metastasis formation in this preclinical model are not suppressed by immunosurveillance mechanisms, nor promoted by the adaptive immune system.

The data in **Chapter 2** reveal that absence of the complete adaptive immune system does not impact mammary tumorigenesis in MMTV-*NeuT* mice. An important question that our work leaves open is whether individual (sub)populations of adaptive immune cells play a role during cancer formation and metastasis in MMTV-*NeuT* mice. By using $Rag^{-/-}$ deficient mice, in which T and B cells are depleted from birth on, we cannot exclude the existence of opposing roles of individual components of the adaptive immune system in our model *e.g.*, Tregs, $CD8^+$ T or γδ T cells. For instance, HER2-positive breast tumors are frequently infiltrated by Tregs [12,13]**.** Increased numbers of $FOXP3^+$ Tregs in tumors generally correlate with worse patient outcomes [14,15] and Treg accumulation within sentinel lymph nodes is a predictor of disease progression and metastatic spread in breast cancer [16]. Furthermore, a distinct group of T cell receptor-expressing innate lymphoid cells, termed ILTC1, were found to have a critical role in cancer immunosurveillance in MMTV-PyMT mice [17]. The generation of these lymphocytes is dependent on the cytokine IL-15 [17]. Interestingly, IL-15 deficiency in MMTV-*NeuT* mice has resulted in accelerated tumor growth compared to wild-type (WT) MMTV-*NeuT* mice [18]. Since all T cells require RAG to develop, the MMTV-*NeuT* mice used in **Chapter 2** also lacked the ILTC1 cell population. Future experiments targeting one specific subset of T cells such as Tregs or ILTC1 cells before and during tumor development will help answer whether distinct adaptive immune cells are important in HER2-positive breast cancer.

It is surprising that we did not find a clear role for the adaptive immune system while based on the immunosurveillance hypothesis [19], we would have expected impact. In fact, preclinical and clinical data in HER2-positive breast cancer have suggested that the endogenous adaptive immune cell repertoire is not completely lacking tumor-specific immune cells and could potentially be involved in immunosurveillance mechanisms. For example, two studies found $CD4^+$ T cell responses directed against HER2 (neu or ErbB2) in the MMTV-*NeuT* mouse model during the pre-malignant phase [20,21] and HER2-specific $CD4^+$ and $CD8^+$ T cell responses have been described in patients with $HER2^+$ breast cancer [22,23]. In addition, antibody-mediated depletion of T cells in MMTV-*NeuT* mice resulted in a momentary and minimal increase in tumor multiplicity [24]. Although T cells and Neu-specific T cells are present in MMTV-*NeuT* mice and $HER2^+$ breast cancer patients, it is very likely that immunosuppression is at play. With the advancing stages of HER2-positive cancer, the infiltrating cell composition is prone to changes like what has been seen in many cancers; the effector cells become fewer and less activated, while the TME becomes dominated by cells with regulatory and immunosuppressive activities [25,26]. The immune-editing process in its most complete manifestation is composed of three sequential phases of tumor "elimination," "equilibrium," and "escape" and is illustrated by studies showing that carcinogen-induced sarcomas and spontaneous epithelial carcinomas were more immunogenic when induced in mice lacking lymphocytes as compared to immunocompetent mice [19]. Indeed, ex vivo expanded HER-2/neu-specific T cells failed to reject transplanted $Her2^+$ tumor cells [27], but neu-specific antibody responses were restored in these transplanted $Her2^+$ tumors with the depletion of MDSC's [27]. Besides MDSCs, regulatory T cells and regulatory dendritic cells have been found to suppress anti-tumor T cell immune responses in MMTV-*NeuT* mice [27-29]. Thus likely, tumor antigen-specific $CD8^+$ T cell responses are induced but their activities are restrained from inducing effective cancer immunosurveillance by their immunosuppressive environment.

The treatment with HER2-targeting therapeutic antibodies has significantly improved the survival of patients with HER2-positive breast cancer [30]. Similar results have been found in MMTV-*NeuT* mice [**31-33**]. Importantly, preclinical studies in transplantation models for Her2-positive breast cancer showed that PD-1 and CTLA-4 inhibition improves HER2-targeted therapies through activation of $CD8^+$ T cells [32,34]. These data have provided a basis for

the clinical use of immune checkpoint inhibitors for the treatment of HER2$^+$ breast cancer patients and their combination with HER2-targeted treatments [30].

In conclusion, we found that absence of the complete adaptive immune does not impact mammary tumorigenesis in MMTV-*NeuT* mice. Further research is needed to determine what the exact immunosuppressive networks are to engage anti-tumor immunity. Moreover, increasing immunity towards HER2$^+$ tumors with immunotherapy may overcome the unresponsiveness of the adaptive immune system and result in effective tumor inhibition.

In contrast to our findings in **Chapter 2**, Tan and colleagues found that metastatic spread of orthotopically transplanted mammary tumors derived from the MMTV-*NeuT* transgenic mouse model was reduced in *Rag1*$^{-/-}$ and *CD4*$^{-/-}$ recipient mice as compared to WT recipients [10]**.** How can a promoting effect versus no effect of the adaptive immune system on metastasis formation be obtained from two independent studies that focus on the same subtype of breast cancer *i.e*., Her2$^+$ -positive mammary tumors? There are three fundamental differences between these two studies:

1. Tan *et al*. used transgenic mice expressing the WT Her2 receptor, whereas in **Chapter 2** transgenic mice expressing an activated form of Her2 are used.
2. Tan *et al.* used mice on the FVB/N background, whereas in **Chapter 2** studies are performed on the Balb/c background.
3. Tan *et al*. performed their studies in mice that were orthotopically transplanted with freshly isolated tumor cells or cell lines from MMTV-*NeuT* transgenic mice, whereas in **Chapter 2** spontaneous mammary tumorigenesis was studied in transgenic MMTV-*NeuT mice*.

Due to somatic mutations within the *Her2* transgene, mammary tumors from MMTV-*NeuT* transgenic mice expressing WT *Her2* display activation of intrinsic Her2 receptor tyrosine kinase activity [35,36]. Therefore, both MMTV-*NeuT* mammary tumor models express activated Her2, and it is thus unlikely that the different results can be attributed to the activation status of the *Her2* transgene.

It is possible that the genetic variation between mouse inbred strains can provide the basis for fundamentally different mechanisms underlying metastasis formation. In the PyMT mouse model for breast carcinoma, PyMT *Rag1*$^{-/-}$ mice had significantly reduced tumor latency compared with PyMT WT mice in the C57BL/6 background, a result that was not seen when using PyMT mice on the FVB/NJ background [37]. Certainly, evaluation of metastasis formation in MMTV-*NeuT* transgenic mice on the FVB/N background intercrossed with *Rag1*$^{-/-}$ mice can help to resolve the impact of the genetic background versus the impact of *de novo* tumorigenesis on the influence of the adaptive immune system on metastatic HER2^{+} breast cancer. However, it is also conceivable that the different outcome between both studies can be explained by the use of transplanted HER2-positive mammary tumors in the Tan *et al*. study, versus spontaneous HER2-positive mammary tumors in **Chapter 2**. Transplantation models, based on engraftment of cultured cells or freshly isolated single-cell suspension models, as used in the Tan *et al* study, derived from end-stage tumors have shown to not fully recapitulate *de novo* tumor formation with co-evolving tumor-host interactions and an immunosuppressive microenvironment [38]. Other disadvantages are derangement of the normal tumor architecture, compared to spontaneous tumors, and cancer cell lines are generally poor predictors of clinical response [39]. In addition, mammary epithelial cells in the MMTV-*NeuT* mouse model disseminate already during the premalignant phase and this early dissemination is not recapitulated in tumor transplantation models as the premalignant phase is bypassed [38,40,41]. Other evidence of discordant results between the MMTV-*NeuT* allograft model versus the MMTV-*NeuT* spontaneous tumor model comes from Gonzalez-Suarez and colleagues who by using spontaneous MMTV-*NeuT* mice on the FVB/N background showed that RANKL is expressed in mammary epithelial cells before tumor onset, but not in epithelial or stromal cells of *de novo* adenocarcinomas [42]. RANKL inhibition in MMTV-*NeuT* mice resulted in decreased spontaneous mammary tumorigenesis [42]. Consistently, RANKL expression by breast cancer cells was also seen in a recent study on human estrogen receptor-positive/HER2- breast cancer cells and patients [43]. In contrast, in the Tan *et al*. study, RANKL expression was predominantly detected in Tregs infiltrating transplanted Her2^{+} tumors and RANKL inhibition only affected primary tumor outgrowth marginally [10].

In conclusion, given these discordant findings with transplanted Her2^{+} tumors versus HER2^{+} patient data and two *de novo* models of Her2^{+} tumors

on different backgrounds it is most likely that the observed promoting effect in the Tan *et al.* study versus no effect of the adaptive immune system on metastasis formation in our study (**Chapter 2**) is caused by using transplanted Her2^{+} mammary tumors versus spontaneous Her2+ mammary tumors.

