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Chapter 14

Discussion Part II - Tumor immune Suppression

Sjoerd T.T. Schetters

Chapter 14 Discussion – Tumor immune suppression

14.1. Cellular components of immune suppression and its response to checkpoint blockade

In Part II of this thesis we have investigated the tumor microenvironment, its cellular components and several modes of immune suppression. Cancer immunotherapy was pronounced as "Breakthrough of the Year" by the journal Science in 2013. Since my own start at 2014, the field of tumor immunology has grown exponentially and discussing all new aspects is not only unfeasible, but also difficult to structure. The current scientific viepoint is challenging to unify as most research has until now been performed in parallel and does not build on verified common principles of tumor immunity. In other words, in the current publication landscape seeing the forest for the trees is challenging. As such, I will focus on my own findings in their respective niches and discuss next steps in dealing with immune suppression.

First, the innate and adaptive cellular dynamics of PD-1 blockade was investigated in Chapter 10. It was originally hypothesized PD-1 blocking antibodies could simply block the static interaction of PD1-expressing exhausted T cell with PDL1-expressing tumor cells[1]. For example, data from early clinical trials showed an relationship between PDL1 expression in the tumor with objective clinical responses[2]. While tumor evolution is one explanation of PDL1 overexpression in tumor, a pre-existing $CD8^+$ T cell response to neoantigens has been proposed to underlie the PDL1 expression in tumor cells[3]. In this scenario, neoantigens produced by tumors with a high mutational burden are recognized by $CD8^+$ T cells, resulting in the production of IFNγ, which in turn upregulates MHCI and PDL1 in tumor cells[4,5]. Two studies using genome-wide CRISPR screens verified the tumor-intrinsic need for PDL1, IFNγ signaling and antigen presentation for successful immunotherapy[6,7]. Both pre-existing and evolved forms of defective IFNγ signaling can be found in patients that show no response to checkpoint blockade[8,9], underscoring the importance of this cytokine in tumor immunity. Since the presence of (dysfunctional) neoantigen-specific $CD8^+$ T cells in the microenvironment is critical for tumor immunity, this could explain the wide reported correlations between PD-1 blockade and characteristics like mutational burden, PDL1 expression and T cell infiltration[10]. However, recent research on the mode of action of PD-1 blockade has suggested a more complex interplay of tumor, innate and adaptive immune cells (see Chapter 9.2). For example, MHCII-expressing cells were critical for PD-1 blockade and MHCII-expressing monocytes in the blood correlated with clinical response to PD-1 blockade[11,12]. Our findings described in Chapter 10 suggest that monocytes are continuously infiltrating the tumor and may follow a differentiation pathway toward $MHCII^+$ antigen-presenting cells expressing the co-stimulatory molecules needed for TIL restimulation. This is corroborated by other studies, including an elegant study from the lab of Robert Schreiber showing an IFNγ-dependent bimodal differentiation of tumor-infiltrating monocytes[13]. These findings are distinct from the common hypothesis that tumor-associated macrophages (TAM) have an established (often pro-tumorigenic) phenotype, which could be switched by targeted therapy[14]. It is perhaps more likely, and interesting in terms of therapeutic potential, that TAM phenotypes are largely imprinted by the microenvironment at the moment of monocyte-to-macrophage differentiation. Indeed, the niche-imprinted hypothesis could be true for TAMs[15], but especially for inflammation-/therapy-induced monocyte recruitment to the tumor. For example, monocyte differentiation into macrophages is typically driven by CSF1/CSF1R signaling and CSF1R-blocking antibodies are shown to "reprogram" TAMs[16,17]. Also, pro-tumor macrophages are enriched with a CSF1-driven gene signature in breast cancer patients[18] and CSF1 produced by anti-tumor $CD8^+$ T cells leads to resistance to PD1 blockade[19]. Therefore, we hypothesize that the differentiation of monocytes entering tumor tissue shows plasticity that is and can be modulated by the microenvironment (Figure 1). In a similar fashion, CD40-expressing monocytes can be triggered to differentiate into iNOS-producing monocyte-derived cells, moDCs, inflammatory DCs or tip-DCs (depending on the person doing the classification) capable of restimulating anti-tumor $CD8^+$ T cells[20]. It is known that myeloid dendritic cells are critical for many forms of immunotherapy[21,22]. The question which dendritic cell presents antigen to tumor-reactive $CD8^+$ T cells is perhaps the most difficult to

