Orchestrating the immune system to initiate adaptive anti-tumor immunity

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Chapter 9

Introduction Part II - Tumor immune suppression

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9.1 Tumor immune suppression as inevitable outcome of tumor evolution

The tumor does not actively suppress the immune system. Instead, immunological selective pressure favors immune-suppressive tumor cells

Initially presumed to be insignificant[1], immune suppression is now recognized as a key characteristic of virtually all cancer types[2]. Avoiding cytotoxic destruction by the immune system has obvious benefits for the tumor. The main question frequently asked is "how does the tumor suppress the immune system?" This anthropomorphic is misleading, since the tumor is not the driver of immune suppression but the consequence of selective pressure from the immune system, resulting in tumor evolution. For evolution theory to work, three conditions need to be met; 1) Heredity of genotypes to offspring, 2) Variation within the population, 3) Selection by factors limiting reproduction. Since cancer cells clonally expand and transfer their genes to progeny cells, their propagation meets the heredity condition. Because of genomic instability, cancer cells continuously acquire genetic alterations, resulting in variation between cancer cells within a tumor. However, cancer cell growth is limited by the amount of oxygen, nutrients, growth factors and presence of apoptotic signaling[2]. These limitations act as selective pressures for tumor growth, meeting the third condition of evolution theory (Figure 1). As a result, the occurrence of a tumor is the consequence of natural selection of clones capable of best dealing with these limitations (survival of the fittest). A loss of one of these conditions leads to an inability of tumors to adapt and prevents outgrowth. The theory of different modes of tumor evolution is reviewed in detail by Davis and colleagues[3]. The evolutionary view of carcinogenesis has several important implications in terms of immune suppression. Indeed, the widespread suppression of the immune system suggests that the immune system is a limiting factor in tumor growth. Also, the fact that variation occurs through the stochastic accumulation of mutations results in increased intra-tumor heterogeneity, a key contributing factor to therapeutic failure and drug resistance[4]. Importantly, it seems tumors are initially recognized by the immune system as harm-causing to which an immune response is appropriate. How does the immune system apply these selective pressures?

Adaptive immune recognition | Cancer cells are of self-origin and therefore originally thought to escape adaptive immunity. However, cancer cells frequently express germline proteins like cancer/testis antigens (for example MAGEs/NY-ESO-1) that are normally confined to immune-privileged tissues[5,6]. While these antigens are expressed in the thymus and subject to central tolerance, the expression in adult tissue is limited to immune-privileged sites and therefore not subject to peripheral

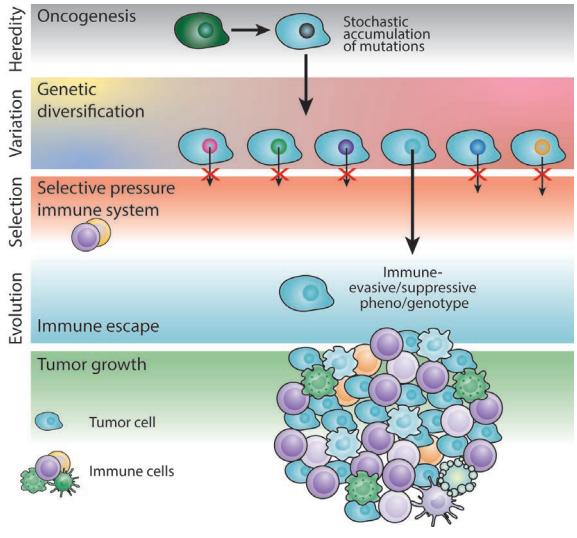

Figure 1 | Evolutionary view of immune-driven tumorigenesis Normal cells can become malignant through acquired genetic mutations and defective DNA repair. Also, malignant cells proliferate uncontrollably, generating new cancer cells with the same mutated genotype. As a result, tumor cells become increasingly diverse in mutated genetic makeup and provide the variation required for Darwinian evolution. The immune system applies selective pressure on immunogenic lineages, resulting in a proliferate advantage of tumor cells that are less immunogenic. Hence, large tumors are the result of a "survival of the fittest", ending in immune escape and immune suppression.

tolerance[6–8]. Therefore, these antigens can be somewhat immunogenic and used as tumor vaccine targets[5]. Additionally, we now known that tumors produce slight variations of normal proteins because of mutations (genetic instability), leading to expression of neo-antigens in MHC class I and II complexes[9,10]. In fact, reduction in DNA repair results in higher mutational burden, increased neo-antigens and increased immunogenicity[11]. Since these neoantigens are completely foreign to the host, neoantigens are not expressed in the thymus and are not subject to central tolerance[12]. Importantly, since neoantigens derived from stochastic genomic instability, the exact immunogenic neoantigen is highly polymorphic and patient-specific[12]. Also, the mutational burden differs between tumor types, with tumors exposed to carcinogenic influences (cigarette smoke, UV exposure etc) exhibiting higher mutational burden[13,14]. Hence, the detection and bioinformatics prediction of neoantigens requires genomic screening of individual tumors[15,16]. Immunogenic neoantigens generating T cell responses have now been identified in both mouse and human tumors[10,17–19]. These T cell responses towards neoantigens have been found in growing tumors, indicative of intact recognition. What is the evidence for T cell driven tumor evolution? It should be mentioned here that the cancer-immunity interplay can be visualized as a continuous battle between the immune system and the tumor, termed the cancer-immunity cycle (Figure 2).