While the findings in **Chapter 2** represent a negative finding, it is a surprising result considering that other breast cancer subtypes are dependent on the adaptive immune system for metastasis formation [9-11]. One possible explanation is that the genetic driver of mammary tumorigenesis in MMTV-*NeuT* mice, the activation of the HER2 oncogene, influences the composition and the activation status of the immune landscape differently compared to other driver mutations that are active in the other breast cancer subtypes. In fact, the idea that genetic events, activation of oncogenes, or loss of tumor suppressor genes (TSGs) in cancer cells, shape the immune landscape is emerging [1,44]. This concept was further investigated in a recent study from our group where 16 mouse models for breast cancer with different tissue-specific mutations were used, revealing that loss of p53 shapes the local immune composition of primary breast tumors to drive pro-metastatic systemic inflammation [8]. Thus, genetic aberrations in tumors influence the immune composition, activation states and therefore different immune responses, including therapy response [45,46]. MMTV-*NeuT* tumors are characterized by the overexpression of an activated form of the epidermal growth factor receptor (EGFR) family member HER2 [47], which does not require ligand binding for receptor activation. Instead, in the MMTV-PyMT mice it was shown that CD4^{+} T cells instructed TAMs to produce EGF to stimulate EGFR-dependent metastasis formation [9]. Furthermore, our group demonstrated that mammary tumors from the genetically engineered *K14cre; Cdh1$^{F/F}$; Trp53$^{F/F}$* (KEP) mouse model for invasive lobular carcinoma (ILC), driven by loss of p53, activate systemic pro-metastatic inflammation in a Wnt-dependent manner [8]. Thus, in other breast cancer subtypes where there is no cell-autonomous EGFR family member activation, tumors may rely on immune cells to drive metastasis.

In conclusion, the metastatic capacity of NeuT-overexpressing tumors might be a cancer cell-autonomous trait. Our findings indicate that it is essential to investigate the impact of the adaptive immune system in other breast cancer subtypes as they can have a different role. Furthermore, to optimally harness an effective anti-tumor immune response and improve therapy outcomes in

HER2-positive patients we need to obtain a deeper understanding of the immunosuppressive pathways.

Impact of the adaptive immune system on chemotherapy response

Chemotherapy is frequently used to treat cancer patients. Although most tumors initially respond to chemotherapeutic drugs, tumors develop mechanisms of resistance to the treatment. Cancer cell-intrinsic factors like resistance to apoptosis or overexpression of drug transporter proteins have been identified as causes of therapy resistance [48]. However, also cancer cell-extrinsic processes underlying poor chemotherapy response have been recognized [48-51]. Experimental studies in highly immunogenic tumor models, *e.g.*, cancer cell line inoculation models and chemically-induced sarcomas such as the 3-methylcholanthrene (MCA) fibrosarcoma model, have indicated that T cells can contribute to the anti-cancer efficacy of certain chemotherapeutics [52-56]. Cytotoxic drugs, such as doxorubicin, oxaliplatin, cyclophosphamide, epothilone B, mitoxantrone, and melphalan have been reported to lose their therapeutic efficacy on tumor cell line outgrowths in mice with a defective adaptive immune cell function, including *Rag*$^{-/-}$ mice [52-54,57]. The success of these chemotherapy treatments is dependent on the stimulation of immunogenic tumor cell death (ICD), as initially proposed by Dr. Zitvogel and Dr. Kroemer [58], which is a type of regulated cell death that stimulates CD8^{+} T-dependent tumor killing responses via damage-associated molecular patterns (DAMPs) emission such as calreticulin, nuclear protein high mobility group box 1 (HMGB1) and adenosine tri-phosphate (ATP)[52-54,59].

Considering that engraftment of cultured cells derived from end-stage tumors do not fully recapitulate *de novo* tumor formation with co-evolving tumor-host interactions and an immunosuppressive microenvironment [38], and that the *de novo* MCA-induced tumours are highly immunogenic, we hypothesized that in established spontaneous tumors that are relatively poorly immunogenic, like breast cancer, chemotherapy might not be powerful enough to activate adaptive immunity. In **Chapter 3** we have tested this hypothesis and describe that the adaptive immune system does not contribute to the therapeutic efficacy of three different chemotherapy drugs in two independent clinically relevant *de novo* mammary tumor models *i.e.*, MMTV-*NeuT* mice for HER2-positive breast cancer and *K14cre; Cdh1*$^{F/F;}$

Trp53$^{F/F}$ (KEP) mice for invasive lobular carcinoma (ILC). Cisplatin, oxaliplatin or doxorubicin were equally effective in inhibiting the growth of *de novo* mammary tumors in T cell– and B cell–deficient MMTV-*NeuT*;*Rag2*$^{-/-}$ mice as in MMTV-*NeuT*;*Rag2*$^{+/-}$ mice. Similarly, the therapeutic benefit of cisplatin and oxaliplatin was the same in *KEP;Rag1*$^{+/-}$ *and KEP;Rag1*$^{-/-}$ mice. In addition, we performed CD8^{+} T cell depletion alone or in combination with oxaliplatin in tumor-bearing *KEP;Rag1*$^{+/-}$ mice and did not see a change in the therapeutic efficacy of oxaliplatin. Thus, the adaptive immune system does not dictate the therapeutic efficacy of chemotherapy in these two *de novo* mouse models. Our data in **Chapter 3** stand in contrast with previous experimental studies in highly immunogenic tumor models where the adaptive immune system dictates the therapeutic efficacy of certain chemotherapeutics [52-54,59]. Several differences between these studies and our study may explain the difference in findings. For example, different cancer (sub)types, different backgrounds, different chemotherapy regimens and the use of different mouse models *i.e.*, cancer cell line inoculation models versus *de novo* mouse models. Several important distinctions between these two types of mouse tumor models have been described. For example, spontaneous tumors were found to have different chemotherapy response profiles compared to inoculated tumor cells isolated from these spontaneous tumors [60]. Furthermore, immunotherapy efficacy exhibited enhanced sensitivity in mice with subcutaneously implanted tumors compared to mice bearing orthotopic tumors from a genetically similar pool of tumor cells, indicating that the host normal tissue has an enormous impact on the tumor microenvironment and therefore on endogenous T cell responses [61]. Hence, it is most conceivable that the differences between our findings from **Chapter 3** and previously described experiments by Zitvogel and Kroemer are caused by the fact that we employed spontaneous mammary tumor models in **Chapter 3** instead of tumor cell line transplantation models or the immunogenic MCA fibrosarcoma model [62]. To test this concept experimentally, we generated a tumor cell line from a KEP tumor and conducted an analogous experiment as previously described in several papers [52-54,57]. Consistent with previous findings in cancer cell line inoculation models and in contrast to our findings in the transgenic KEP model (**Chapter 3**), we observed that tumor outgrowths from a KEP tumor cell line inoculated in *Rag1*$^{+/-}$ mice responded to oxalipatin treatment while tumor outgrowths from the same KEP tumor cell line inoculated in *Rag1*$^{-/-}$ mice did not respond to oxaliplatin treatment (Fig.1; unpublished). Though this experiment should be reproduced with more cell lines, different chemotherapeutics and with MMTV-*ErbB2* tumor cell lines, we here report

that oxaliplatin loses its therapeutic efficacy on KEP tumor cell line outgrowths in mice with a defective adaptive immune cell function. Thus, **Chapter 3** and these unpublished data illustrate the distinction in impact of the adaptive immune system on chemotherapy response between *de novo* tumor models and tumor transplantation models.

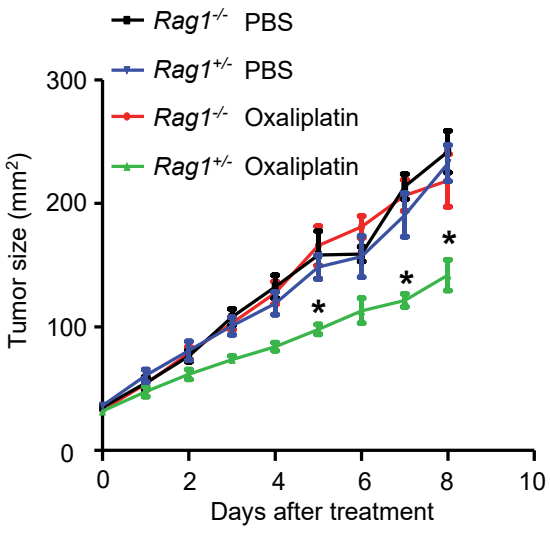


Figure 1. Impact of the adaptive immune system on the efficacy of oxaliplatin in a KEP mammary cell line transplantation model. Mice (*Rag1*$^{+/-}$ or *Rag1*$^{-/-}$) were injected s.c in the flank with 3 million KEP tumor cells. When the tumors reached 30 mm^2 in size, mice were treated with PBS or oxaliplatin (6mg/kg, i.v) at day 0. Tumor growth in oxaliplatin-treated *Rag1*$^{+/-}$ mice compared to untreated *Rag1*$^{+/-}$ mice was significantly different at 3 time-points, *p<0.05 by Mann-Whitney test. Each treatment group included 8 mice and was repeated two times with identical results.