answer. Many DC types have been implicated in boosting efficacy of cancer immunotherapy, although it remains unclear where this restimulation occurs and which DC subset does what[23]. All genetic mouse models that selectively deplete DCs or subsets of DCs do not distinguish between the tumor-draining lymph node (where priming occurs) and tumor microenvironment (where restimulation takes place). Also, these influences may change over time as the tumor-immunity cycle progresses (Chapter 9). These spatiotemporal differences will need to be address to support rational combination design that aim to boost innate and adaptive immune
responses.

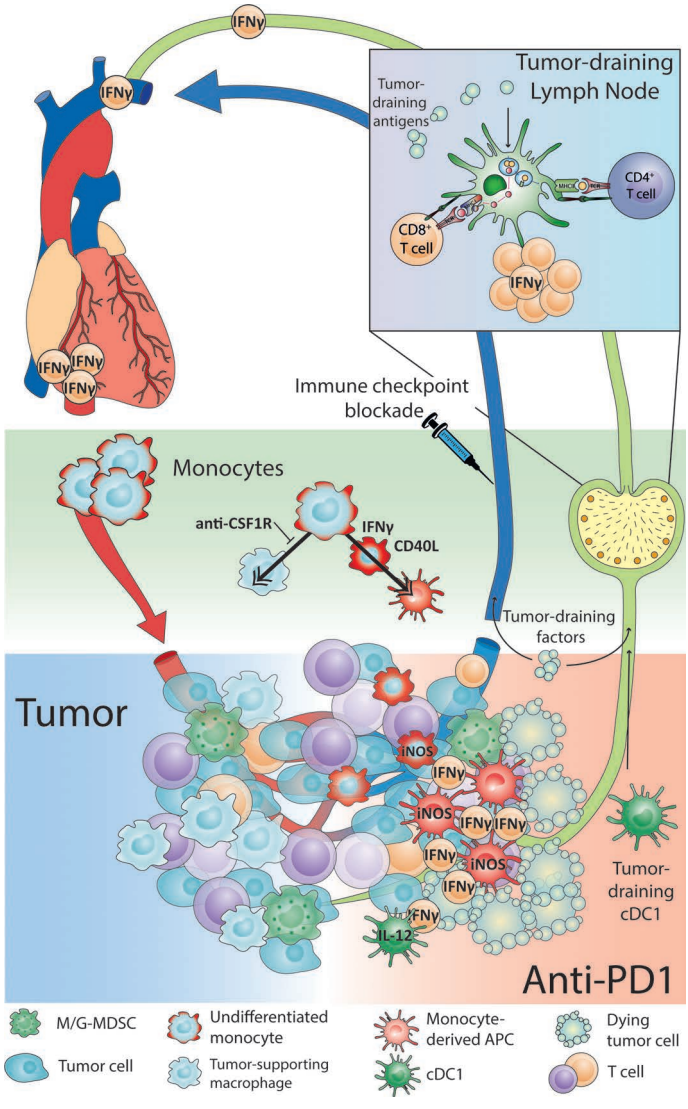

Figure 1 | Monocyte recruitment and differentiation in the tumor and the link with T cell activation during PD1 ICB. Antagonistic PD1 immune checkpoint blockade (ICB) releases the brake of tumor-resident CD8⁺ T cells, which need local TCR triggering by tumor peptide:MHCI complex and co-stimulation via CD28. Tumor-resident DCs and tumor-infiltrating moDCs may provide co-stimulation and produce iNOS and IL12. Tumor-resident cDC1s may take up tumor antigens, migrate to the tumor-draining lymph node and prime CD8⁺ T cells. In parallel, dying tumor cells release tumor antigens that drain to the lymph node where priming of CD8⁺ and CD4⁺ T cells may occur. Lymph node-primed T cells enter the circulation and accumulate in the tumor to further induce tumor regression. Monocytes infiltrating the tumor generally differentiate into TAMs, but may be skewed (by local inflammation or administration of CSF1R blockade or CD40 agonists) towards monocyte-derived APCs for the restimulation of T cells.

