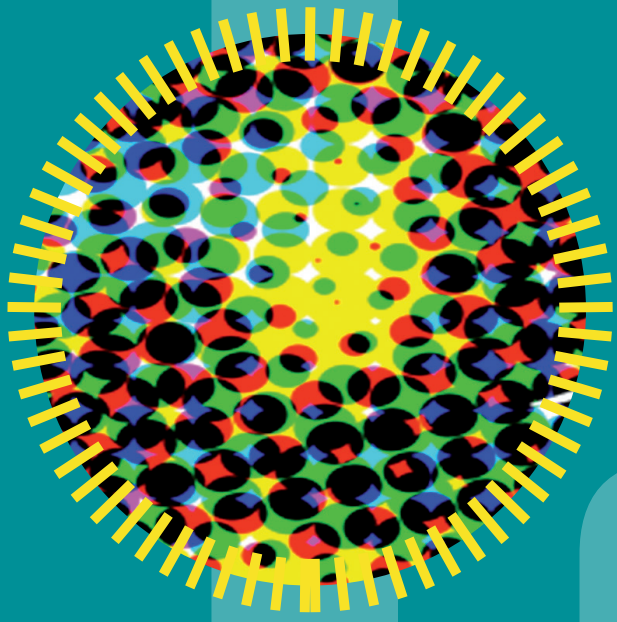
Breaking barriers: unraveling response mechanisms to immunotherapy in breast cancer

Blomberg, O.S.



RESPONSE

RESISTANCE

NEW OPPORTUNITIES

Introduction & Scope of thesis

Breast cancer remains the leading cause of cancer-related deaths in women worldwide, with most deaths resulting from metastatic disease. Although survival rates have improved in recent years due to advances in treatment options and earlier detection due to screening programs, breast cancer incidence is on the rise and patients diagnosed with advanced metastatic disease remain, with rare exceptions, incurable. Breast cancer is comprised of different subtypes driven by heterogeneity in pathology, genomic alternations, hormone receptor status, and gene-expression profiles[1]. More recently, another factor of heterogeneity is recognized to be a crucial determinant of clinical behavior and response to therapy: the tumor microenvironment (TME). Tumors are complex ecosystems containing not only malignant carcinoma cells, but also a diverse range endothelial, stromal, and immune cells, which together comprise the TME[2]. During tumor initiation and progression, cancerous cells are in constant crosstalk with these tumor infiltrating non-cancerous cells. The immune system is of particular interest, as it plays a dual role in cancer initiation and metastatic spread.

Immune cells as guardians of homeostasis

Before considering the role of the immune system in cancer, it is important to realize the fundamental tasks of immune cells as guardians of homeostasis. The immune system is equipped to rapidly respond to numerous types of enemies, ranging from intracellular viruses to large parasites. The first line of defense is typically elicited by innate immune cells, which are essential for both protective immunity and tissue regeneration after the insult. These initial responders also play a crucial role in recruiting and activating adaptive immune cells that provide the second line of defense by producing targeted antigen-specific responses and provide immunological memory, protecting the body against future infections. Equally important however, is the proper resolution of the inflammatory response upon clearance of these pathogens to protect the integrity of the body's tissues. In addition, the immune system must prevent mounting responses against harmless environmental antigens or autoimmune responses against self-antigens. Cancer draws many parallels with chronic viral infections and autoimmune diseases[3,4]. Chronic infections, autoimmunity, and cancer lead to unresolved inflammatory responses, which cause damage to the host tissue. The immune system uses various mechanisms such as upregulation of immune checkpoint molecules and expansion of immunoregulatory cells to limit this damage. However, the persistent presence of tumor antigens and the aberrant activation of the immune system in patients with cancer leads to a chronically inflamed but immunosuppressed state which facilitates cancer progression and metastasis.