How can we explain that there is no role for the adaptive immune system in chemotherapy response of spontaneous mouse tumor models? From earlier studies by others we know that subcutaneous inoculation of cancer cell suspensions results in massive tumor cell necrosis and early release of tumor antigens which could trigger acute adaptive immune responses, whereas spontaneously arising tumors that take months to develop often are known to trigger a more chronic inflammatory response that prevents acute T cell priming (immunosuppression) [63-65]. This could explain why the adaptive immune system contributed to the chemotherapy response of injected tumors, but not of established spontaneous tumors. Similarly, both T and B cells in the MCA-induced sarcoma model have been demonstrated as a critical factor in suppressing tumor initiation [66], suggesting that in immunogenic tumor models expressing strong antigens, chemotherapy-induced ICD is effective in activating CD8$^+$ T cells to contribute to the chemotherapy response [62]. We hypothesize that in established spontaneous tumors, chemotherapy is not able to activate adaptive immunity that is powerful enough to overcome the immunosuppressive networks in the microenvironment of *de novo* tumors. This hypothesis has been proven correct by others [67-69] and in **Chapter 4** in which we show that targeting

macrophages and neutrophils in combination with chemotherapy improved survival of KEP mice in a $CD8^+$ T cell-dependent mechanism. Our study in **Chapter 4** demonstrates that to boost an adaptive immune response in the KEP model during platinum-containing chemotherapy it is pivotal to create a type I interferons (IFNs)-enriched TME. Whether ICD is induced in KEP tumor models upon targeting of macrophages and neutrophils during platinum-containing chemotherapy remains unknown. Adaptive immunity has been engaged by synergistic effects of chemotherapy and immunotherapy in clinical settings and in *de novo* cancer mouse models [70-74] including MMTV-*NeuT* mice [75] and our KEP mouse model (unpublished). It is possible that ICD is important for these synergistic effects. Lastly, although we did not detect major changes in intra-tumoral CD4/$CD8^+$ T cell ratio or proportion of $FoxP3^+$ cells after chemotherapy treatment of *MMTV-NeuT; Rag2*$^{+/-}$ and *KEP; Rag1*$^{+/-}$ mice, we cannot exclude that other distinct adaptive immune populations such as Tregs or γδ T cells have opposing roles during chemotherapy.

The ICD concept has been established in hundreds of publications based on transplantation models [76], yet GEMMs have shown to represent human tumors better than transplantation models [77], We (**Chapter 3**) and others [67-69] have not seen evidence for a contributing role of the adaptive immune system upon chemotherapy treatment in *de novo* mouse tumor models. Thus, our study continues to urge for a careful analysis of the involvement of the adaptive immune system in chemotherapy response in a larger set of *de novo* tumor models that represent different solid human cancer types and extend these findings to the clinical situation.

Targeting macrophages as anti-cancer therapy

The TME of solid tumors contains many cell types of which macrophages are frequently the largest population. For many cancer types, including breast cancer, macrophage presence in tumors is a negative prognostic factor [78-81]. Indeed, our group recently showed that a gene signature derived from tumor-associated macrophages (TAMs) from KEP mammary tumors could be used to predict poor survival in two separate cohorts of ILC patients [82]. Preclinical studies have established that macrophages contribute to the various cancer hallmarks including cancer proliferation, suppression of anti-tumor immune responses, angiogenesis and migration [83,84]. Therapeutic approaches targeting TAMs focus on inhibiting pro-tumor macrophage

function via depletion, blockade of their recruitment or repolarization of macrophages towards an anti- tumor phenotype [84]. Blocking the CSF-1/CSF-1R signaling pathway, essential for macrophage survival, has proven to be an attractive strategy to eliminate or reprogram macrophages and suppress tumor growth in preclinical studies [85]. This has resulted in the development and clinical testing of CSF-1R signaling pathway inhibitors, including antibodies against the receptor (anti-CSF-1R), the ligand (anti-CSF-1), and inhibitors of the tyrosine kinase domain of CSF-1R [34,85-89]. However, monotherapy treatment with CSF-1R inhibitors does not exert anti-tumor effects in several models [90], including in the KEP mouse model (**Chapter 4**). Differences in anti-tumor effects of CSF-1/CSF-1R pathway targeting are likely caused by different cancer (sub)types and cancer mouse models with their different TME, use of a different type of inhibitor, doses and timing of the initiation of treatment.

CSF-1R blockade was tolerated well during phase I and II clinical trials but has shown only marginal therapeutic benefit [85]. Therefore, current clinical and experimental- efforts are focused on finding the right combination partners for TAM targeting [85]. These combination partners may vary from immune checkpoint blockade, adoptive T cell transfer, radiotherapy to chemotherapy. In **Chapter 4** we set out to obtain a better understanding of the mechanisms of action of anti-CSF-1R *in vivo* and to identify the optimal combination partner among existing anti-cancer therapies to enhance their efficacy. CSF-1R pathway targeting has shown to enhance the cytotoxic efficacy of chemotherapy in various experimental tumor models [68,69,91-94], including in the KEP model, as described in **Chapter 4** of this thesis. However, our study reveals a distinct mechanism of how therapeutic targeting of macrophages enhances chemotherapy efficacy. In **Chapter 4** we demonstrated that anti-CSF-1R induces type I IFN signaling in KEP mammary tumors, which acts synergistically with cisplatin to prevent tumor outgrowth and to prolong survival. Furthermore, we showed that anti-CSF-1R synergized with platinum-containing drugs, *i.e*. cisplatin and oxaliplatin, but not with the taxane docetaxel, though IFNα was induced.

The exact mechanism that induces the type I IFN expression in cisplatin/anti-CSF-1R-treated mice is mostly unknown. While our data showed that CSF-1R blockade depletes 80% of intratumoral macrophages, we noted a small population of remaining TAMs expressing high levels of IFNα. These TAMs are most likely causative of the increased IFNα levels in the tumors. Of note, as CSF-1R expression was significantly lower in the

remaining TAMs, it could explain their resistance to the anti-CSF-1R therapy. Moreover, our study shows that circulating monocytes can infiltrate into the tumor of anti-CSF-1R-treated mice, suggesting that these IFNα expressing TAMs are either newly recruited monocytes or remaining TAMs. It is unclear from our study whether the remaining macrophages upon anti-CSF1R treatment are repolarized, though noteworthy, three studies using either CSF-1R neutralizing antibodies or CSF-1R small molecule inhibitors discovered that TAMs were repolarized towards a tumor-inhibiting state in a preclinical pancreatic cancer, glioblastoma and lung cancer mouse model [95-97]. Induction of type I IFN expression by targeting macrophage function has not only been seen by us in **Chapter 4** but also by others; for instance type I IFNs were also increased in macrophages of the pancreatic cancer model after CSF-1R neutralizing antibodies [95]. Furthermore, we also noticed IFNα upregulation in anti-CSF-1R treated MC38 colon adenocarcinoma tumors, indicating that anti-CSF-1R unleashes type I IFN signaling in other cancers besides breast cancer. Lastly, a study targeting macrophages via their MerTK receptor resulted in the accumulation of apoptotic cells within transplanted MC38 colon carcinoma tumors and was associated with circulating cell-free tumor-derived DNA which triggered a type I interferon response by macrophages [98]. It is therefore likely that in our KEP model the dying cancer cells and/or dying macrophages released cytosolic DNA, which is scavenged by the remaining macrophages and activates the cGAS-STING pathway which triggers IFNα expression by these macrophages.

There is a vital interest in the development of clinically more effective combination therapies that combine IFN-I based therapies with for instance immune checkpoint inhibitors or chemotherapy [99-102]. The type I IFN family includes 13 different IFNα proteins (14 in mice), one IFNβ protein and others less well defined family members such as IFNε and IFNω [103]. Type I IFN molecules bind to their receptor that is composed of IFNAR1 and IFNAR2 subunits in a heterodimer or an IFNAR1 homodimer [103]. IFNs activate the kinases Janus kinase 1 (JAK1) and tyrosine kinase 2 (TYK2) which phosphorylate STAT1 and STAT2 to promote the expression of type I IFN-stimulated genes (ISGs). These innate immune signals enhance tumor antigen presentation and thereby augment the antigen-specific $CD8^+$ T cells response [104,105]. Indeed, type I IFN gene signatures have shown to correlate with increased bone metastasis-free survival or with metastasis-free survival in general in breast cancer patients [106-108]. Furthermore, type I IFN signaling is essential for the function and survival of cytotoxic T cells [109] and NK

cells[110]. Notably, impaired type I IFN signaling is a feature of immune dysfunction in patients with cancer and is linked with poor prognosis [109,111,112]. We detected an increase in ISGs in advanced solid tumor biopsies of cancer patients treated with emactuzumab, a humanized anti-human CSF-1R monoclonal antibody, compared to their reference levels, which is in line with our findings in the KEP mouse model (**Chapter 4**). Thus, the data shown in **Chapter 4** highlight that CSF-1R blockade may be used as a strategy to induce an intra-tumoral type I IFNs response.

An important question that our work leaves open is what the molecular mechanisms are of how type I IFN employs its anti-cancer efficacy in cisplatin/anti-CSF-1R-treated mice. Surprisingly, $CD8^+$ T cells were not unleashed upon cisplatin/anti-CSF-1R-treatment and $CD8^+$ T cell depletion did not influence the survival in cisplatin/anti-CSF-1R-treated mice. Though additional studies investigating other cytotoxic cells are required, these data indicate that another mechanism is responsible for the anti-cancer efficacy. Type I IFNs can have a direct effect on tumor progression by blocking proliferation or inducing apoptosis in cancer cells [113]. Concomitant, our *in vitro* work in **Chapter 4** of this thesis demonstrated that high concentrations of IFNα, subtype IFNα1, has a direct inhibitory effect on KEP cancer cells. Of note, it will be interesting to evaluate other IFNα molecules and IFNβ as their influence against viral infections [114] and their anti-proliferative effects on cancer cells [115] have shown to differ and could be cumulative. However, no increase of apoptotic cells was found in tumors of cisplatin/anti-CSF-1R-treated mice (**Chapter 4**), suggesting that a different mechanism such as necroptosis, an inflammatory programmed form of necrosis, or senescence might at play. In this regard, studies have shown that both cisplatin [116,117] and type I IFNs [118,119] can induce senescence in cancer cells, suggesting that senescence in cisplatin/anti-CSF-1R-treated tumors could perhaps explain the reduced proliferation and lack of apoptosis. Since the majority of breast cancer deaths are caused by metastatic disease [120], it will be of great value to study whether type I IFNs influences metastasis formation upon anti-CSF-1R with platinum based chemotherapy [13]. Monotherapy of CSF-1R blockade did not affect the metastasis-specific survival in the KEP-based model of spontaneous breast metastasis (**Chapter 4**).