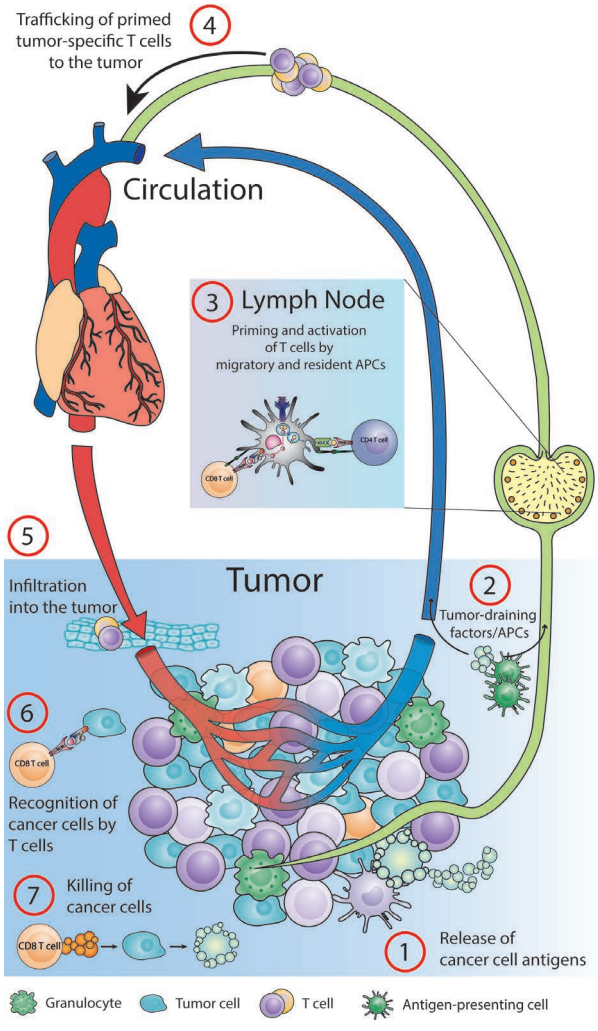

Figure 2 | The cancer-immunity cycle When cancer cells die, antigens are released (1), which can be taken up by dendritic cells in the tumor microenvironment (2) or drain to the tumor-draining lymph node where both resident and migratory DCs are able to present tumor antigens to naïve T cells (3). In the draining lymph node, tumor-specific effector T cells are generated, which travel via the blood to the highly vascularized tumor (4). T cells can enter the tumor by attaching to the endothelial cells and migrating into the tumor parenchyma (5). Here, effector T cells are able to recognize tumor-specific antigens presented by cancers (6). When T cells are not highly suppressed and MHC class I expression is high enough, tumor cells can be killed by cytotoxic T cells (7). This leads to another release of antigens that resets the cancer-immunity cycle[53].

Clonal and subclonal tumor evolution | Recent advances in genomic sequencing have allowed not only the identification and prediction of neo-antigens, but also assess the genetic heterogeneity within and between tumors[20–23]. Using this information, a retrospective evolution tree of clonal (stem) and subclonal (branches) mutations can be constructed[20,24,25]. For example, in non-small-cell lung cancer (NSCLC) driver mutations in EGFR, MET, BRAF and TP53 were mostly clonal, while mutations in PIK3CA and NF1 occurred later in evolution[26]. In glioblastoma, subclones may have diverged and survived in parallel for decades before diagnosis and treatment[27]. However, tracking back the selective pressures that generated the subclones is difficult; in absence of selection, heterogeneity can still arise as a function of time, error rates and random genetic drift[22]. In terms of immune-driven selective pressure, it was shown that there is an interplay between cancer subclones and local immune microenvironment[28,29]. More specifically, within a single ovarian cancer patient treated with chemotherapy, the progressing metastases were found to be devoid of immune cells, whereas regressing and stable metastases were enriched with expanding $CD8^+$ and $CD4^+$ T cells[28]. The highest neoantigen load combined with the most abundant $CD8^+$ T cell infiltrates correlated with long-term survival in pancreatic ductal adenocarcinoma (PDAC) patients[30]. Similarly, Angelova and colleagues showed that the amount of immunoediting (immune-driven sequence of cancer cell elimination, equilibrium and escape), rather than the mutational load, was associated with an active immune response[29]. Branched tumor evolution could be traced back to a failure of the immune system to eliminate adapted clones, leading to immune-escape. So, genetic instability leads to increased neoantigens with immunogenic capacity, which in turn needs to be evaded by immunosuppression. Like any genetic mutation, neoantigens can also be ordered by clonality and differentially affects tumor growth and response to therapy. In fact, the number of neoantigen subclones decreased specifically in immune-infiltrated early-stage NSCLC tumor regions[31]. This study showed that the immune system applied strong selection pressure in early-stage tumors, which also correlated with poor disease-free survival. Alternatively, reducing the presence of T cell reactive antigens by downregulating MHC class I complexes is a common adaptation to T cell-mediated selective pressure[32,33]. However, this adaptation does not come without a cost. In fact, loss of one of the two HLA haplotypes (encoding MHC class I) in tumor subclones increases the neoantigen burden, mutagenesis and PDL1 positivity[34]. Hence, loss of HLA is an immune escape mechanism that is subject to strong selective pressures.