Dual role of the immune system in cancer

To mount an effective anti-tumor immune response, a complex sequence of interactions between multiple cell types must occur[5]. To initiate an anti-tumor T cell response, cancer cells need to release tumor antigens, which are taken up by professional antigen-presenting cells such as dendritic cells (DCs). Upon appropriate maturation signals provided by the TME, activated DCs migrate to the tumor-draining lymph nodes where they present the antigens on MHC-I and MHC-

II molecules to T cells. If T cells are in turn properly activated with co-stimulatory signals provided by DCs and $CD4^+$ T_{helper} cells, they migrate back to the tumor, where they can recognize cancer cells via T cell receptor (TCR)-MHC interactions, leading to cancer cell killing. Besides T cells, other immune cell populations may exert anti-tumor functions. Natural Killer (NK) cells can directly kill cancer cells in absence of MHC-I expression on tumor cells. Moreover, depending on tumor type and stage, tumor-infiltrating myeloid populations, including eosinophils, neutrophils, and macrophages, can contribute to anti-tumor immunity, either via direct tumor cell killing through, amongst others, production of reactive oxygen species and secretion of cytokines, or indirectly, by mediating T cell and NK cell recruitment and activation.

However, the mere presence of anti-tumor T cells or NK cells in the TME is not sufficient for tumor control. Cancer cells employ a plethora of mechanisms to evade anti-tumor immunity. Paradoxically, the presence of an anti-tumor immune response exacerbates these immune evasion tactics, with those cancer cells surviving that succeed to withstand the constant immune pressure in a process called immunoediting[6]. Consequently, it is frequently these immuno-edited cells that attain the ability to escape the primary tumor and disseminate to distant organs. Mutational adaptation, downregulation of MHC-I expression, reduced IFN sensitivity, and upregulation of inhibitory ligands such as PD-L1 that inhibit the recognition and prevent the killing of cancer cells by T cells are among the many cancer cell-intrinsic mechanisms of immune evasion[7]. However, an equally important mechanism of immune evasion is the establishment of an immunosuppressive environment that inhibits the development or effectiveness of the anti-tumor immune response[2]. Immune cells display immense diversity and plasticity, responding to specific environmental cues by acquiring either anti- or pro-tumorigenic phenotypes. Cancer cell-derived cytokines such as TGFβ and IL-1β skew immune cells into tumor-promoting phenotypes. These tumor-educated immune cells, in particular tumor-associated macrophages (TAMs) and neutrophils, inhibit anti-tumor immunity via expression of T cell inhibitory ligands, production of immunosuppressive cytokines, consumption of essential amino acids from the TME, and production of reactive oxygen species[8,9].

Moreover, tumors affect the immune system far beyond the TME[3]. Again, a duality in the role of the immune system exists. On the one hand, systemic immunity is required to mount effective anti-tumor responses, especially to prevent metastatic spread[10]. Conversely, tumors secrete mediators that signal to the bone marrow to influence hematopoiesis, skewing immune cell development towards increased myelopoiesis and polarization of immunosuppressive cells[3], and preparing distant organs for arrival of metastatic cells by inducing (pre-)metastatic niche formation[11]. A high neutrophil-to-lymphocyte ratio in the circulation is associated with poor survival across solid tumor types[12]. Similarly, increased levels of regulatory T cells (T_{regs}) have been found in the circulation of breast cancer patients[13,14], and their *ex vivo* immunosuppressive capacity is a predictor for cancer recurrence[15]. Tumor-educated immune cells may further promote metastasis by additional means that go beyond the suppression of anti-tumor immunity, aiding extravasation, cancer cell survival, setting up of a favorable (pre-)metastatic niche, supporting extravasation

and the outgrowth of a metastatic lesion[2]. The tumor's fate is ultimately determined by the balance between anti-tumor immunity and tumor-promoting inflammation and immunosuppression. Understanding the mechanisms that govern the interplay between cancer and the immune system underlying these processes is crucial to develop new therapies and improve patient outcomes.

Immunotherapy for breast cancer

The goal of immunotherapy is to tip the balance in favor of anti-tumor immunity. Immune checkpoint blockade (ICB) aims to improve the priming, expansion, and effector functions of anti-tumor T cells to mount an effective anti-tumor immune response. The introduction of CTLA-4 blockade in 2011, followed by the addition of PD-1 blockade a few years later, drastically improved survival of patients with unresectable metastatic melanoma, a patient cohort that was previously untreatable[16,17]. This major success quickly revolutionized the treatment landscape for patients with other metastatic cancers. In addition to metastatic melanoma, checkpoint inhibitors are now approved for the treatment of many other cancer types including metastatic PD-L1$^+$ triple-negative breast cancer (TNBC), non-small cell lung cancer, renal cell carcinoma, gastric cancer and head and neck squamous cell carcinoma, and all metastatic solid tumors with high microsatellite instability or deficiency in DNA mismatch repair[18,19].