Studies in preclinical cancer models and patients have described that chemotherapeutic drugs, such as anthracyclines and cyclophosphamide, induce type I IFN production, which is required for their therapeutic efficacy as blockade of type I IFN signaling results in loss of the anti-cancer efficacy

[99,121]. However, in our study in **Chapter 4** cisplatin response was not affected by blockade of type I IFN signaling. Only the combination of cisplatin/anti-CSF-1R induced a type I IFNs response that led to enhanced survival. Interestingly, the increase in type I IFN during anti-CSF-1R therapy did not enhance the efficacy of the taxane docetaxel. What could have caused the synergy of CSF-1R blockade with cisplatin and oxaliplatin but not with docetaxel? The two conventional chemotherapeutics have a different mode of action: while platinum-based anticancer drugs cause crosslinks in the DNA and prompt apoptosis, taxanes affect cell division through stabilization of microtubules. In line with this notion, a comprehensive study into the mutagenic impact of common chemotherapeutics found that cisplatin induces the highest amounts of single nucleotide variant (SNV)'s, indels and deletions compared to several other standard cytotoxics, including the taxane paclitaxel [122]. It is now well-known that distinct cytotoxic drugs differentially affect immune cells and the influence of the immune system on chemo-responsiveness has shown to depend on the type of chemotherapeutic drug and dosing [49,123,124]. Especially cisplatin has shown to induce antitumor immunomodulation in multiple preclinical and clinical studies [125-128]. Hence, it is conceivable that platinum-based anticancer drugs create a milieu in KEP tumors that is preventing type 1 IFN signaling. In line with this notion, cisplatin response was not affected by blockade of type I IFN signaling (**Chapter 4**). To this end, it will be interesting to investigate whether alterations effecting genes or pathways of the IFN signaling cascade are present in KEP tumors after cisplatin and docetaxel treatments. Furthermore, two clinical trials recently combined paclitaxel with CSF-1R blockade; emactuzumab in patients with advanced/metastatic solid tumors [129] and pexidartinib (PLX3397) in patients with refractory solid tumors [130]. Only the combination of paclitaxel with pexidartinib noted an objective response rate of 16% [130], while no anti-tumor activity alone or in combination with paclitaxel was found with emactuzumab [129]. Based on our data, cisplatin may have been a more optimal combination chemotherapeutic drug. However, synergistic affects with CSF-1R blockade may also depend on the tumor (sub)type, stage, prior treatments and CSF-1R blockade drug. Future studies should expand tumor models, numbers and types of chemotherapeutic agents used in the clinic to examine synergistic effects with anti-CSF-1R and choose the optimal cytotoxic drug to maximize the effects of CSF-1R targeting agents.

Targeting neutrophil-dependent immunosuppression further improves cisplatin/anti-CSF-1R efficacy

In contrast to cancer cells which develop mechanisms of resistance to therapies, immune cells are not under the same mutational pressure and thus unlikely to develop therapy resistance. However, bidirectional feedback between cancer cells and their microenvironment can induce resistance of the tumor microenvironment to immuno-modulation of CSF-1R targeting. In several models, resistance to CSF-1R targeting or macrophage inhibition was seen by the recruitment of tumor-promoting neutrophils [131-135]. These newly recruited neutrophils embodied similar pro-tumor mechanisms as the depleted TAMs, such as regulating proccesses like immunosuppression and angiogenesis [134,135]. However, different than those studies, neutrophils did not take over the function of macrophages upon CSF-1R blockade and cisplatin in KEP tumors, but unlike macrophages, neutrophils exhibited immunomodulatory functions (**Chapter 4**). In the poorly immunogenic KEP model, we targeted the immunosuppressive neutrophils in cisplatin/anti-CSF-1R-treated mice to obtain an effective $CD8^+$ T cell response that contributed to tumor control and extended survival (**Chapter 4**). Moreover, antibody-mediated depletion of NK cells resulted in a partial los of the benefit of neutrophil depletion, suggesting that not only $CD8^+$ T cells but also NK cells are necessary to engage anti-tumor immunity upon neutrophil depletion in cisplatin/anti-CSF-1R-treated mice. Engagement of anti-tumor immunity upon macrophage and neutrophil targeting was also seen in a mouse model for pancreatic cancer [133], but whether type I IFN signaling was induced is unknown. However, since we did not observe an increase in neutrophil recruitment by absolute neutrophil numbers unlike other studies have noted, it is likely that a different mechanism influenced neutrophil function in the KEP mouse model upon macrophage targeting.

The exact TME signals that instructed neutrophils to acquire pro-tumor functions upon CSF-1R blockade are unknown, although it is plausible that prolonged type I IFN signaling could have led to immunosuppressive circuits. While several studies have suggested that type I IFNs induce anti-tumor properties in neutrophils [136-138], other studies in chronic infections such as malaria-infected hosts and patients with active tuberculosis found that a type I IFN transcriptional signature in neutrophils is correlated with tissue damage and disease pathogenesis [139,140]. Moreover, negative-feedback mechanisms were reported in studies on chronic viral infections when type I IFN signaling persisted and lead for example to the generation of an

immunosuppressive environment [93,141,142]. In fact, higher levels of PD-L1 were found on type I IFN-producing macrophages upon anti-CSF-1R treatment in KEP mice (**Chapter 4**), suggesting that perhaps an autocrine mechanism was present to resolve the inflammatory responses. Whether sustained type I IFN signaling can rewire neutrophils in cisplatin/anti-CSF-1R-treated mice should be addressed in future studies. Furthermore, RNA-sequencing analysis on neutrophils isolated from cisplatin/anti-CSF-1R-treated mouse tumors displayed elevated expression levels of type I IFN-stimulated genes compared to neutrophils in tumors of cisplatin/control antibody-treated mice (**Chapter 4**). It is unclear whether the type I IFN signaling of neutrophils promotes their immunosuppressive abilities.

How neutrophils exert their immunosuppressive functions needs to be further elucidated. Interestingly, a correlation was recently found between type I IFN signaling and ROS production of neutrophils in a melanoma model [138]. Immunosuppressive- pro-metastatic neutrophils in the KEP mouse model have previously shown to express high levels of inducible nitric oxide synthase (iNOS) [11,143]. By influencing conformational changes in TCR recognition, iNOS prevents specific peptide recognition by T cells [144]. To elucidate whether neutrophils employ iNOS to prevent an anti-tumor immune response in cisplatin/anti-CSF-1R-treated mice additional studies are required.

Since neutrophils have shown to influence various tumor-promoting processes, neutrophils have become interesting putative targets for therapeutic intervention [145]. Moreover, a high neutrophil-to-lymphocyte ratio in the circulation of multiple cancers is linked to poor prognosis in patients [146]. Currently, the chemokine receptors CXCR1 and CXCR2 that are important for neutrophil recruitment are under clinical evaluation [147,148]. Our study shows that the therapeutic efficacy of targeting macrophages and neutrophils in cisplatin-treated KEP mice is mediated by the induction of type I IFNs and by unleashing anti-tumor responses. To this end, it will be important to evaluate the development of protumor functions by neutrophils in patients that receive combinational therapy of chemotherapy with anti-CSF-1R or type I IFN-stimulating drugs and the subsequent testing of neutrophil-targeting therapy efficacy.

Concluding remarks and future perspectives

The work presented in this thesis focuses on obtaining a better understanding of the adaptive immune system in breast cancer initiation, progression, metastasis and chemotherapy response. In addition, this thesis focuses on maximizing the success of immunomodulatory agents targeting myeloid cells using genetically engineered mouse models. This thesis demonstrates that unlike other breast cancer mouse models [9-11], the adaptive immune system is not involved in primary tumor and metastasis formation in a *de novo* tumor mouse model of HER2-positive breast cancer (**Chapter 2**). To harness successful anti-tumor immunity and increase therapy outcomes in HER2-positive patients, future research should be aimed at understanding the immunosuppressive networks in HER2-positive breast cancer. Furthermore, while the endogenous adaptive immune system has shown to play an important role during chemotherapy response of immunogenic cancer models [76], our research shows that the adaptive immune system is not important during chemotherapy response in two *de novo* breast tumor mouse models. Remarkably, by performing studies with CSF-1R blockade to target macrophages, we demonstrate that the use of agents that trigger type I IFN responses enhances the anti-cancer efficacy of chemotherapy. This thesis further elucidates that engagement of anti-tumor immunity can be reached with the addition of neutrophil depletion during chemotherapy and CSF-1R blockade. Thus, these data suggest that a combination strategy triggering the removal of the immunosuppressive TME networks and subsequent type I IFN response is the mechanism of action to acquire a proficient adaptive immune response in the less immunogenic ILC mouse model upon chemotherapy treatment. This thesis reveals that investigating the function of the adaptive immune system during tumor development and chemotherapy in a larger set of solid breast cancer subtypes is essential for the development of immunomodulatory approaches. Lastly, the data in this thesis describe that the synergy of combined chemotherapy and CSF-1R blockade is chemotherapy dependent as we found that only platinum drugs, but not docetaxel, synergized with CSF-1R blockade and increased survival further. These data indicate that therapeutic approaches using type I IFN-inducing agents such as CSF-1R-targeting drugs or STING agonists are important for successful anti-cancer therapy, however future research should obtain more insights into the synergistic effects of combinatorial therapies with myeloid targeting and evaluate immunomodulatory drug-induced resistance. Considering the realization that cancer subtype, the genetic background of the tumors, disease stage and treatment history affect anti-cancer immunity, the vision for immunomodulatory therapies must change to a more personalized treatment.