Therapy-driven immune-tumor evolution | Therapy has a significant effect on tumor evolution by increasing or redirecting selective pressure, with new subclones gaining proliferative advantages and adapting to the therapy[21,35]. In fact, modeling tumor responses to therapeutic interventions has shown that tumors adapt broadly similar to rapidly evolving pathogens[36]. Treatment of stage IV melanoma patients with adoptive T cell transfer resulted in loss of neoantigens over time[37]. T cell-driven anti-tumor immunity through immune checkpoint blockade (ICB; Chapter 9.2) is positively correlated with the tumor mutational burden and neo-antigen expression[36,38–44]. The effectivity of neoantigen-directed anti-tumor immunity is further demonstrated by the loss of neoantigenic mutations in patients on therapy[45]. However, the clonality of neoantigens affects the efficacy of immunotherapy. McGranahan and colleagues have shown that the heterogeneity in neoantigens (higher number of neoantigen subclones) negatively impacts ICB[19]. Indeed, clonal neoantigen-specific $CD8^+$ T cell were enriched in patients showing durable clinical responses. Similarly, in glioblastoma patients treated with ICB it was shown that non-responders had a greater diversity of T cells[46]. Perhaps the majority of subclonal neoantigens leads to impaired or overextended T cell responses (antigen competition) in combination with a higher probability of immune escape of highly variable subclones.

In summary, the adaptive immune system applies selective pressure on genetically diverse dividing tumor cells, which can be enhanced by immunotherapy. In terms of therapeutic settings, the adaptation before treatment is referred to as primary resistance, while the adaptation to therapy is referred to as adaptive (or acquired) resistance[47]. The modes of suppression can be tumor cell-intrinsic (for example downregulation of MHC class I) leading to immune evasion or tumor cell extrinsic (upregulation of checkpoints) leading to suppression of anti-tumor immunity. For the latter mode of immune suppression effective therapies have been designed; immune checkpoint blockers.

9.2 Immune checkpoint blockade – PD1 as an example

Any mechanism that suppresses or avoids the immune system will provide a survival benefit for cancer cells and can be therapeutically exploited

At this stage it is important to consider that tumor cells are not selective in the mode of immune suppression, but are preyed upon by immune cells. Instead, literally any form of genetic mutation that confers an immunosuppressive benefit to its phenotype will be less likely eradicated and therefore persists. Because of this, new immunosuppressive mechanisms are still being recognized and probed for possible therapeutic intervention. Not surprisingly, many of these immunosuppressive mechanisms involve regulatory mechanisms originally described as immunological checkpoints placed to prevent hyperactivation and auto-immunity[48]. In particular, immune checkpoints expressed by adaptive immune cells with cytolytic activity like $CD8^+$ T cells are common escape mechanisms. Programmed cell death-1 (PD1) and cytotoxic T-lymphocyte antigen 4 (CTLA4) are currently the best described immune checkpoints and their blockade with antagonistic antibodies has shown unparalleled clinical success in treating many types of cancer[49–51]. Both molecules are members of the co-inhibitory receptor family expressed by T cells[52]. The exact mode of interference using immune checkpoint blockade (ICB) remains unclearly defined, but are best understood for PD1 ICB.

PD1 biology and therapeutic blockade | PD1 was first isolated by the research group of Nobel Prize winner Tasuku Honjo from murine T cell hybridoma and a hematopoietic progenitor cell line undergoing cell death in 1992, hence its name programmed cell death 1 (PD1)[54]. Genetic knockout of PD1 in mice was shown to result in different autoimmune phenotypes, with $PD1^{-/-}$ T cells exhibiting antigen-specific hyperactivity[55,56]. The primary ligand for PD1, PD1 ligand 1 (PDL1), was independently discovered in 1999 and shown to inhibit T cell responses[57,58]. However, PDL1 was found not only to bind PD1, but also CD80, which is expressed by DCs and activated T cells[59,60]. In 2002, it was shown that PDL1 expression was high on a variety of mouse and human tumors and promoted the apoptosis of antigen-specific T cells[61]. Soon after it was shown that antagonistic PDL1 blockade could eliminate tumors in mice if a T cell response was present[62–64]. The expression of PD-1 is low on homeostatic T cells and is temporarily upregulated upon activation[65]. Importantly, when T cell receive antigen-specific signaling over an extended period of time like during a chronic viral infection, they become unresponsive (termed exhaustion) and constitutively express PD1. For example, PD1 is upregulated on exhausted $CD8^+$ T cells during chronic HIV infection[66]. Blocking PD1 in a mouse model of choriomeningitis virus (CMV) resulted in restoration of $CD8^+$ T cell functioning and reduction in viral load[67]. Additional co-inhibitory molecules have since been identified to contribute to the exhaustive phenotype of T cells during chronic viral infection, like TIM-3[68,69]. In parallel, tumor-infiltrating $CD8^+$ T cells exhibit a similar phenotype and can be reversed by PD1 ICB[70–72]. Interestingly, the molecular mode of action of PD1 on T cell suppression was recently shown to be mediate via CD28 on T cells[73,74]. Mechanistically, triggering PD1 leads to dephosphorylation of the CD28 co-receptor by PD1-recruited Shp2 phosphatase[73]. In fact, T cell-intrinsic expression of CD28 is critical for PD1 ICB efficacy[73,74]. This was surprising as most co-stimulatory/inhibitory receptors are thought to affect the threshold of signaling downstream of the TCR directly[52]. This is of importance, because CD28 engagement by CD80 or CD86, expressed by antigen presenting cells (APCs) instead of tumor cells, would be critical for PD1 blockade. Additionally, CD80 expressed by APCs have been shown to block PDL1-PD1 interactions by cis-PDL1/CD80 interactions[75]. In mouse tumor models, the presence of PDL1 on myeloid cells (including myeloid APCs), but not tumor cells is critical for PD1 blockade[76]. Therefore, it seems myeloid APCs are required to provide CD80/86 co-stimulation to CD28 on T cells for successful PD1 blockade, although it is yet unknown whether this needs to happen in the tumor microenvironment, in the tumor-draining lymph node or both.