Notwithstanding its successes, the reality is that only a small proportion of patients shows durable responses to immune checkpoint inhibitors[20], and the efficacy is much lower against less immunogenic cancer types such as breast cancer[21]. Indeed, durable responses to ICB as monotherapy are only achieved in around 5% of patients with metastatic breast cancer[22] and are mainly limited to TNBC[23]. Although response rates are improved by selecting patients on PD-L1$^+$ tumors[24], most metastatic breast cancer patients fail to respond to ICB as monotherapy, emphasizing the need to better understand the mechanisms governing immunotherapy response and resistance.

Combining immunotherapy with chemotherapy has further increased response rates in breast cancer patients[25] and led to the FDA approval of pembrolizumab (aPD-1) plus chemotherapy as first-line treatment for patients with PD-L1$^+$ metastatic TNBC. More recently, the successes of neoadjuvant ICB in melanoma and mismatch repair deficient colorectal cancers[26,27] have spurred the clinical implementation of neoadjuvant ICB in other cancer types including breast cancer[28-30]. In the Keynote-522 trial in early-phase TNBC patients, neoadjuvant pembrolizumab and chemotherapy not only induced high rate of pathological complete responses (pCR), but also improved event-free survival, leading to FDA approval of this treatment regimen[29,31]. The Gepar-Nuevo trial found only a modest increase in pCR rate upon durvalumab (aPD-L1) plus chemotherapy in early-stage TNBC patients, yet these patients had similar event-free survival as those in the Keynote-522 trial[32], highlighting that the absence of a pCR in the primary tumor does not exclude prevention of metastatic disease. The latter observation exemplifies that we are still far from completely understanding what mechanisms govern response to immunotherapy.

Breast tumors have relatively low number of mutations and low level of

neoantigen expression[33,34]. TNBC is considered the most immunogenic breast cancer subtype, characterized by the highest tumor mutational burden, most extensive lymphocyte infiltration, and increased PD-L1 expression[35-37]. These three features have been identified as biomarkers for immunotherapy response in recent years, however, our understanding of the biology behind immunotherapy response remains rather limited. Research into the mechanisms of ICB response has predominantly focused on T cells. However, effective immune responses rely on tightly regulated crosstalk between innate and adaptive immune cells[5]. Indeed, growing evidence suggests that a major barrier to successful cancer immunotherapy is the tumor microenvironment, where, as outlined above, chronic inflammation and immunosuppression limit the development or effectiveness of anti-tumor immunity. Moreover, mounting an effective anti-tumor immune response requires coordinated responses across different tissues[10,38], particularly in the context of metastatic disease. Therefore, to improve our mechanistic understanding of the biology behind immunotherapy response and resistance in breast cancer, it will be critical to take a holistic approach, studying the crosstalk between innate – adaptive immune cells in the tumor micro- and macro-environment.

Modeling immunotherapy responses in breast cancer

The complexity of the cancer – immune crosstalk during cancer progression and metastasis and upon immunotherapy, and the lack of accurate tumor models that capture all the features of the metastatic cascade, make research in this field challenging. In this thesis, I argue that the genetically engineered mouse models are a valuable tool to study the mechanisms of immunotherapy response. Although these model systems are slow and costly, and our inability to manipulate them easily limits the mechanistic studies we can perform, the mechanisms we do uncover oftentimes hold strong translational relevance due to the high fidelity of the chronic inflammation and immunosuppression present in the tumor micro- and macro-environment[39].

In this thesis, we predominantly make use of the lowly immunogenic *Keratin14-Cre;Cdh1*$^{F/F}$*;Trp53*$^{F/F}$ (KEP) mouse model of *de novo* mammary tumorigenesis, in which spontaneous invasive lobular carcinomas develop around 6-8 months age in concert with tumor-induced chronic inflammation which typifies cancer patients[40,41]. In addition, we use the KEP-based mastectomy model for multi-organ metastatic disease, which faithfully recapitulates the steps of the metastatic cascade[42]. KEP tumors and KEP-derived metastases are characterized by a highly immunosuppressive tumor microenvironment, which is dominated by macrophages[43] as well as infiltrated by immunosuppressive neutrophils[41,44] and T_{regs}[45], recapitulating the tumor immune landscape of breast cancer patients[46,47]. Using these two models allows the side-by-side comparison of the mechanisms of response and resistance to immunotherapy in primary and metastatic breast cancer. As described in the following chapters, KEP tumors and KEP-derived metastases are resistant to immune checkpoint blockade strategies such as combined anti-PD-1 + anti-CTLA-4 therapy when used as monotherapy, but anti-tumor immune responses can be unleashed by combining immunotherapy with chemotherapy, thus resembling the clinical experience in breast cancer patients outlined above[22,24].