Second, we looked into the immune composition of murine glioblastoma multiforme (GBM) using a syngeneic orthotopic mouse model (Chapter 11). Since checkpoint blockade requires both innate and adaptive immune cells, as well as the active expression of checkpoint receptors on T cell, we explored the cellular composition of the brains of glioblastoma-bearing mice. Adaptive immune cells like T cells are largely excluded from the physiological brain and are mostly present in conditions

that disrupt or alter the blood-brain-barrier[24]. Since the origin of glioblastoma in the subventricular zone of the brain is immune privileged, it is mostly limited to immune surveillance by microglia, the brain's resident macrophage[25]. Additionally, glioblastoma is highly invasive outside of the tumor bulk, exhibiting diffuse tumor cell infiltration beyond the tumor margin[26]. The infiltrative nature of glioblastoma complicates therapeutic approaches, since infiltrating cells cannot be surgically removed and the radiation therapy cannot be applied beyond malignant tissue. An attractive approach to battle infiltrating brain tumor cells would be to recruit selective cytotoxic T cells able to recognize and eliminate single tumor cells beyond the bulk tumor. T cell infiltration in the tumor bulk increased in high grade glioblastoma tumors and correlated with increased patient survival[27]. As we have discussed in Chapter 10 and above, immune checkpoint blockade has the capability to selectively kill tumor cells when several preconditions are favorable. In Chapter 11 we explored these preconditions in the brain hemisphere contralateral to the main tumor-bearing hemisphere. We found a significant increase of tumor-specific PD1$^+$ CD8$^+$ T cells in the contralateral hemisphere, a favorable prognostic marker of PD1 ICB efficacy in melanoma[5]. In parallel, we found activated microglia in the contralateral hemisphere, marked by the robust upregulation of MHC class II. We have previously hypothesized that under inflammatory conditions microglia can assume a DC-like phenotype and reciprocally interact with brain-infiltrating T cells[28]. In fact, microglia from neurodegenerative diseases assume a similar reactive phenotype as GBM-derived microglia[28]. In contrast, microglia and macrophages from the GL261 tumor model show distinct transcriptional profiles, suggestive of different functions[29,30]. In the case of the main bulk of the tumor infiltrating monocytes and macrophages are more abundant and dominate the myeloid cell pool, whereas in the contralateral hemisphere microglia are more abundant and is more likely to affect infiltrating tumor-specific T cells. It will be of interest to investigate the microglia-T cell interactions in the contralateral brain where diffuse tumor cells infiltrate. Regardless, after active vaccination, the induction and presence of anti-glioma CD8$^+$ T cells resulted in increased survival in GBM patients[31–33]. While early trials using PD1 ICB alone have shown only minor effects on patient survival, local immunity was increased and neo-antigens were lost, indicative of successful PD1 blockade[34–36]. Interestingly, a personalized neo-antigen based vaccine induced robust CD4$^+$ T cells with a Th1 phenotype against predicted neo-antigens, suggesting a beneficial role for CD4$^+$ T cell immunity in GBM patients[33]. Combined with PD1 ICB, GBM patients may stand a chance in the future.

Third, continuing recent findings from our lab on the glyco-code in pancreatic ductal adenocarcinoma (PDAC), we investigated the expression of sialic acids in PDAC tumor tissue and several mouse models of PDAC (Chapter 13). We have previously shown that sialylated antigens could be using in a vaccination strategy to induce immune suppression through *de novo* generation of regulatory T cells[37]. After discovering the widespread expression of sialic acids in tumor tissue from PDAC patients and the relationship with TAMs, we hypothesized a sialic acid-driven "glyco-code" by PDAC tumors (Chapter 12). Surprisingly, we found host-derived stromal cells, and not tumor cells, to be primary contributors of sialic acids. Glycosylation is the most abundant post-translational modification of proteins and one of the most ancient forms of "self/non-self" discrimination. In fact, the glycan-binding lectin receptors can be traced back in evolution as far as plants (Chapter 1.1.2)[#]. Lectin receptors capable of binding sialic acids are termed Siglecs and are differentially expressed by many cells of the immune system. In particular, inhibitory Siglecs containing a cytoplasmic ITIM domain are known to suppress immune responses when triggered by sialic acids as a form of "self" identification[38]. We have argued that any mutation or adaptive change that suppresses part of the immune system, would provide a selective advantage if the tumor is under selective pressure. It is therefore not surprising that in tumors glycosylation is selected to suppress or evade the immune system. This effect can be indirect as may be possible for the observed recruitment of stromal cells expressing the sialic acid-rich extracellular matrix that has the potential to suppress Siglec E-expressing cells, including infiltrating monocytes (Chapter 13). The data discussed in Chapter 13 on stroma-derived glycosylated extracellular matrix (ECM) is preliminary, but exciting. It is known for some time that high levels of fibrosis shapes the