Even though PD1 ICB has shown impressive clinical efficacy, only a minority of patients show a clinical response and a large portion of patients exhibit tumor cell extrinsic acquired or adaptive resistance even after initially effective ICB[47]. Therefore, understanding the mechanism of action and clinical

factors affecting PD1 checkpoint blockade is crucial to facilitate rational design of combination therapies. The effector phase of the anti-tumor immune response induced by anti-PD1 (αPD1) ICB is dependent on cytotoxic CD8$^+$ T cells recognizing tumor cells expressing peptide-MHC class I complexes[49]. Tumor intrinsic properties such as mutational load[41,77], neoantigen load[19,36], metabolism[78] and genetic subtype affect the response to ICB. Also, the exhaustion status of tumor-infiltrating CD8$^+$ T cells and their abundance relative to tumor cells affects PD1 ICB[79,80]. Interestingly, several indirect factors affect PD1 blockade, including gut microbiome[81–83] and the antigen:MHCII/CD4$^+$ T cell axis, and may be crucial in selecting suitable patients.

APCs and CD4$^+$ T cells affect PD1 ICB | Since APCs, providing co-stimulation, are critical for PD1 ICB and CD4$^+$ T cells are hypothesized to be essential for proper CD8$^+$ T cell immunity, a special role and therapeutic target may exist for MHCII$^+$ APCs. Also, CD4$^+$ T cells have been shown to mediate anti-tumor immunity, even in the absence of CD8$^+$ T cells[84–86]. The importance of CD4$^+$ T cell-mediated selective pressure through MHC class II antigen presentation in the TME was reported across many cancer types[87]. Additionally, MHC class II expression in the TME predicted a positive clinical response to PD1 ICB when MHC class I expression was compromised[88]. When MHC class I on tumor cells is lost, NK cells provide cytotoxic anti-tumor immunity through the loss of MHCI-"self"[89,90]. These NK cells can in turn be stimulated by local APCs or recruit conventional DCs[91–94]. It was recently shown that MHCII-mediated neoantigen presentation in the tumor microenvironment is critical for anti-tumor immunity[95,96]. In fact, APCs and not tumor cells were the main drivers of MHCII-mediated antigen presentation in the tumor microenvironment[97]. A wide variety of APCs has now been identified in human and murine tumors, although their exact role, accumulation and development remains unclear[98]. However, it was recently shown that the number of peripheral MHCII$^+$ monocytes pre-treatment was associated with increased efficacy of PD1 ICB[99]. These monocytes may infiltrate the tumor and provide MHCII-mediated support to anti-tumor immune responses during PD1 ICB.
In summary, the physiological PD1-PDL1 axis has evolved as an immune-regulatory mechanism in T cells, functioning to prevent auto-immunity. As T cells provide selective pressure to the heterogeneous population of tumor cells, tumor cells modulating this axis have a selective advantage in a "survival of the fittest". Components of the innate immune system, including APCs, are critical players in the immune-suppressive end product of tumor evolution and therefore provide a therapeutic target for PD1 ICB. Many other forms of immune checkpoints have now been described as a result of immune-driven selective pressure, completely changing the way cancer therapies will be designed[49,100]. Of note, ICB therapy is not without some side-effects and this needs to be taken into account, especially when combination therapies are considered[101].

9.3 Outline of Part II

Immune suppression of the tumor is the result of Darwinian evolution of heterogeneous tumor cells experiencing differential levels of immune selective pressures. In other words, tumor eventually escape the immune system through evolution, which can be investigated and exploited for therapy.

In **Chapter 10** we investigate the innate and adaptive immune response to PD1 ICB in two mouse tumor models. We show that successful anti-tumor immunity induced by PD1 ICB involves the recruitment of monocytes and differentiation into monocyte-derived DCs. Tumor-infiltrating moDCs correlated with the PD1-induced increase in effector CD8$^+$ T cells. Importantly, moDC differentiation was observed in melanoma patients undergoing PD1 ICB and moDCs were significantly enriched in responding patients compared to non-responding patients. MoDCs expressed high levels of CD40 in mouse models, which could be targeted by agonistic CD40 antibodies, augmenting PD1 efficacy. The infiltration of monocytes and differentiation to CD40-expressing moDCs provide a target for rational combination therapy.

While immune-driven tumor evolution occurs via the same principles of Darwinian natural selection,

the resulting form of immune suppression is dependent on many factors, including the physical location. The anti-tumor immune response in brain tumors, especially glioblastoma (GBM), is somewhat unique because of its development in an immune-privileged organ. In **Chapter 11**, we investigate the immune components in a mouse model of GBM, with a focus on T cell-expressed immune checkpoint receptors and their ligands. GBM is characterized by highly infiltrative tumor cells far beyond the bulk of the primary tumor, hampering therapeutic interventions like surgery and radiotherapy. We show extensive infiltration of tumor-specific T cells in the brain hemisphere contralateral to the hemisphere where the primary tumor is located. Additionally, myeloid cell infiltration is high in the primary tumor and significantly elevated in the contralateral hemispher e. The resident myeloid cell, the microglia, has been suggested to interact with T cells in the brain through MHC class II during neurodegeneration and brain tumor development[102]. In brain tumor-bearing mice, the expression of MHC class II was elevated on microglia in the contralateral hemisphere. Hence, we propose significant involvement of immune suppression outside of the primary tumor and may predict the beneficial efficacy of ICB beyond the primary tumor.