These features make these models excellent tools to address the research questions I aimed to answer in this thesis.

Scope of thesis

As outlined above, the immunotherapy field is rapidly progressing, and immunotherapy has now become part of standard patient care. However, our mechanistic understanding of the parameters driving response and resistance to immunotherapy is lagging behind. In this thesis, I aimed to unravel the complex mechanisms of immunotherapy response and resistance in primary and metastatic breast cancer focusing on the interconnectivity between innate and adaptive immune cells in the tumor micro- and macro-environment. The main questions I address in this thesis are:

1) What are the cellular determinants for response and resistance to immunotherapy in breast cancer?
2) How can we rationally exploit these mechanisms to improve immunotherapy response in breast cancer?

In **chapter 2**, I describe immune regulation of metastasis, highlighting how immune cells affect every step of the metastatic cascade from primary tumor growth, invasion and intravasation, survival in the circulation, (pre-)metastatic niche formation, extravasation, and colonization. I introduce the major players in anti-tumor immunity, tumor-induced inflammation and immunosuppression, and describe how these immune cells with opposing functions can be influenced by immunotherapy. I focus on the complex crosstalk between the cancer and immune system and describe the interconnectivity of different immune cell types that in turn dictate the tumor's fate. I postulate that through gaining mechanistic insights in immune control of metastasis, we can identify novel therapeutic opportunities to combat metastasis.

In **chapter 3** we aim to dissect the mechanisms of immune checkpoint blockade response in breast cancer by combining unbiased analyses of the systemic immune landscape upon ICB therapy in patients with metastatic breast cancer with mechanistic studies in the spontaneous mouse models for primary and metastatic breast cancer introduced above. We uncover a critical role for eosinophils during ICB response and elucidate the molecular mechanisms that lead to eosinophil differentiation, systemic expansion, and tumor infiltration. Our data provide proof-of-principle that therapeutic engagement of eosinophils may improve immunotherapy responses in breast cancer.

In **chapters 4** and **5** we examine the negative regulators of anti-tumor immunity and immunotherapy response in breast cancer. **Chapter 4** describes how regulatory T cells, which express the same immune checkpoint molecules as $CD8^+$ T cells, are unintended targets for immunotherapy. Combined aPD-1/CTLA-4 therapy promoted T_{reg} proliferation and activation, limiting its efficacy. Depletion of T_{regs} during neoadjuvant ICB changed the TME into a state favorable for ICB response and induced persistent and robust systemic $CD8^+$ T cell activation. While neoadjuvant ICB and T_{reg}-depletion did not affect primary tumor growth, it prolonged metastasis-related survival after primary tumor resection. These data emphasize that neoadjuvant ICB can be empowered by simultaneous targeting of T_{regs}, extending metastasis-related survival independent of a primary tumor response.

In **chapter 5** we describe how tumor progression and metastatic spread induces the expansion and polarization of immunosuppressive neutrophils. We observed that ICB combined with neutrophil depletion provides modest, but significant survival benefit against primary tumors via promoting $CD8^+$ T cell activation. Considering that neutrophil depletion was only effective for about a week, our data emphasize the dominant immunosuppressive function of neutrophils in the TME, where they pose a barrier for ICB response. Our data suggest that neutrophil modulating strategies may improve responses to immunotherapeutic strategies in breast cancer.