References

1 Wellenstein, M. D. & de Visser, K. E. Cancer-Cell-Intrinsic Mechanisms Shaping the Tumor Immune Landscape. *Immunity* **48**, 399-416, doi:10.1016/j.immuni.2018.03.004 (2018).

2 Busch, S. E. *et al.* Lung Cancer Subtypes Generate Unique Immune Responses. *The Journal of Immunology* **197**, 4493-4503, doi:10.4049/jimmunol.1600576 (2016).

3 Tekpli, X. *et al.* An independent poor-prognosis subtype of breast cancer defined by a distinct tumor immune microenvironment. *Nature Communications* **10**, 5499, doi:10.1038/s41467-019-13329-5 (2019).

4 Casanovas, O., Hicklin, D. J., Bergers, G. & Hanahan, D. Drug resistance by evasion of antiangiogenic targeting of VEGF signaling in late-stage pancreatic islet tumors. *Cancer Cell* **8**, 299-309 (2005).

5 de Visser, K. E., Korets, L. V. & Coussens, L. M. De novo carcinogenesis promoted by chronic inflammation is B lymphocyte dependent. *Cancer Cell* **7**, 411-423 (2005).

6 Andreu, P. *et al.* FcRgamma activation regulates inflammation-associated squamous carcinogenesis. *Cancer Cell* **17**, 121-134, doi:10.1016/j.ccr.2009.12.019 (2010).

7 Haybaeck, J. *et al.* A lymphotoxin-driven pathway to hepatocellular carcinoma. *Cancer Cell* **16**, 295-308, doi:S1535-6108(09)00294-3 [pii]
10.1016/j.ccr.2009.08.021 (2009).

8 Wellenstein, M. D. *et al.* Loss of p53 triggers WNT-dependent systemic inflammation to drive breast cancer metastasis. *Nature*, doi:10.1038/s41586-019-1450-6 (2019).

9 DeNardo, D. G. *et al.* CD4(+) T cells regulate pulmonary metastasis of mammary carcinomas by enhancing protumor properties of macrophages. *Cancer Cell* **16**, 91-102, doi:S1535-6108(09)00216-5 [pii]
10.1016/j.ccr.2009.06.018 (2009).

10 Tan, W. *et al.* Tumour-infiltrating regulatory T cells stimulate mammary cancer metastasis through RANKL-RANK signalling. *Nature* **470**, 548-553, doi:10.1038/nature09707 (2011).

11 Coffelt, S. B. *et al.* IL-17-producing gammadelta T cells and neutrophils conspire to promote breast cancer metastasis. *Nature* **522**, 345-348, doi:10.1038/nature14282 (2015).

12 Glajcar, A., Szpor, J., Hodorowicz-Zaniewska, D., Tyrak, K. E. & Okoń, K. The composition of T cell infiltrates varies in primary invasive breast cancer of different molecular subtypes as well as according to tumor size and nodal status. *Virchows Arch* **475**, 13-23, doi:10.1007/s00428-019-02568-y (2019).

13 Garner, H. & de Visser, K. E. Immune crosstalk in cancer progression and metastatic spread: a complex conversation. *Nat Rev Immunol* **20**, 483-497, doi:10.1038/s41577-019-0271-z (2020).

14 Sasada, T., Kimura, M., Yoshida, Y., Kanai, M. & Takabayashi, A. CD4+CD25+ regulatory T cells in patients with gastrointestinal malignancies: possible involvement of regulatory T cells in disease progression. *Cancer* **98**, 1089-1099, doi:10.1002/cncr.11618 (2003).

15 Nishikawa, H. & Sakaguchi, S. Regulatory T cells in tumor immunity. *Int J Cancer* **127**, 759-767, doi:10.1002/ijc.25429 (2010).

16 Nakamura, R. *et al.* Accumulation of regulatory T cells in sentinel lymph nodes is a prognostic predictor in patients with node-negative breast cancer. *Eur J Cancer* **45**, 2123-2131, doi:10.1016/j.ejca.2009.03.024 (2009).

17 Dadi, S. *et al.* Cancer Immunosurveillance by Tissue-Resident Innate Lymphoid Cells and Innate-like T Cells. *Cell* **164**, 365-377, doi:10.1016/j.cell.2016.01.002 (2016).

18 Croci, S. *et al.* Interleukin-15 is required for immunosurveillance and immunoprevention of HER2/neu-driven mammary carcinogenesis. *Breast Cancer Res* **17**, 70, doi:10.1186/s13058-015-0588-x (2015).

19 Shankaran, V. *et al.* IFNÎ³, and lymphocytes prevent primary tumour development and shape tumour immunogenicity. *Nature* **410**, 1107-1111 (2001).

20 Takeuchi, N. *et al.* Anti-HER-2/neu immune responses are induced before the development of clinical tumors but declined following tumorigenesis in HER-2/neu transgenic mice. *Cancer Res* **64**, 7588-7595, doi:10.1158/0008-5472.CAN-04-1081 (2004).

21 Kmieciak, M. *et al.* Danger signals and nonself entity of tumor antigen are both required for eliciting effective immune responses against HER-2/neu positive mammary carcinoma: implications for vaccine design. *Cancer Immunol Immunother* **57**, 1391-1398, doi:10.1007/s00262-008-0475-8 (2008).

22 Disis, M. L. *et al.* Immunity to the HER-2/neu oncogenic protein. *Ciba Found Symp* **187**, 198-207; discussion 207-111, doi:10.1002/9780470514672.ch13 (1994).

23 Peoples, R., Milatovich, A. & Francke, U. Hemizygosity at the insulin-like growth factor I receptor (IGF1R) locus and growth failure in the ring chromosome 15 syndrome. *Cytogenet Cell Genet* **70**, 228-234, doi:10.1159/000134040 (1995).

24 Park, J. M., Terabe, M., Donaldson, D. D., Forni, G. & Berzofsky, J. A. Natural immunosurveillance against spontaneous, autochthonous breast cancers revealed and enhanced by blockade of IL-13-mediated negative regulation. *Cancer Immunol Immunother* **57**, 907-912, doi:10.1007/s00262-007-0414-0 (2008).

25 Hanahan, D. & Coussens, L. M. Accessories to the crime: functions of cells recruited to the tumor microenvironment. *Cancer cell* **21**, 309-322, doi:10.1016/j.ccr.2012.02.022 (2012).

26 Joyce, J. A. & Fearon, D. T. T cell exclusion, immune privilege, and the tumor microenvironment. *Science* **348**, 74-80, doi:10.1126/science.aaa6204 (2015).

27 Morales, J. K. *et al.* Adoptive transfer of HER2/neu-specific T cells expanded with alternating gamma chain cytokines mediate tumor regression when combined with the depletion of myeloid-derived suppressor cells. *Cancer Immunol Immunother* **58**, 941-953, doi:10.1007/s00262-008-0609-z (2009).

28 Ambrosino, E. *et al.* Immunosurveillance of Erbb2 carcinogenesis in transgenic mice is concealed by a dominant regulatory T-cell self-tolerance. *Cancer Res* **66**, 7734-7740, doi:10.1158/0008-5472.CAN-06-1432 (2006).

29 Norian, L. A. *et al.* Tumor-infiltrating regulatory dendritic cells inhibit CD8+ T cell function via L-arginine metabolism. *Cancer Res* **69**, 3086-3094, doi:10.1158/0008-5472.CAN-08-2826 (2009).

30 Costa, R. L. B. & Czerniecki, B. J. Clinical development of immunotherapies for HER2(+) breast cancer: a review of HER2-directed monoclonal antibodies and beyond. *NPJ Breast Cancer* **6**, 10, doi:10.1038/s41523-020-0153-3 (2020).

31 Park, S. *et al.* The therapeutic effect of anti-HER2/neu antibody depends on both innate and adaptive immunity. *Cancer Cell* **18**, 160-170, doi:10.1016/j.ccr.2010.06.014 (2010).

32 Stagg, J. *et al.* Anti-ErbB-2 mAb therapy requires type I and II interferons and synergizes with anti-PD-1 or anti-CD137 mAb therapy. *Proc Natl Acad Sci U S A* **108**, 7142-7147, doi:10.1073/pnas.1016569108 (2011).

33 Conti, L. *et al.* Immunotargeting of the xCT Cystine/Glutamate Antiporter Potentiates the Efficacy of HER2-Targeted Immunotherapies in Breast Cancer. *Cancer Immunol Res* **8**, 1039-1053, doi:10.1158/2326-6066.CIR-20-0082 (2020).

34 Cassier, P. A. *et al.* CSF1R inhibition with emactuzumab in locally advanced diffuse-type tenosynovial giant cell tumours of the soft tissue: a dose-escalation and dose-expansion phase 1 study. *The Lancet. Oncology* **16**, 949-956, doi:10.1016/S1470-2045(15)00132-1 (2015).