[#] The modern Siglecs as we describe them here seem to have evolved later in animal evolution, with several expansion events during key developmental moments like the appearance of lactation[93].

heterogeneity of PDAC and contributes to therapy resistance[39,40]. However, therapeutic approaches aimed at depleting stromal cells in the PDAC microenvironment have shown conflicting results[41]. Stromal cells seem to restrain PDAC growth and depleting stromal cells leads to increased tumor invasion and decreased survival[42,43]. Single cell analysis of PDAC tumors recently showed the existence of at least 2 separate fibroblast populations, next to the stellate cells (the main pancreas-resident stromal cell)[44]. In may be possible that functional heterogeneity exists within the stromal compartment which differentially affect PDAC progression[45]. Regardless, cancer-associated fibroblasts were equally abundant as the stellate cells, indicating recruitment of accessory stromal cells in human PDAC reminiscent of our observation that stromal cells producing sialic acids are recruited to the SLC35A1-KO tumor (Chapter 13). The stromal compartment in PDAC produces high levels of ECM resulting in a fibrotic stroma that negatively impacts patient survival[46]. Proteomics analysis of the ECM has shown that the composition is largely collagen, glycoproteins and proteoglycans[47]. Akin to the stromal cells, targeting the tumor ECM has been suggested as a viable therapeutic strategy[48]. The effect of the PDAC tumor stroma on the immune system has been demonstrated by several studies. A small subset of stromal cells expressing fibroblast activation protein (FAP) has been shown to contribute to desmoplasia[49], immune suppression[50] and production of immune suppressive chemokine CXCL12[51]. Alternatively, using a quantitative microscopy tissue microarray approach, Mahajan et al. showed that high expression of immune markers like CD3 (for T cells) and CD206 (for macrophages) correlated with high progression-free survival[52]. Interestingly, the stroma architecture was correlated with expression of T cell and macrophage markers, suggesting a relationship between stroma and immune composition[52,53]. Another study using a somewhat similar microscopy approach showed that decreased physical distance between cytotoxic T cells and tumor cells correlated with increased overall patient survival[54]. Indeed, it has been suggested that the desmoplastic stroma surrounding the tumor beds act as physical barriers for cytotoxic $CD8^+$ T cells, preventing tumor cell killing[55]. However, the expression of collagen-I (ECM) and α-SMA (stromal cells) did not correlate with the paucity of T cell accumulation around the tumor beds, suggesting additional factors preventing T cell localization to the tumor[54]. Rodriguez et al. showed that the sialic acid glyco-code is related to enriched immune suppression, TAM formation and poor patient outcome in PDAC. We hypothesize that the enrichment of sialic acids on stromal-derived ECM components provide immune suppressive signaling to Siglec-expressing innate immune cells. Further experiment will need to be performed to answer the following questions: 1) How are ECM-producing fibroblasts recruited to the tumor and aid in ECM deposition 2) which ECM protein is highly sialylated and why, 3) which receptor may transduce inhibitory signaling upon sialic acid binding and 4) whether we can reduce tumor sialylation through ECM-targeted sialidases.