In **chapter 6** we investigate a new immunomodulatory agent, a PD-1-targeted IL-2 variant (PD1-IL2v), that showed encouraging results in preclinical pancreatic cancer models expressing strong tumor antigens[48-50]. We set out to put murinized (mu)PD1-IL2v to the test in our lowly immunogenic, immunotherapy-resistant breast cancer mouse model. Although muPD1-IL2v therapy in KEP mice reproduced many of the favorable changes in T cells that were previously reported[48-50], it failed to control mammary tumor growth, emphasizing that other immunosuppressive features were still in place limiting its efficacy. Combining muPD1-IL2v with the chemotherapeutic cisplatin additionally induced, amongst others, anti-tumor polarization of macrophages and unleashed anti-tumor immunity to improve tumor control and survival. Our data highlight that combining muPD1-IL2v therapy with cisplatin is a powerful approach to induce broad activation of systemic and intratumoral adaptive and innate immunity, resulting in effective immunotherapy responses in KEP mice.

The mechanisms of immunotherapy response and resistance that we identified in **chapter 3 – 6** are discussed in **chapter 7** of this thesis. I discuss the importance of innate – adaptive immune cell crosstalk in tumor micro- and macro-environment that dictates immunotherapy response. I put these concepts into a broader perspective, discuss future research directions and highlight potential new therapeutic strategies that may enhance immunotherapy efficacy in breast cancer patients in the future.

References

1. Nolan, E., Lindeman, G.J., and Visvader, J.E. (2023). Deciphering breast cancer: from biology to the clinic. Cell *186*, 1708-1728.
2. de Visser, K.E., and Joyce, J.A. (2023). The evolving tumor microenvironment: From cancer initiation to metastatic outgrowth. Cancer Cell *41*, 374-403.
3. Garner, H., and de Visser, K.E. (2020). Immune crosstalk in cancer progression and metastatic spread: a complex conversation. Nat Rev Immunol *20*, 483-497.
4. Shalapour, S., and Karin, M. (2019). Pas de Deux: Control of Anti-tumor Immunity by Cancer-Associated Inflammation. Immunity *51*, 15-26.
5. Chen, D.S., and Mellman, I. (2013). Oncology meets immunology: the cancer-immunity cycle. Immunity *39*, 1-10.
6. Angelova, M., Mlecnik, B., Vasaturo, A., Bindea, G., Fredriksen, T., Lafontaine, L., Buttard, B., Morgand, E., Bruni, D., Jouret-Mourin, A., et al. (2018). Evolution of Metastases in Space and Time under Immune Selection. Cell *175*, 751-765.e716.
7. O'Donnell, J.S., Teng, M.W.L., and Smyth, M.J. (2019). Cancer immunoediting and resistance to T cell-based immunotherapy. Nat Rev Clin Oncol *16*, 151-167.
8. Coffelt, S.B., Wellenstein, M.D., and de Visser, K.E. (2016). Neutrophils in cancer: neutral no more. Nat Rev Cancer *16*, 431-446.
9. DeNardo, D.G., and Ruffell, B. (2019). Macrophages as regulators of tumour immunity and immunotherapy. Nat Rev Immunol *19*, 369-382.
10. Hiam-Galvez, K.J., Allen, B.M., and Spitzer, M.H. (2021). Systemic immunity in cancer. Nat Rev Cancer *21*, 345-359.