Since immune selective pressure drives immune escape of tumors, it is not surprising that some tumors express inhibitory ligands beyond the typical inhibitory T cell receptor. One archaic form of immune modulation is the expression of specific glycans via post-translational modification of membrane proteins. In **Chapter 12** we propose that tumors express specific immune-suppressive glycan patterns as immune escape mechanism, termed the glyco-code. Aberrant tumor glycosylation can modify the local immune response via glycan-binding receptors. Specifically, we have found that sialic acids are highly expressed in pancreatic ductal carcinoma tumors and this correlated with the presence of immune-suppressive macrophages and poor survival outcome (Rodriguez et al.).

In **Chapter 13**, we investigate the specific expression of sialic acids in mouse PDAC tumor models and tumors from PDAC patients. We observed highly distinct expression patterns of sialic acids in human PDAC patients that where mimicked in mouse models. Tumor-specific knockout of the sialic acid transporter SLC35A1 using CRISPR-Cas9 abrogated sialic acid expression *in vitro* but did not deplete sialic acids *in vivo*. Instead, sialic acids colocalizes with extracellular matrix proteins typically produced by stromal cells and host-derived stromal cells in PDAC mouse models expressed high levels of sialic acids. Sialic acid-binding receptors were found to be expressed by tumor-resident myeloid cells in human and mouse models, indicating putative sialic acid-mediated immune suppression through the recruitment of stromal cells producing sialic acid-rich ECM.

Finally, in **Chapter 14**, we discuss our results in light of the rapidly evolving field of tumor immunology. Cancer vaccines are typically therapeutic for patients with established disease (apart from the HPV vaccine[103,104]). In fact, at the moment most cancer vaccines are in clinical trials in late stage cancer patients and are required to eradicate large tumors. The immunological status of those patients is completely different from healthy controls, both at the level of the tumor microenvironment and systemically[53]. The immunological status of the tumor and the patient as a whole will have to be taken into account to develop and choose effective treatment strategies[105]. Combination therapy will be needed at the level of the individual patient to most effectively cure patients.

References

1. Hanahan, D. & Weinberg, R. A. The Hallmarks of Cancer. Cell 100, 57–70 (2000).
2. Hanahan, D. & Weinberg, R. a. Hallmarks of cancer: The next generation. Cell 144, 646–674 (2011).
3. Davis, A., Gao, R. & Navin, N. Tumor evolution: Linear, branching, neutral or punctuated? Biochim. Biophys. Acta - Rev. Cancer 1867, 151–161 (2017).
4. Greaves, M. Evolutionary determinants of cancer. Cancer Discov. 5, 806–820 (2015).
5. Simpson, A. J. G., Caballero, O. L., Jungbluth, A., Chen, Y.-T. & Old, L. J. Cancer/testis antigens, gametogenesis and cancer. Nat. Rev. Cancer 5, 615–625 (2005).
6. Coulie, P. G., Van den Eynde, B. J., van der Bruggen, P. & Boon, T. Tumour antigens recognized by T lymphocytes: at the core of cancer immunotherapy. Nat. Rev. Cancer 14, 135 (2014).