35 Guy, C. T. *et al.* Expression of the neu protooncogene in the mammary epithelium of transgenic mice induces metastatic disease. *Proc Natl Acad Sci U S A* **89**, 10578-10582, doi:10.1073/pnas.89.22.10578 (1992).

36 Siegel, P. M., Dankort, D. L., Hardy, W. R. & Muller, W. J. Novel activating mutations in the neu proto-oncogene involved in induction of mammary tumors. *Mol Cell Biol* **14**, 7068-7077, doi:10.1128/mcb.14.11.7068 (1994).

37 Gross, E. T. *et al.* Immunosurveillance and immunoediting in MMTV-PyMT-induced mammary oncogenesis. *Oncoimmunology* **6**, e1268310, doi:10.1080/2162402X.2016.1268310 (2017).

38 Ruiter, J. R. d., Wessels, L. F. A. & Jonkers, J. Mouse models in the era of large human tumour sequencing studies. *Open Biology* **8**, 180080, doi:doi:10.1098/rsob.180080 (2018).

39 de Ruiter, J. R., Wessels, L. F. A. & Jonkers, J. Mouse models in the era of large human tumour sequencing studies. *Open Biol* **8**, doi:10.1098/rsob.180080 (2018).

40 Zoi, I., Karamouzis, M. V., Adamopoulos, C. & Papavassiliou, A. G. RANKL Signaling and ErbB Receptors in Breast Carcinogenesis. *Trends Mol Med* **22**, 839-850, doi:10.1016/j.molmed.2016.07.009 (2016).

41 Harper, K. L. *et al.* Mechanism of early dissemination and metastasis in Her2+ mammary cancer. *Nature* **540**, 588-592, doi:10.1038/nature20609 (2016).

42 Gonzalez-Suarez, E. *et al.* RANK ligand mediates progestin-induced mammary epithelial proliferation and carcinogenesis. *Nature* **468**, 103-107, doi:10.1038/nature09495 (2010).

43 Gomes, I. *et al.* Expression of receptor activator of NFkB (RANK) drives stemness and resistance to therapy in ER+HER2- breast cancer. *Oncotarget* **11**, 1714-1728, doi:10.18632/oncotarget.27576 (2020).

44 Chen, D. S. & Mellman, I. Oncology meets immunology: the cancer-immunity cycle. *Immunity* **39**, 1-10, doi:10.1016/j.immuni.2013.07.012 (2013).

45 Li, B., Cui, Y., Nambiar, D. K., Sunwoo, J. B. & Li, R. The Immune Subtypes and Landscape of Squamous Cell Carcinoma. *Clinical Cancer Research* **25**, 3528-3537, doi:10.1158/1078-0432.Ccr-18-4085 (2019).

46 Xu, F. *et al.* Analysis of Lung Adenocarcinoma Subtypes Based on Immune Signatures Identifies Clinical Implications for Cancer Therapy. *Molecular Therapy - Oncolytics* **17**, 241-249, doi:10.1016/j.omto.2020.03.021 (2020).

47 Yarden, Y. & Sliwkowski, M. X. Untangling the ErbB signalling network. *Nat Rev Mol Cell Biol* **2**, 127-137, doi:10.1038/35052073 (2001).

48 Vasan, N., Baselga, J. & Hyman, D. M. A view on drug resistance in cancer. *Nature* **575**, 299-309, doi:10.1038/s41586-019-1730-1 (2019).

49 Shiao, S. L., Ganesan, A. P., Rugo, H. S. & Coussens, L. M. Immune microenvironments in solid tumors: new targets for therapy. *Genes & development* **25**, 2559-2572, doi:25/24/2559 [pii] 10.1101/gad.169029.111 (2011).

50 de Visser, K. E. & Jonkers, J. Towards understanding the role of cancer-associated inflammation in chemoresistance. *Curr Pharm Des* **15**, 1844-1853, doi:10.2174/138161209788453239 (2009).

51 Coffelt, S. B. & de Visser, K. E. Immune-mediated mechanisms influencing the efficacy of anticancer therapies. *Trends Immunol* **36**, 198-216, doi:10.1016/j.it.2015.02.006 (2015).

52 Apetoh, L. *et al.* Toll-like receptor 4-dependent contribution of the immune system to anticancer chemotherapy and radiotherapy. *Nature medicine* **13**, 1050-1059, doi:10.1038/nm1622 (2007).

53 Obeid, M. *et al.* Calreticulin exposure dictates the immunogenicity of cancer cell death. *Nature medicine* **13**, 54-61, doi:nm1523 [pii] 10.1038/nm1523 (2007).

54 Ghiringhelli, F. *et al.* Activation of the NLRP3 inflammasome in dendritic cells induces IL-1beta-dependent adaptive immunity against tumors. *Nature medicine* **15**, 1170-1178, doi:nm.2028 [pii] 10.1038/nm.2028 (2009).

55 Vacchelli, E., Prada, N., Kepp, O. & Galluzzi, L. Current trends of anticancer immunochemotherapy. *Oncoimmunology* **2**, e25396, doi:10.4161/onci.25396 (2013).

56 Ma, Y. *et al.* Anticancer chemotherapy-induced intratumoral recruitment and differentiation of antigen-presenting cells. *Immunity* **38**, 729-741, doi:10.1016/j.immuni.2013.03.003 (2013).

57 Bezu, L. *et al.* Combinatorial strategies for the induction of immunogenic cell death. *Front Immunol* **6**, 187, doi:10.3389/fimmu.2015.00187 (2015).

58 Casares, N. *et al.* Caspase-dependent immunogenicity of doxorubicin-induced tumor cell death. *Journal of Experimental Medicine* **202**, 1691-1701, doi:10.1084/jem.20050915 (2005).

59 Galluzzi, L. *et al.* Consensus guidelines for the definition, detection and interpretation of immunogenic cell death. *Journal for ImmunoTherapy of Cancer* **8**, e000337, doi:10.1136/jitc-2019-000337 (2020).

60 Olive, K. P. *et al.* Inhibition of Hedgehog signaling enhances delivery of chemotherapy in a mouse model of pancreatic cancer. *Science* **324**, 1457-1461, doi:1171362 [pii] 10.1126/science.1171362 (2009).

61 Devaud, C. *et al.* Tissues in different anatomical sites can sculpt and vary the tumor microenvironment to affect responses to therapy. *Molecular therapy : the journal of the American Society of Gene Therapy* **22**, 18-27, doi:10.1038/mt.2013.219 (2014).

62 Mattarollo, S. R. *et al.* Pivotal role of innate and adaptive immunity in anthracycline chemotherapy of established tumors. *Cancer Res* **71**, 4809-4820, doi:0008-5472.CAN-11-0753 [pii] 10.1158/0008-5472.CAN-11-0753 (2011).

63 Schreiber, K., Rowley, D. A., Riethmuller, G. & Schreiber, H. Cancer immunotherapy and preclinical studies: why we are not wasting our time with animal experiments. *Hematology/oncology clinics of North America* **20**, 567-584, doi:10.1016/j.hoc.2006.03.001 (2006).

64 Yu, P., Rowley, D. A., Fu, Y. X. & Schreiber, H. The role of stroma in immune recognition and destruction of well-established solid tumors. *Curr Opin Immunol* **18**, 226-231 (2006).

65 Willimsky, G. *et al.* Immunogenicity of premalignant lesions is the primary cause of general cytotoxic T lymphocyte unresponsiveness. *J Exp Med* **205**, 1687-1700, doi:jem.20072016 [pii] 10.1084/jem.20072016 (2008).

66 Shankaran, V. *et al.* IFNgamma and lymphocytes prevent primary tumour development and shape tumour immunogenicity. *Nature* **410**, 1107-1111 (2001).

67 Denardo, D. G. *et al.* Leukocyte complexity predicts breast cancer survival and functionally regulates response to chemotherapy. *Cancer Discovery* **1**, 54-55 (2011).

68 Mitchem, J. B. *et al.* Targeting tumor-infiltrating macrophages decreases tumor-initiating cells, relieves immunosuppression, and improves chemotherapeutic responses. *Cancer Res* **73**, 1128-1141, doi:10.1158/0008-5472.Can-12-2731 (2013).

69 Ruffell, B. *et al.* Macrophage IL-10 blocks CD8+ T cell-dependent responses to chemotherapy by suppressing IL-12 expression in intratumoral dendritic cells. *Cancer Cell* **26**, 623-637, doi:10.1016/j.ccell.2014.09.006 (2014).

70 Nistico, P. *et al.* Chemotherapy enhances vaccine-induced antitumor immunity in melanoma patients. *Int J Cancer* **124**, 130-139, doi:10.1002/ijc.23886 (2009).

71 Ramakrishnan, R. *et al.* Chemotherapy enhances tumor cell susceptibility to CTL-mediated killing during cancer immunotherapy in mice. *J Clin Invest* **120**, 1111-1124, doi:40269 [pii] 10.1172/JCI40269 (2010).

72 Robert, C. *et al.* Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. *N Engl J Med* **364**, 2517-2526, doi:10.1056/NEJMoa1104621 (2011).

73 Schmid, P. *et al.* Atezolizumab and Nab-Paclitaxel in Advanced Triple-Negative Breast Cancer. *N Engl J Med* **379**, 2108-2121, doi:10.1056/NEJMoa1809615 (2018).