11. Patras, L., Shaashua, L., Matei, I., and Lyden, D. (2023). Immune determinants of the pre-metastatic niche. Cancer Cell *41*, 546-572.
12. Templeton, A.J., McNamara, M.G., Seruga, B., Vera-Badillo, F.E., Aneja, P., Ocana, A., Leibowitz-Amit, R., Sonpavde, G., Knox, J.J., Tran, B., et al. (2014). Prognostic role of neutrophil-to-lymphocyte ratio in solid tumors: a systematic review and meta-analysis. J Natl Cancer Inst *106*, dju124.
13. Decker, T., Fischer, G., Bücke, W., Bücke, P., Stotz, F., Grüneberger, A., Gropp-Meier, M., Wiedemann, G., Pfeiffer, C., Peschel, C., and Götze, K. (2012). Increased number of regulatory T cells (T-regs) in the peripheral blood of patients with Her-2/neu-positive early breast cancer. J Cancer Res Clin Oncol *138*, 1945-1950.
14. Perez, S.A., Karamouzis, M.V., Skarlos, D.V., Ardavanis, A., Sotiriadou, N.N., Iliopoulou, E.G., Salagianni, M.L., Orphanos, G., Baxevanis, C.N., Rigatos, G., and Papamichail, M. (2007). CD4+CD25+ regulatory T-cell frequency in HER-2/neu (HER)-positive and HER-negative advanced-stage breast cancer patients. Clin Cancer Res *13*, 2714-2721.
15. Wang, L., Simons, D.L., Lu, X., Tu, T.Y., Solomon, S., Wang, R., Rosario, A., Avalos, C., Schmolze, D., Yim, J., et al. (2019). Connecting blood and intratumoral T(reg) cell activity in predicting future relapse in breast cancer. Nat Immunol *20*, 1220-1230.
16. Hodi, F.S., O'Day, S.J., McDermott, D.F., Weber, R.W., Sosman, J.A., Haanen, J.B., Gonzalez, R., Robert, C., Schadendorf, D., Hassel, J.C., et al. (2010). Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med *363*, 711-723.
17. Topalian, S.L., Hodi, F.S., Brahmer, J.R., Gettinger, S.N., Smith, D.C., McDermott, D.F., Powderly, J.D., Carvajal, R.D., Sosman, J.A., Atkins, M.B., et al. (2012). Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med *366*, 2443-2454.
18. Alexander, W. (2016). The Checkpoint Immunotherapy Revolution: What Started as a Trickle Has Become a Flood, Despite Some Daunting Adverse Effects; New Drugs, Indications, and Combinations Continue to Emerge. P T *41*, 185-191.
19. Lee, J.B., Kim, H.R., and Ha, S.J. (2022). Immune Checkpoint Inhibitors in 10 Years: Contribution of Basic Research and Clinical Application in Cancer Immunotherapy. Immune Netw *22*, e2.
20. Larkin, J., Chiarion-Sileni, V., Gonzalez, R., Grob, J.J., Cowey, C.L., Lao, C.D., Schadendorf, D., Dummer, R., Smylie, M., Rutkowski, P., et al. (2015). Combined Nivolumab and Ipilimumab or Monotherapy in Untreated Melanoma. N Engl J Med *373*, 23-34.
21. Debien, V., De Caluwé, A., Wang, X., Piccart-Gebhart, M., Tuohy, V.K., Romano, E., and Buisseret, L. (2023). Immunotherapy in breast cancer: an overview of current strategies and perspectives. NPJ Breast Cancer *9*, 7.
22. Winer, E.P., Lipatov, O., Im, S.A., Goncalves, A., Muñoz-Couselo, E., Lee, K.S., Schmid, P., Tamura, K., Testa, L., Witzel, I., et al. (2021). Pembrolizumab versus investigator-choice chemotherapy for metastatic triple-negative breast cancer (KEYNOTE-119): a randomised, open-label, phase 3 trial. Lancet Oncol *22*, 499-511.
23. Dirix, L.Y., Takacs, I., Jerusalem, G., Nikolinakos, P., Arkenau, H.T., Forero-Torres, A., Boccia,