Several caveats of the studies in Chapter 10, 11 and 13 exist because of differences in the evolutionary time line between mouse and man. Where human tumors often develop over several years or decades (although primary glioblastoma and PDAC tumors may progress faster), evolution has been able to create heterogeneity in overall subclones and neoantigens. In mice, clonally cultured tumor cells are mostly injected subcutaneously or orthotopically and develop over weeks instead of years, with little chance of evolutionary selection. It was recently shown that the myeloid compartment infiltrating lung cancer is fairly similar between mouse models and humans[56]. Whether shaping the T cell landscape occurs in mice in a similar fashion is questionable and needs to be kept in mind.

14.2 Tumor evolution and immune escape

14.2.1 Evolutionary adaptation to therapy

Many patients exhibit adaptive resistance to treatment, regardless of the initial clinical response. For example, metastatic breast cancer patients treated with the PI(3)Kα inhibitor BYL719 showed an initial clinical response, but relapsed and eventually died of lung metastases[57]. Analysis of metastatic lesions showed that loss of PTEN was a convergent evolutionary adaptation to the selective pressures applied by PI(3)Kα inhibition[57]. Similarly, metastatic colorectal patients refractory to anti-EFGR treatment showed the development of new RAS/BRAF mutations, regardless of pre-existing mutations in the

pathway[58]. Similar evolutionary adaptation can be found as a result of immunotherapy[59]. For example, after successful PD1 blockade, tumors arise with increased expression of alternative checkpoint receptors[60]. After T cell immunotherapy, a subset of tumor cells called tumor-initiating stem cells (TSCs) exhibit enhanced resistance via TGFβ[61]. Alternatively, tumor cells may lose the expression of MHC class I antigen presentation as an evolutionary escape mechanism[62]. Future therapeutics will need to understand and anticipate convergent evolutionary escape mechanisms and improve combination regiments[63].

For example, Glioblastoma (GBM) provide an example of evolutionary escape mechanisms that hamper adequate therapeutic approaches. GBM is a highly heterogeneous tumor type and subclones arising after treatment cause relapse with poor outcome[64–66]. Interestingly, the dominant subclone at the moment of clinical diagnosis is often not the subclone that dominates during relapse after treatment[65]. In fact, the dominating clone at relapse is generally diverged from a common ancestor shared with the pre-treatment dominant clone decades before diagnosis in most patients[65]. It seems that therapy selects for pre-existing subclones within a heterogeneous tumor that has developed for over a long period over time accumulating parallel propagating subclones. This poses an obstacle for therapy design, since the prediction of treatment efficacy cannot be established based on the overall genetic makeup of the tumor pre-treatment. Non-invasive methods to track the emergence of resistant subclones during and after treatment would help guide therapy decisions. Recently, it was shown that tumor evolution of GBM could be tracked by sequencing the circulating tumor DNA in the cerebrospinal fluid of patients[67]. However, the detection of single point mutations to investigate tumor evolution may not be sufficient as tumors often harbor extrachromosomal DNA elements (ecDNA) that are differently inherited throughout clones[68]. Similar ecDNA elements were found in gliobastoma and related to pathology and drug resistance[69–72]. Hence, therapeutic approaches will need to be selected based on genotype-specific compositions and tumor evolutionary escape mechanisms monitored. However, it remains unclear at which level the immune system is involved.

14.2.2 Evolutionary view of immune suppression; golden bullet or precision medicine

The evolutionary nature of tumor growth adds a layer of complexity and uncertainty to a singular definition of tumor immune escape/suppression. Because cancer cells accumulate mutations in a stochastic manner and the immune system restricts the growth of susceptible clones, the manner of immune suppression and evasion can assume many forms (for example, MHCI downregulation, PDL1 upregulation or aberrant glycosylation) and be unpredictable, even within the same patient. Additional factors that affect the "fitness" of tumor cells like oxygen and nutrients are also applying selective pressure. Therefore, the most immune-suppressive clone may not survive if the mutations are at the expense of the cell's capability to deal with oxygen and nutrient shortage. What the relative contribution of all selective pressures is among different types of tumors is difficult to define, but will shape the most successful clones (ie. the emerging tumors in patients arriving in the clinic). Also, selective pressures may be different even within the same patient or the same tumor. For example, the tumor cells close to blood vessels have ample access oxygen and nutrients, while more distal tumor cells are selected to cope with lower levels of oxygen and nutrient. However, tumor cells close to blood vessels may also encounter cytotoxic $CD8^+$ T cells at a higher frequency, resulting in selection of clones more resistant to cytotoxic killing.