74 (https://ClinicalTrials.gov/show/NCT03515798).

75 Workenhe, S. T. *et al.* De novo necroptosis creates an inflammatory environment mediating tumor susceptibility to immune checkpoint inhibitors. *Commun Biol* **3**, 645, doi:10.1038/s42003-020-01362-w (2020).

76 Galluzzi, L. *et al.* Consensus guidelines for the definition, detection and interpretation of immunogenic cell death. *J Immunother Cancer* **8**, doi:10.1136/jitc-2019-000337 (2020).

77 Guerin, M. V., Finisguerra, V., Van den Eynde, B. J., Bercovici, N. & Trautmann, A. Preclinical murine tumor models: a structural and functional perspective. *eLife* **9**, e50740, doi:10.7554/eLife.50740 (2020).

78 Zhang, Q. W. *et al.* Prognostic significance of tumor-associated macrophages in solid tumor: a meta-analysis of the literature. *PLoS One* **7**, e50946, doi:10.1371/journal.pone.0050946 (2012).

79 Zhao, X. *et al.* Prognostic significance of tumor-associated macrophages in breast cancer: a meta-analysis of the literature. *Oncotarget* **8**, 30576-30586, doi:10.18632/oncotarget.15736 (2017).

80 Yuan, X. *et al.* Prognostic significance of tumor-associated macrophages in ovarian cancer: A meta-analysis. *Gynecol Oncol* **147**, 181-187, doi:10.1016/j.ygyno.2017.07.007 (2017).

81 Yagi, T. *et al.* Tumour-associated macrophages are associated with poor prognosis and programmed death ligand 1 expression in oesophageal cancer. *Eur J Cancer* **111**, 38-49, doi:10.1016/j.ejca.2019.01.018 (2019).

82 Tuit, S. *et al.* Transcriptional Signature Derived from Murine Tumor-Associated Macrophages Correlates with Poor Outcome in Breast Cancer Patients. *Cell Rep* **29**, 1221-1235 e1225, doi:10.1016/j.celrep.2019.09.067 (2019).

83 Mantovani, A., Marchesi, F., Malesci, A., Laghi, L. & Allavena, P. Tumour-associated macrophages as treatment targets in oncology. *Nat Rev Clin Oncol* **14**, 399-416, doi:10.1038/nrclinonc.2016.217 (2017).

84 Zhou, J. *et al.* Tumor-Associated Macrophages: Recent Insights and Therapies. *Frontiers in Oncology* **10**, doi:10.3389/fonc.2020.00188 (2020).

85 Kowal, J., Kornete, M. & Joyce, J. A. Re-education of macrophages as a therapeutic strategy in cancer. *Immunotherapy* **11**, 677-689, doi:10.2217/imt-2018-0156 (2019).

86 Ries, C. H. *et al.* Targeting tumor-associated macrophages with anti-CSF-1R antibody reveals a strategy for cancer therapy. *Cancer Cell* **25**, 846-859, doi:10.1016/j.ccr.2014.05.016 (2014).

87 Ries, C. H., Hoves, S., Cannarile, M. A. & Ruttinger, D. CSF-1/CSF-1R targeting agents in clinical development for cancer therapy. *Curr Opin Pharmacol* **23**, 45-51, doi:10.1016/j.coph.2015.05.008 (2015).

88 von Tresckow, B. *et al.* An Open-Label, Multicenter, Phase I/II Study of JNJ-40346527, a CSF-1R Inhibitor, in Patients with Relapsed or Refractory Hodgkin Lymphoma. *Clin Cancer Res* **21**, 1843-1850, doi:10.1158/1078-0432.CCR-14-1845 (2015).

89 Cannarile, M. A. *et al.* Colony-stimulating factor 1 receptor (CSF1R) inhibitors in cancer therapy. *J Immunother Cancer* **5**, 53, doi:10.1186/s40425-017-0257-y (2017).

90 DeNardo, D. G. *et al.* Leukocyte complexity predicts breast cancer survival and functionally regulates response to chemotherapy. *Cancer Discov* **1**, 54-67, doi:10.1158/2159-8274.Cd-10-0028 (2011).

91 Paulus, P., Stanley, E. R., Schäfer, R., Abraham, D. & Aharinejad, S. Colony-stimulating factor-1 antibody reverses chemoresistance in human MCF-7 breast cancer xenografts. *Cancer Res* **66**, 4349-4356, doi:10.1158/0008-5472.Can-05-3523 (2006).

92 Olson, O. C., Kim, H., Quail, D. F., Foley, E. A. & Joyce, J. A. Tumor-Associated Macrophages Suppress the Cytotoxic Activity of Antimitotic Agents. *Cell Rep* **19**, 101-113, doi:10.1016/j.celrep.2017.03.038 (2017).

93 Weizman, N. *et al.* Macrophages mediate gemcitabine resistance of pancreatic adenocarcinoma by upregulating cytidine deaminase. *Oncogene* **33**, 3812-3819, doi:10.1038/onc.2013.357 (2014).

94 Alhudaithi, S. S. *et al.* Local Targeting of Lung-Tumor-Associated Macrophages with Pulmonary Delivery of a CSF-1R Inhibitor for the Treatment of Breast Cancer Lung Metastases. *Mol Pharm*, doi:10.1021/acs.molpharmaceut.0c00983 (2020).

95 Zhu, Y. *et al.* CSF1/CSF1R blockade reprograms tumor-infiltrating macrophages and improves response to T-cell checkpoint immunotherapy in pancreatic cancer models. *Cancer Res* **74**, 5057-5069, doi:10.1158/0008-5472.Can-13-3723 (2014).

96 Pyonteck, S. M. *et al.* CSF-1R inhibition alters macrophage polarization and blocks glioma progression. *Nature medicine* **19**, 1264-1272, doi:10.1038/nm.3337 (2013).

97 Zhang, H., Almuqbil, R. M., Alhudaithi, S. S., Sunbul, F. S. & da Rocha, S. R. P. Pulmonary administration of a CSF-1R inhibitor alters the balance of tumor-associated macrophages and supports first-line chemotherapy in a lung cancer model. *Int J Pharm* **598**, 120350, doi:10.1016/j.ijpharm.2021.120350 (2021).

98 Zhou, Y. *et al.* Blockade of the Phagocytic Receptor MerTK on Tumor-Associated Macrophages Enhances P2X7R-Dependent STING Activation by Tumor-Derived cGAMP. *Immunity* **52**, 357-373.e359, doi:10.1016/j.immuni.2020.01.014 (2020).

99 Sistigu, A. *et al.* Cancer cell-autonomous contribution of type I interferon signaling to the efficacy of chemotherapy. *Nat Med* **20**, 1301-1309, doi:10.1038/nm.3708 (2014).

100 Vacchelli, E. *et al.* Autocrine signaling of type 1 interferons in successful anticancer chemotherapy. *Oncoimmunology* **4**, e988042, doi:10.4161/2162402X.2014.988042 (2015).

101 Arico, E., Castiello, L., Capone, I., Gabriele, L. & Belardelli, F. Type I Interferons and Cancer: An Evolving Story Demanding Novel Clinical Applications. *Cancers (Basel)* **11**, doi:10.3390/cancers11121943 (2019).

102 Benci, J. L. *et al.* Tumor Interferon Signaling Regulates a Multigenic Resistance Program to Immune Checkpoint Blockade. *Cell* **167**, 1540-1554 e1512, doi:10.1016/j.cell.2016.11.022 (2016).

103 McNab, F., Mayer-Barber, K., Sher, A., Wack, A. & O'Garra, A. Type I interferons in infectious disease. *Nat Rev Immunol* **15**, 87-103, doi:10.1038/nri3787 (2015).

104 Deng, L. *et al.* STING-Dependent Cytosolic DNA Sensing Promotes Radiation-Induced Type I Interferon-Dependent Antitumor Immunity in Immunogenic Tumors. *Immunity* **41**, 843-852, doi:10.1016/j.immuni.2014.10.019 (2014).

105 Corrales, L. *et al.* Direct Activation of STING in the Tumor Microenvironment Leads to Potent and Systemic Tumor Regression and Immunity. *Cell Rep* **11**, 1018-1030, doi:10.1016/j.celrep.2015.04.031 (2015).

106 Bidwell, B. N. *et al.* Silencing of Irf7 pathways in breast cancer cells promotes bone metastasis through immune escape. *Nature medicine* **18**, 1224-1231, doi:10.1038/nm.2830 (2012).

107 Snijders, A. M. *et al.* An interferon signature identified by RNA-sequencing of mammary tissues varies across the estrous cycle and is predictive of metastasis-free survival. *Oncotarget* **5**, 4011-4025, doi:10.18632/oncotarget.2148 (2014).

108 Callari, M. *et al.* Subtype-dependent prognostic relevance of an interferon-induced pathway metagene in node-negative breast cancer. *Mol Oncol* **8**, 1278-1289, doi:10.1016/j.molonc.2014.04.010 (2014).

109 Katlinski, K. V. *et al.* Inactivation of Interferon Receptor Promotes the Establishment of Immune Privileged Tumor Microenvironment. *Cancer Cell* **31**, 194-207, doi:10.1016/j.ccell.2017.01.004 (2017).

110 Swann, J. B. *et al.* Type I IFN contributes to NK cell homeostasis, activation, and antitumor function. *J Immunol* **178**, 7540-7549, doi:10.4049/jimmunol.178.12.7540 (2007).