R., Lippman, M.E., Somer, R., Smakal, M., et al. (2018). Avelumab, an anti-PD-L1 antibody, in patients with locally advanced or metastatic breast cancer: a phase 1b JAVELIN Solid Tumor study. Breast Cancer Res Treat *167*, 671-686.
24. Adams, S., Loi, S., Toppmeyer, D., Cescon, D.W., De Laurentiis, M., Nanda, R., Winer, E.P., Mukai, H., Tamura, K., Armstrong, A., et al. (2019). Pembrolizumab monotherapy for previously untreated, PD-L1-positive, metastatic triple-negative breast cancer: cohort B of the phase II KEYNOTE-086 study. Ann Oncol *30*, 405-411.
25. Cortes, J., Cescon, D.W., Rugo, H.S., Nowecki, Z., Im, S.A., Yusof, M.M., Gallardo, C., Lipatov, O., Barrios, C.H., Holgado, E., et al. (2020). Pembrolizumab plus chemotherapy versus placebo plus chemotherapy for previously untreated locally recurrent inoperable or metastatic triple-negative breast cancer (KEYNOTE-355): a randomised, placebo-controlled, double-blind, phase 3 clinical trial. Lancet *396*, 1817-1828.
26. Chalabi, M., Fanchi, L.F., Dijkstra, K.K., Van den Berg, J.G., Aalbers, A.G., Sikorska, K., Lopez-Yurda, M., Grootscholten, C., Beets, G.L., Snaebjornsson, P., et al. (2020). Neoadjuvant immunotherapy leads to pathological responses in MMR-proficient and MMR-deficient early-stage colon cancers. Nat Med *26*, 566-576.
27. Blank, C.U., Rozeman, E.A., Fanchi, L.F., Sikorska, K., van de Wiel, B., Kvistborg, P., Krijgsman, O., van den Braber, M., Philips, D., Broeks, A., et al. (2018). Neoadjuvant versus adjuvant ipilimumab plus nivolumab in macroscopic stage III melanoma. Nat Med *24*, 1655-1661.
28. Maio, M., Blank, C., Necchi, A., Di Giacomo, A.M., Ibrahim, R., Lahn, M., Fox, B.A., Bell, R.B., Tortora, G., and Eggermont, A.M.M. (2021). Neoadjuvant immunotherapy is reshaping cancer management across multiple tumour types: The future is now! Eur J Cancer *152*, 155-164.
29. Schmid, P., Cortes, J., Dent, R., Pusztai, L., McArthur, H., Kümmel, S., Bergh, J., Denkert, C., Park, Y.H., Hui, R., et al. (2022). Event-free Survival with Pembrolizumab in Early Triple-Negative Breast Cancer. N Engl J Med *386*, 556-567.
30. Mittendorf, E.A., Zhang, H., Barrios, C.H., Saji, S., Jung, K.H., Hegg, R., Koehler, A., Sohn, J., Iwata, H., Telli, M.L., et al. (2020). Neoadjuvant atezolizumab in combination with sequential nab-paclitaxel and anthracycline-based chemotherapy versus placebo and chemotherapy in patients with early-stage triple-negative breast cancer (IMpassion031): a randomised, double-blind, phase 3 trial. Lancet *396*, 1090-1100.
31. Schmid, P., Cortes, J., Pusztai, L., McArthur, H., Kummel, S., Bergh, J., Denkert, C., Park, Y.H., Hui, R., Harbeck, N., et al. (2020). Pembrolizumab for Early Triple-Negative Breast Cancer. N Engl J Med *382*, 810-821.
32. Loibl, S., Schneeweiss, A., Huober, J., Braun, M., Rey, J., Blohmer, J.U., Furlanetto, J., Zahm, D.M., Hanusch, C., Thomalla, J., et al. (2022). Neoadjuvant durvalumab improves survival in early triple-negative breast cancer independent of pathological complete response. Ann Oncol *33*, 1149-1158.
33. Alexandrov, L.B., Nik-Zainal, S., Wedge, D.C., Aparicio, S.A., Behjati, S., Biankin, A.V., Bignell, G.R., Bolli, N., Borg, A., Børresen-Dale, A.L., et al. (2013). Signatures of mutational processes in human cancer. Nature *500*, 415-421.
34. Schumacher, T.N., and Schreiber, R.D. (2015). Neoantigens in cancer immunotherapy. Science *348*, 69-74.
35. Stanton, S.E., Adams, S., and Disis, M.L. (2016). Variation in the Incidence and Magnitude of Tumor-Infiltrating Lymphocytes in Breast Cancer Subtypes: A Systematic Review. JAMA Oncol *2*, 1354-1360.
36. Bianchini, G., De Angelis, C., Licata, L., and Gianni, L. (2022). Treatment landscape of triple-negative breast cancer - expanded options, evolving needs. Nat Rev Clin Oncol *19*, 91-113.
37. Denkert, C., von Minckwitz, G., Darb-Esfahani, S., Lederer, B., Heppner, B.I., Weber, K.E., Budczies, J., Huober, J., Klauschen, F., Furlanetto, J., et al. (2018). Tumour-infiltrating lymphocytes and prognosis in different subtypes of breast cancer: a pooled analysis of 3771 patients treated with neoadjuvant therapy. Lancet Oncol *19*, 40-50.
38. Spitzer, M.H., Carmi, Y., Reticker-Flynn, N.E., Kwek, S.S., Madhireddy, D., Martins, M.M., Gherardini, P.F., Prestwood, T.R., Chabon, J., Bendall, S.C., et al. (2017). Systemic Immunity Is Required for Effective Cancer Immunotherapy. Cell *168*, 487-502.e415.
39. Wakefield, L., Agarwal, S., and Tanner, K. (2023). Preclinical models for drug discovery for metastatic disease. Cell *186*, 1792-1813.
40. Derksen, P.W., Liu, X., Saridin, F., van der Gulden, H., Zevenhoven, J., Evers, B., van Beijnum, J.R., Griffioen, A.W., Vink, J., Krimpenfort, P., et al. (2006). Somatic inactivation of E-cadherin and p53 in mice leads to metastatic lobular mammary carcinoma through induction of anoikis resistance and angiogenesis. Cancer Cell *10*, 437-449.
41. Coffelt, S.B., Kersten, K., Doornebal, C.W., Weiden, J., Vrijland, K., Hau, C.S., Verstegen, N.J.M., Ciampricotti, M., Hawinkels, L., Jonkers, J., and de Visser, K.E. (2015). IL-17-producing