7. Zippelius, A. et al. Thymic Selection Generates a Large T Cell Pool Recognizing a Self-Peptide in Humans. J. Exp. Med. 195, 485 LP – 494 (2002).
8. Gotter, J., Brors, B., Hergenhahn, M. & Kyewski, B. Medullary Epithelial Cells of the Human Thymus Express a Highly Diverse Selection of Tissue-specific Genes Colocalized in Chromosomal Clusters. J. Exp. Med. 199, 155 LP – 166 (2004).
9. van Rooij, N. et al. Tumor Exome Analysis Reveals Neoantigen-Specific T-Cell Reactivity in an Ipilimumab-Responsive Melanoma. J. Clin. Oncol. 31, e439–e442 (2013).
10. Robbins, P. F. et al. Mining exomic sequencing data to identify mutated antigens recognized by adoptively transferred tumor-reactive T cells. Nat. Med. 19, 747 (2013).
11. Germano, G. et al. Inactivation of DNA repair triggers neoantigen generation and impairs tumour growth. Nature 552, 116 (2017).
12. Schumacher, T. N., Scheper, W. & Kvistborg, P. Cancer Neoantigens. Annu. Rev. Immunol. 37, 173–200 (2018).
13. Alexandrov, L. B. et al. Signatures of mutational processes in human cancer. Nature 500, 415 (2013).
14. Alexandrov, L. B. et al. Mutational signatures associated with tobacco smoking in human cancer. Science (80-.). 354, 618 LP – 622 (2016).
15. Yadav, M. et al. Predicting immunogenic tumour mutations by combining mass spectrometry and exome sequencing. Nature 515, 572 (2014).
16. Brown, S. D. et al. Neo-antigens predicted by tumor genome meta-analysis correlate with increased patient survival. Genome Res. 24, 743–750 (2014).
17. Matsushita, H. et al. Cancer exome analysis reveals a T-cell-dependent mechanism of cancer immunoediting. Nature 482, 400 (2012).
18. Castle, J. C. et al. Exploiting the Mutanome for Tumor Vaccination. Cancer Res. 72, 1081 LP – 1091 (2012).
19. McGranahan, N. et al. Clonal neoantigens elicit T cell immunoreactivity and sensitivity to immune checkpoint blockade. Science (80-.). 351, 1463 LP – 1469 (2016).
20. Gerlinger, M. et al. Intratumor Heterogeneity and Branched Evolution Revealed by Multiregion Sequencing. N. Engl. J. Med. 366, 883–892 (2012).
21. Zhang, J. et al. Intratumor heterogeneity in localized lung adenocarcinomas delineated by multiregion sequencing. Science (80-.). 346, 256–259 (2014).
22. Shibata, D. Heterogeneity and Tumor History. Science (80-.). 336, 304–305 (2012).
23. Oesper, L., Mahmoody, A. & Raphael, B. J. THetA: inferring intra-tumor heterogeneity from high-throughput DNA sequencing data. Genome Biol. 14, R80 (2013).
24. McGranahan, N. & Swanton, C. Clonal Heterogeneity and Tumor Evolution: Past, Present, and the Future. Cell 168, 613–628 (2017).
25. Turajlic, S. & Swanton, C. Metastasis as an evolutionary process. Science (80-.). 352, 169 LP – 175 (2016).
26. Jamal-Hanjani, M. et al. Tracking the Evolution of Non–Small-Cell Lung Cancer. N. Engl. J. Med. 376, 2109–2121 (2017).
27. Wang, J. et al. Clonal evolution of glioblastoma under therapy. Nat. Genet. 48, 768 (2016).
28. Jiménez-Sánchez, A. et al. Heterogeneous Tumor-Immune Microenvironments among Differentially Growing Metastases in an Ovarian Cancer Patient. Cell 170, 927-938.e20 (2017).
29. Angelova, M. et al. Evolution of Metastases in Space and Time under Immune Selection. Cell 175, 751-765.e16 (2018).
30. Balachandran, V. P. et al. Identification of unique neoantigen qualities in long-term survivors of pancreatic cancer. Nature 551, 512 (2017).
31. Rosenthal, R. et al. Neoantigen-directed immune escape in lung cancer evolution. Nature 567, 479–485 (2019).
32. Garrido, F., Aptsiauri, N., Doorduijn, E. M., Garcia Lora, A. M. & van Hall, T. The urgent need to recover MHC class I in cancers for effective immunotherapy. Curr. Opin. Immunol. 39, 44–51 (2016).
33. Rooney, M. S., Shukla, S. A., Wu, C. J., Getz, G. & Hacohen, N. Molecular and Genetic Properties of Tumors Associated with Local Immune Cytolytic Activity. Cell 160, 48–61 (2015).
34. McGranahan, N. et al. Allele-Specific HLA Loss and Immune Escape in Lung Cancer Evolution. Cell 171, 1259-1271.e11 (2017).
35. Johnson, B. E. et al. Mutational Analysis Reveals the Origin and Therapy-Driven Evolution of Recurrent Glioma. Science (80-.). 343, 189–193 (2014).
36. Łuksza, M. et al. A neoantigen fitness model predicts tumour response to checkpoint blockade immunotherapy. Nature 551, 517 (2017).
37. Verdegaal, E. M. E. et al. Neoantigen landscape dynamics during human melanoma–T cell interactions. Nature 536, 91 (2016).
38. Samstein, R. M. et al. Tumor mutational load predicts survival after immunotherapy across multiple cancer types. Nat. Genet. 51, 202–206 (2019).
39. Snyder, A. et al. Genetic Basis for Clinical Response to CTLA-4 Blockade in Melanoma. N. Engl. J. Med. 371, 2189–2199 (2014).
40. Van Allen, E. M. et al. Genomic correlates of response to CTLA-4 blockade in metastatic melanoma. Science (80-.). 350, 207 LP – 211 (2015).
41. Rizvi, N. A. et al. Mutational landscape determines sensitivity to PD-1 blockade in non–small cell lung cancer. Science (80-.). 348, 124–129 (2016).
42. Rosenberg, J. E. et al. Atezolizumab in patients with locally advanced and metastatic urothelial carcinoma who have progressed following treatment with platinum-based chemotherapy: a single-arm, multicentre, phase 2 trial. Lancet 387, 1909–1920 (2016).
43. Hugo, W. et al. Genomic and Transcriptomic Features of Response to Anti-PD-1 Therapy in Metastatic Melanoma. Cell 165, 35–44 (2016).
44. Mandal, R. et al. Genetic diversity of tumors with mismatch repair deficiency influences anti–PD-1 immunotherapy