111 Critchley-Thorne, R. J. *et al.* Impaired interferon signaling is a common immune defect in human cancer. *Proc Natl Acad Sci U S A* **106**, 9010-9015, doi:10.1073/pnas.0901329106 (2009).

112 Castiello, L. *et al.* Disruption of IFN-I Signaling Promotes HER2/Neu Tumor Progression and Breast Cancer Stem Cells. *Cancer Immunol Res* **6**, 658-670, doi:10.1158/2326-6066.CIR-17-0675 (2018).

113 Parker, B. S., Rautela, J. & Hertzog, P. J. Antitumour actions of interferons: implications for cancer therapy. *Nat Rev Cancer* **16**, 131-144, doi:10.1038/nrc.2016.14 (2016).

114 Koyama, T. *et al.* Divergent activities of interferon-alpha subtypes against intracellular hepatitis C virus replication. *Hepatol Res* **34**, 41-49, doi:10.1016/j.hepres.2005.10.005 (2006).

115 Tanimoto, T. *et al.* The combination of IFN-alpha2 and IFN-alpha8 exhibits synergistic antiproliferative activity on renal cell carcinoma (RCC) cell lines through increased binding affinity for IFNAR-2. *J Interferon Cytokine Res* **27**, 517-523, doi:10.1089/jir.2007.0155 (2007).

116 Wang, X. *et al.* Evidence of cisplatin-induced senescent-like growth arrest in nasopharyngeal carcinoma cells. *Cancer Res* **58**, 5019-5022 (1998).

117 Chatterjee, N. *et al.* REV1 inhibitor JH-RE-06 enhances tumor cell response to chemotherapy by triggering senescence hallmarks. *Proc Natl Acad Sci U S A* **117**, 28918-28921, doi:10.1073/pnas.2016064117 (2020).

118 Yang, H., Wang, H., Ren, J., Chen, Q. & Chen, Z. J. cGAS is essential for cellular senescence. *Proc Natl Acad Sci U S A* **114**, E4612-e4620, doi:10.1073/pnas.1705499114 (2017).

119 Dou, Z. *et al.* Cytoplasmic chromatin triggers inflammation in senescence and cancer. *Nature* **550**, 402-406, doi:10.1038/nature24050 (2017).

120 Seyfried, T. N. & Huysentruyt, L. C. On the origin of cancer metastasis. *Crit Rev Oncog* **18**, 43-73, doi:10.1615/critrevoncog.v18.i1-2.40 (2013).

121 Moschella, F. *et al.* Cyclophosphamide induces a type I interferon-associated sterile inflammatory response signature in cancer patients' blood cells: implications for cancer chemoimmunotherapy. *Clin Cancer Res* **19**, 4249-4261, doi:10.1158/1078-0432.Ccr-12-3666 (2013).

122 Szikriszt, B. *et al.* A comprehensive survey of the mutagenic impact of common cancer cytotoxics. *Genome Biol* **17**, 99, doi:10.1186/s13059-016-0963-7 (2016).

123 Shree, T. *et al.* Macrophages and cathepsin proteases blunt chemotherapeutic response in breast cancer. *Genes & development* **25**, 2465-2479, doi:25/23/2465 [pii] 10.1101/gad.180331.111 (2011).

124 Kersten, K., Salvagno, C. & de Visser, K. E. Exploiting the Immunomodulatory Properties of Chemotherapeutic Drugs to Improve the Success of Cancer Immunotherapy. *Front Immunol* **6**, 516, doi:10.3389/fimmu.2015.00516 (2015).

125 de Biasi, A. R., Villena-Vargas, J. & Adusumilli, P. S. Cisplatin-induced antitumor immunomodulation: a review of preclinical and clinical evidence. *Clin Cancer Res* **20**, 5384-5391, doi:10.1158/1078-0432.CCR-14-1298 (2014).

126 Rebe, C., Demontoux, L., Pilot, T. & Ghiringhelli, F. Platinum Derivatives Effects on Anticancer Immune Response. *Biomolecules* **10**, doi:10.3390/biom10010013 (2019).

127 Wang, W. *et al.* Identification of an Immune-Related Signature for Predicting Prognosis in Patients With Pancreatic Ductal Adenocarcinoma. *Front Oncol* **10**, 618215, doi:10.3389/fonc.2020.618215 (2020).

128 Liu, W. *et al.* Cisplatin remodels the tumor immune microenvironment via the transcription factor EB in ovarian cancer. *Cell Death Discov* **7**, 136, doi:10.1038/s41420-021-00519-8 (2021).

129 Gomez-Roca, C. A. *et al.* Phase I study of emactuzumab single agent or in combination with paclitaxel in patients with advanced/metastatic solid tumors reveals depletion of immunosuppressive M2-like macrophages. *Ann Oncol* **30**, 1381-1392, doi:10.1093/annonc/mdz163 (2019).

130 Wesolowski, R. *et al.* Phase Ib study of the combination of pexidartinib (PLX3397), a CSF-1R inhibitor, and paclitaxel in patients with advanced solid tumors. *Ther Adv Med Oncol* **11**, 1758835919854238, doi:10.1177/1758835919854238 (2019).

131 Swierczak, A. *et al.* The promotion of breast cancer metastasis caused by inhibition of CSF-1R/CSF-1 signaling is blocked by targeting the G-CSF receptor. *Cancer Immunol Res* **2**, 765-776, doi:10.1158/2326-6066.Cir-13-0190 (2014).

132 Kumar, V. *et al.* Cancer-Associated Fibroblasts Neutralize the Anti-tumor Effect of CSF1 Receptor Blockade by Inducing PMN-MDSC Infiltration of Tumors. *Cancer Cell* **32**, 654-668.e655, doi:10.1016/j.ccell.2017.10.005 (2017).

133 Nywening, T. M. *et al.* Targeting both tumour-associated CXCR2(+) neutrophils and CCR2(+) macrophages disrupts myeloid recruitment and improves chemotherapeutic responses in pancreatic ductal adenocarcinoma. *Gut* **67**, 1112-1123, doi:10.1136/gutjnl-2017-313738 (2018).

134 Rivera, L. B. *et al.* Intratumoral myeloid cells regulate responsiveness and resistance to antiangiogenic therapy. *Cell Rep* **11**, 577-591, doi:10.1016/j.celrep.2015.03.055 (2015).

135 Pahler, J. C. *et al.* Plasticity in tumor-promoting inflammation: impairment of macrophage recruitment evokes a compensatory neutrophil response. *Neoplasia* **10**, 329-340, doi:10.1593/neo.07871 (2008).

136 Pylaeva, E., Lang, S. & Jablonska, J. The Essential Role of Type I Interferons in Differentiation and Activation of Tumor-Associated Neutrophils. *Front Immunol* **7**, 629, doi:10.3389/fimmu.2016.00629 (2016).

137 Andzinski, L. *et al.* Type I IFNs induce anti-tumor polarization of tumor associated neutrophils in mice and human. *Int J Cancer* **138**, 1982-1993, doi:10.1002/ijc.29945 (2016).

138 Kalafati, L. *et al.* Innate Immune Training of Granulopoiesis Promotes Anti-tumor Activity. *Cell* **183**, 771-785.e712, doi:10.1016/j.cell.2020.09.058 (2020).

139 Berry, M. P. *et al.* An interferon-inducible neutrophil-driven blood transcriptional signature in human tuberculosis. *Nature* **466**, 973-977, doi:10.1038/nature09247 (2010).

140 Rocha, B. C. *et al.* Type I Interferon Transcriptional Signature in Neutrophils and Low-Density Granulocytes Are Associated with Tissue Damage in Malaria. *Cell Rep* **13**, 2829-2841, doi:10.1016/j.celrep.2015.11.055 (2015).

141 Sivick, K. E. *et al.* Magnitude of Therapeutic STING Activation Determines CD8(+) T Cell-Mediated Anti-tumor Immunity. *Cell Rep* **29**, 785-789, doi:10.1016/j.celrep.2019.09.089 (2019).

142 Spaapen, R. M. *et al.* Therapeutic activity of high-dose intratumoral IFN-β requires direct effect on the tumor vasculature. *J Immunol* **193**, 4254-4260, doi:10.4049/jimmunol.1401109 (2014).

143 Kersten, K. *et al.* Mammary tumor-derived CCL2 enhances pro-metastatic systemic inflammation through upregulation of IL1beta in tumor-associated macrophages. *Oncoimmunology* **6**, e1334744, doi:10.1080/2162402X.2017.1334744 (2017).

144 Nagaraj, S. *et al.* Altered recognition of antigen is a mechanism of CD8+ T cell tolerance in cancer. *Nature medicine* **13**, 828-835, doi:10.1038/nm1609 (2007).

145 Jaillon, S. *et al.* Neutrophil diversity and plasticity in tumour progression and therapy. *Nat Rev Cancer* **20**, 485-503, doi:10.1038/s41568-020-0281-y (2020).

146 Shaul, M. E. & Fridlender, Z. G. Tumour-associated neutrophils in patients with cancer. *Nat Rev Clin Oncol* **16**, 601-620, doi:10.1038/s41571-019-0222-4 (2019).

147 Schott, A. F. *et al.* Phase Ib Pilot Study to Evaluate Reparixin in Combination with Weekly Paclitaxel in Patients with HER-2-Negative Metastatic Breast Cancer. *Clin Cancer Res* **23**, 5358-5365, doi:10.1158/1078-0432.Ccr-16-2748 (2017).

148 (https://ClinicalTrials.gov/show/NCT03177187).