gammadelta T cells and neutrophils conspire to promote breast cancer metastasis. Nature *522*, 345-348.
42. Doornebal, C.W., Klarenbeek, S., Braumuller, T.M., Klijn, C.N., Ciampricotti, M., Hau, C.S., Hollmann, M.W., Jonkers, J., and de Visser, K.E. (2013). A preclinical mouse model of invasive lobular breast cancer metastasis. Cancer Res *73*, 353-363.
43. Salvagno, C., Ciampricotti, M., Tuit, S., Hau, C.S., van Weverwijk, A., Coffelt, S.B., Kersten, K., Vrijland, K., Kos, K., Ulas, T., et al. (2019). Therapeutic targeting of macrophages enhances chemotherapy efficacy by unleashing type I interferon response. Nat Cell Biol *21*, 511-521.
44. Wellenstein, M.D., Coffelt, S.B., Duits, D.E.M., van Miltenburg, M.H., Slagter, M., de Rink, I., Henneman, L., Kas, S.M., Prekovic, S., Hau, C.S., et al. (2019). Loss of p53 triggers WNT-dependent systemic inflammation to drive breast cancer metastasis. Nature *572*, 538-542.
45. Kos, K., Aslam, M.A., van de Ven, R., Wellenstein, M.D., Pieters, W., van Weverwijk, A., Duits, D.E.M., van Pul, K., Hau, C.S., Vrijland, K., et al. (2022). Tumor-educated T(regs) drive organ-specific metastasis in breast cancer by impairing NK cells in the lymph node niche. Cell Rep *38*, 110447.
46. Gentles, A.J., Newman, A.M., Liu, C.L., Bratman, S.V., Feng, W., Kim, D., Nair, V.S., Xu, Y., Khuong, A., Hoang, C.D., et al. (2015). The prognostic landscape of genes and infiltrating immune cells across human cancers. Nat Med *21*, 938-945.
47. Kim, I.S., Gao, Y., Welte, T., Wang, H., Liu, J., Janghorban, M., Sheng, K., Niu, Y., Goldstein, A., Zhao, N., et al. (2019). Immuno-subtyping of breast cancer reveals distinct myeloid cell profiles and immunotherapy resistance mechanisms. Nat Cell Biol *21*, 1113-1126.
48. Codarri Deak, L., Nicolini, V., Hashimoto, M., Karagianni, M., Schwalie, P.C., Lauener, L., Varypataki, E.M., Richard, M., Bommer, E., Sam, J., et al. (2022). PD-1-cis IL-2R agonism yields better effectors from stem-like CD8+ T cells. Nature *610*, 161-172.
49. Tichet, M., Wullschleger, S., Chryplewicz, A., Fournier, N., Marcone, R., Kauzlaric, A., Homicsko, K., Deak, L.C., Umaña, P., Klein, C., and Hanahan, D. (2023). Bispecific PD1-IL2v and anti-PD-L1 break tumor immunity resistance by enhancing stem-like tumor-reactive CD8+ T cells and reprogramming macrophages. Immunity *56*, 162-179.e166.
50. Piper, M., Hoen, M., Darragh, L.B., Knitz, M.W., Nguyen, D., Gadwa, J., Durini, G., Karakoc, I., Grier, A., Neupert, B., et al. (2023). Simultaneous targeting of PD-1 and IL-2Rβγ with radiation therapy inhibits pancreatic cancer growth and metastasis. Cancer Cell *41*, 950-969.e6.