response. Science (80-.). 364, 485 LP – 491 (2019).
45. Riaz, N. et al. Tumor and Microenvironment Evolution during Immunotherapy with Nivolumab. Cell 171, 934-949.e15 (2017).
46. Zhao, J. et al. Immune and genomic correlates of response to anti-PD-1 immunotherapy in glioblastoma. Nat. Med. 25, 462–469 (2019).
47. Sharma, P., Hu-Lieskovan, S., Wargo, J. A. & Ribas, A. Primary, Adaptive, and Acquired Resistance to Cancer Immunotherapy. Cell 168, 707–723 (2017).
48. Pardoll, D. M. The blockade of immune checkpoints in cancer immunotherapy. Nat. Rev. Cancer 12, 252–264 (2012).
49. Ribas, A. & Wolchok, J. D. Cancer immunotherapy using checkpoint blockade. Science (80-.). 359, 1350 LP – 1355 (2018).
50. Wei, S. C., Duffy, C. R. & Allison, J. P. Fundamental Mechanisms of Immune Checkpoint Blockade Therapy. Cancer Discov. 8, 1069 LP – 1086 (2018).
51. Topalian, S. L., Drake, C. G. & Pardoll, D. M. Immune Checkpoint Blockade: A Common Denominator Approach to Cancer Therapy. Cancer Cell 27, 450–461 (2015).
52. Chen, L. & Flies, D. B. Molecular mechanisms of T cell co-stimulation and co-inhibition. Nat. Rev. Immunol. 13, 227–42 (2013).
53. Chen, D. S. & Mellman, I. Oncology Meets Immunology: The Cancer-Immunity Cycle. Immunity 39, 1–10 (2013).
54. Ishida, Y., Agata, Y., Shibahara, K. & Honjo, T. Induced expression of PD-1, a novel member of the immunoglobulin gene superfamily, upon programmed cell death. EMBO J. 11, 3887–3895 (1992).
55. Nishimura, H., Nose, M., Hiai, H., Minato, N. & Honjo, T. Development of lupus-like autoimmune diseases by disruption of the PD-1 gene encoding an ITIM motif-carrying immunoreceptor. Immunity 11, 141–151 (1999).
56. Nishimura, H. et al. Autoimmune Dilated Cardiomyopathy in PD-1 Receptor-Deficient Mice. Science (80-.). 291, 319–322 (2001).
57. Dong, H., Zhu, G., Tamada, K. & Chen, L. B7-H1, a third member of the B7 family, co-stimulates T-cell proliferation and interleukin-10 secretion. Nat. Med. 5, 1365–1369 (1999).
58. Freeman, G. J. et al. Engagement of the Pd-1 Immunoinhibitory Receptor by a Novel B7 Family Member Leads to Negative Regulation of Lymphocyte Activation. J. Exp. Med. 192, 1027 LP – 1034 (2000).
59. Park, J.-J. et al. B7-H1/CD80 interaction is required for the induction and maintenance of peripheral T-cell tolerance. Blood 116, 1291–1298 (2010).
60. Butte, M. J., Keir, M. E., Phamduy, T. B., Sharpe, A. H. & Freeman, G. J. Programmed Death-1 Ligand 1 Interacts Specifically with the B7-1 Costimulatory Molecule to Inhibit T Cell Responses. Immunity 27, 111–122 (2007).
61. Dong, H. et al. Tumor-associated B7-H1 promotes T-cell apoptosis: A potential mechanism of immune evasion. Nat. Med. 8, 793–800 (2002).
62. Strome, S. E. et al. B7-H1 Blockade Augments Adoptive T-Cell Immunotherapy for Squamous Cell Carcinoma. Cancer Res. 63, 6501 LP – 6505 (2003).
63. Iwai, Y. et al. Involvement of PD-L1 on tumor cells in the escape from host immune system and tumor immunotherapy by PD-L1 blockade. Proc. Natl. Acad. Sci. 99, 12293 LP – 12297 (2002).
64. Curiel, T. J. et al. Blockade of B7-H1 improves myeloid dendritic cell–mediated antitumor immunity. Nat. Med. 9, 562–567 (2003).
65. Agata, Y. et al. Expression of the PD-1 antigen on the surface of stimulated mouse T and B lymphocytes. Int. Immunol. 8, 765–772 (1996).
66. Day, C. L. et al. PD-1 expression on HIV-specific T cells is associated with T-cell exhaustion and disease progression. Nature 443, 350–354 (2006).
67. Barber, D. L. et al. Restoring function in exhausted CD8 T cells during chronic viral infection. Nature 439, 682–687 (2006).
68. Zhao, F. et al. Blockade of osteopontin reduces alloreactive CD8+ T cell-mediated graft-versus-host disease. Blood 117, 1723–1733 (2011).
69. Zhou, Q. et al. Coexpression of Tim-3 and PD-1 identifies a CD8+ T-cell exhaustion phenotype in mice with disseminated acute myelogenous leukemia. Blood 117, 4501–4510 (2011).
70. Fourcade, J. et al. Upregulation of Tim-3 and PD-1 expression is associated with tumor antigen-specific CD8+ T cell dysfunction in melanoma patients. J. Exp. Med. 207, 2175–2186 (2010).
71. Sakuishi, K. et al. Targeting Tim-3 and PD-1 pathways to reverse T cell exhaustion and restore anti-tumor immunity. J. Exp. Med. 207, 2187–2194 (2010).
72. Wherry, E. J. & Kurachi, M. Molecular and cellular insights into T cell exhaustion. Nat. Rev. Immunol. 15, 486 (2015).
73. Kamphorst, A. O. et al. Rescue of exhausted CD8 T cells by PD-1–targeted therapies is CD28-dependent. Science (80-.). 355, 1423 LP – 1427 (2017).
74. Hui, E. et al. T cell costimulatory receptor CD28 is a primary target for PD-1–mediated inhibition. Science (80-.). 355, 1428 LP – 1433 (2017).
75. Sugiura, D. et al. Restriction of PD-1 function by cis-PD-L1/CD80 interactions is required for optimal T cell responses. Science (80-.). 364, 558 LP – 566 (2019).
76. Tang, H. et al. PD-L1 on host cells is essential for PD-L1 blockade–mediated tumor regression. J. Clin. Invest. 128, 580–588 (2018).
77. Hellmann, M. D. et al. Nivolumab plus Ipilimumab in Lung Cancer with a High Tumor Mutational Burden. N. Engl. J. Med. 378, 2093–2104 (2018).
78. Najjar, Y. G. et al. Tumor cell oxidative metabolism as a barrier to PD-1 blockade immunotherapy in melanoma. JCI Insight 4, (2019).
79. Huang, A. C. et al. T-cell invigoration to tumour burden ratio associated with anti-PD-1 response. Nature 545, 60 (2017).
80. Huang, A. C. et al. A single dose of neoadjuvant PD-1 blockade predicts clinical outcomes in resectable melanoma. Nat. Med. 25, 454–461 (2019).