Regardless of these levels of complexity, evolution is often convergent; similar traits evolve independently because there is a finite amount of biological solutions to selective pressures. Many examples can be found in biology like the independent evolution of C4 carbon fixation in plants[73], loss of skin pigmentation in humans[74], plant seed dispersal by ants[75], the ability of powered flight[76], echolocation[77] and the development of eyes[78]. Although some species (like bats and birds in terms of flight) are of completely different origin, encountering similar environmental and physical constraints leads to similar "evolutionary solutions" to the problem of selective pressures. "The Hallmarks of Cancer" that are independently shared across tumors are examples of this type of convergent

evolution[79–81]. Indeed, while genetic heterogeneity has a profound negative effect on the clinical course of cancer patients[82], it is still constrained by selective pressures and results in functional convergence on key traits[83–86,$]. This is especially important when therapy is considered; the tumor-directed killing applies a direct and directional selective pressure on tumors[87]. In fact, evolution theory has been proposed to support predictive models of therapy efficacy[88]. When successful, the treatment is fast and effective enough to prevent tumor adaptation (evolution) and recurrence. It should be noted that genetic convergence seems most prevalent within tumor types and not across tumor types[81]. For example, the VHL gene is mutated in 52,3% of kidney cancers, but only in 6,9% of tumors across 12 cancer types[81]. Of note, extensive evaluation of genetic composition by several research groups has suggested that the majority of mutations can be explained by genetic drift or neutralism; the variation in genotypes is due to stochastic accumulation, not selective pressure[81,89,90]. This is in stark contrast with common evolved phenotypes as described by the "Hallmarks of Cancer" that define commonalities between tumors. It is the search for phenotypic convergence of tumors that drives the search for shared targets and therefore widely applicable therapeutics or golden bullets, like PD1/PDL1 blockade.

14.3. New age of combination therapy

Cancer therapy should target convergent phenotypes and unique immunogenic genotypes

It is becoming apparent that identifying evolutionary adaptations to immunological selective pressure before and during treatment can aid the selection of immunotherapies. A cancer patient is now proposed to first undergo extensive immune profiling before treatment. This inherent immunological status, the immune set-point, can then be used to predict therapeutic potential and select patients[91]. Initially presumed to be a "one gene, one drug" type of problem that could be solved by genotyping tumor biopsies, it is now understood tumors require a longitudinal and multidimensional approach[92]. Understanding convergent immune phenotypes (like PDL1 expression, T cell infiltration and the glyco-code), molecular subtypes and intratumoral clonality, mutational burden and evolutionary changes over time, will significantly aid immunotherapy in the new age. For example, tumor biopsies before treatment may provide information on the mutational burden and immune status (predicting PD1 ICB), the predicted neo-antigens needed for personalized vaccination and the glyco-code for glycosidase targeting. We believe a multi-dimensional approach that targets convergent phenotypes and patient-specific immunogenic genotypes will outcompete the evolutionary capacity of tumors.

References

1. 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).
2. Topalian, S. L. et al. Safety, Activity, and Immune Correlates of Anti–PD-1 Antibody in Cancer. N. Engl. J. Med. 366, 2443–2454 (2012).
3. Ribas, A. & Wolchok, J. D. Cancer immunotherapy using checkpoint blockade. Science (80-.). 359, 1350 LP – 1355 (2018).
4. Tumeh, P. C. et al. PD-1 blockade induces responses by inhibiting adaptive immune resistance. Nature 515, 568–571 (2014).
5. Eroglu, Z. et al. High response rate to PD-1 blockade in desmoplastic melanomas. Nature 553, 347 (2018).

[$] It should be noted here that the "tumor evolution" narrative is as seductive as it is explanatory. Take for example Dr. Pangloss in Voltaire's Candide; "..for as all things have been created for some end, they must necessarily be created for the best end. Observe, for instance, the nose is formed for spectacles, therefore we wear spectacles. Our legs were clearly intended for breeches, and therefore we wear them." While the plausibility of selective pressures is tempting in creating a narrative, defining the agents of evolutionary change should be considered with care. Its continuing use in this thesis is aimed to explain key concepts that bind these pages together; the development of the immune system and its reaction to pathogens, the development of heterogeneous solid tumors and the adaptation of tumors and pathogens to immune responses in a selective environment.