81. Routy, B. et al. Gut microbiome influences efficacy of PD-1–based immunotherapy against epithelial tumors. Science (80-.). 359, 91 LP – 97 (2018).
82. Gopalakrishnan, V. et al. Gut microbiome modulates response to anti–PD-1 immunotherapy in melanoma patients. Science (80-.). 359, 97 LP – 103 (2018).
83. Matson, V. et al. The commensal microbiome is associated with anti–PD-1 efficacy in metastatic melanoma patients. Science (80-.). 359, 104 LP – 108 (2018).
84. Quezada, S. A. et al. Tumor-reactive CD4+ T cells develop cytotoxic activity and eradicate large established melanoma after transfer into lymphopenic hosts. J. Exp. Med. 207, 637–650 (2010).
85. Tran, E. et al. Cancer Immunotherapy Based on Mutation-Specific CD4+ T Cells in a Patient with Epithelial Cancer. Science (80-.). 344, 641 LP – 645 (2014).
86. Spitzer, M. H. et al. Systemic Immunity Is Required for Effective Cancer Immunotherapy. Cell 168, 487-502.e15 (2017).
87. Marty Pyke, R. et al. Evolutionary Pressure against MHC Class II Binding Cancer Mutations. Cell 175, 416-428.e13 (2018).
88. Rodig, S. J. et al. MHC proteins confer differential sensitivity to CTLA-4 and PD-1 blockade in untreated metastatic melanoma. Sci. Transl. Med. 10, (2018).
89. Benci, J. L. et al. Opposing Functions of Interferon Coordinate Adaptive and Innate Immune Responses to Cancer Immune Checkpoint Blockade. Cell 178, 933-948.e14 (2019).
90. Deng, W. et al. A shed NKG2D ligand that promotes natural killer cell activation and tumor rejection. Science (80-.). 348, 136–139 (2015).
91. Fernandez, N. C. et al. Dendritic cells directly trigger NK cell functions: Cross-talk relevant in innate anti-tumor immune responses in vivo. Nat. Med. 5, 405–411 (1999).
92. Böttcher, J. P. et al. NK Cells Stimulate Recruitment of cDC1 into the Tumor Microenvironment Promoting Cancer Immune Control. Cell 172, 1022-1028.e14 (2018).
93. Guillerey, C., Huntington, N. D. & Smyth, M. J. Targeting natural killer cells in cancer immunotherapy. Nat. Immunol. 17, 1025 (2016).
94. Barry, K. C. et al. A natural killer–dendritic cell axis defines checkpoint therapy–responsive tumor microenvironments. Nat. Med. 1–14 (2018). doi:10.1038/s41591-018-0085-8
95. Alspach, E. et al. MHC-II neoantigens shape tumour immunity and response to immunotherapy. Nature (2019). doi:10.1038/s41586-019-1671-8
96. Kreiter, S. et al. Mutant MHC class II epitopes drive therapeutic immune responses to cancer. Nature 520, 692 (2015).
97. Abelin, J. G. et al. Defining HLA-II Ligand Processing and Binding Rules with Mass Spectrometry Enhances Cancer Epitope Prediction. Immunity 51, 766-779.e17 (2019).
98. Veglia, F. & Gabrilovich, D. I. Dendritic cells in cancer: the role revisited. Curr. Opin. Immunol. 45, 43–51 (2017).
99. Krieg, C. et al. High-dimensional single-cell analysis predicts response to anti-PD-1 immunotherapy. Nat. Med. 24, 144 (2018).
100. Topalian, S. L., Drake, C. G. & Pardoll, D. M. Immune checkpoint blockade: A common denominator approach to cancer therapy. Cancer Cell 27, 451–461 (2015).
101. Postow, M. A., Sidlow, R. & Hellmann, M. D. Immune-Related Adverse Events Associated with Immune Checkpoint Blockade. N. Engl. J. Med. 378, 158–168 (2018).
102. Schetters, S. T. T., Gomez-Nicola, D., Garcia-Vallejo, J. J. & Van Kooyk, Y. Neuroinflammation: Microglia and T cells get ready to tango. Front. Immunol. 8, (2018).
103. Joura, E. A. et al. A 9-Valent HPV Vaccine against Infection and Intraepithelial Neoplasia in Women. N. Engl. J. Med. 372, 711–723 (2015).
104. Harper, D. M. Currently approved prophylactic HPV vaccines. Expert Rev. Vaccines 8, 1663–1679 (2009).
105. Chen, D. S. & Mellman, I. Elements of cancer immunity and the cancer–immune set point. Nature 541, 321 (2017).

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