6. Manguso, R. T. et al. *In vivo* CRISPR screening identifies Ptpn2 as a cancer immunotherapy target. Nature 547, 413 (2017).
7. Patel, S. J. et al. Identification of essential genes for cancer immunotherapy. Nature 548, 537 (2017).
8. Shin, D. S. et al. Primary Resistance to PD-1 Blockade Mediated by JAK1/2 Mutations. Cancer Discov. 7, 188 LP – 201 (2017).
9. Sucker, A. et al. Acquired IFNγ resistance impairs anti-tumor immunity and gives rise to T-cell-resistant melanoma lesions. Nat. Commun. 8, 15440 (2017).
10. Cristescu, R. et al. Pan-tumor genomic biomarkers for PD-1 checkpoint blockade–based immunotherapy. Science (80-.). 362, eaar3593 (2018).
11. 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).
12. Krieg, C. et al. High-dimensional single-cell analysis predicts response to anti-PD-1 immunotherapy. Nat. Med. 24, 144 (2018).
13. Gubin, M. M. et al. High-Dimensional Analysis Delineates Myeloid and Lymphoid Compartment Remodeling during Successful Immune-Checkpoint Cancer Therapy. Cell 175, 1014-1030.e19 (2018).
14. Yang, M., McKay, D., Pollard, J. W. & Lewis, C. E. Diverse Functions of Macrophages in Different Tumor Microenvironments. Cancer Res. 78, 5492 LP – 5503 (2018).
15. Guilliams, M., Mildner, A. & Yona, S. Developmental and Functional Heterogeneity of Monocytes. Immunity 49, 595–613 (2018).
16. 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 LP – 5069 (2014).
17. Xu, J. et al. CSF1R Signaling Blockade Stanches Tumor-Infiltrating Myeloid Cells and Improves the Efficacy of Radiotherapy in Prostate Cancer. Cancer Res. 73, 2782–2794 (2013).
18. Cassetta, L. et al. Human Tumor-Associated Macrophage and Monocyte Transcriptional Landscapes Reveal Cancer-Specific Reprogramming, Biomarkers, and Therapeutic Targets. Cancer Cell 35, 588-602.e10 (2019).
19. Neubert, N. J. et al. T cell-induced CSF1 promotes melanoma resistance to PD1 blockade. Sci. Transl. Med. 10, (2018).
20. Marigo, I. et al. T Cell Cancer Therapy Requires CD40-CD40L Activation of Tumor Necrosis Factor and Inducible Nitric-Oxide-Synthase-Producing Dendritic Cells. Cancer Cell 30, 377–390 (2016).
21. Wculek, S. K. et al. Dendritic cells in cancer immunology and immunotherapy. Nat. Rev. Immunol. (2019). doi:10.1038/s41577-019-0210-z
22. Curiel, T. J. et al. Blockade of B7-H1 improves myeloid dendritic cell–mediated antitumor immunity. Nat. Med. 9, 562–567 (2003).
23. Moussion, C. & Mellman, I. The Dendritic Cell Strikes Back. Immunity 49, 997–999 (2018).
24. Engelhardt, B., Vajkoczy, P. & Weller, R. O. The movers and shapers in immune privilege of the CNS. Nat. Immunol. 18, 123–131 (2017).
25. Lee, J. H. et al. Human glioblastoma arises from subventricular zone cells with low-level driver mutations. Nature 560, 243–247 (2018).
26. Cuddapah, V. A., Robel, S., Watkins, S. & Sontheimer, H. A neurocentric perspective on glioma invasion. Nat. Rev. Neurosci. 15, 455 (2014).
27. Lohr, J. et al. Effector T-Cell Infiltration Positively Impacts Survival of Glioblastoma Patients and Is Impaired by Tumor-Derived TGF-β. Clin. Cancer Res. 17, 4296 LP – 4308 (2011).
28. 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, 1905 (2018).
29. Hambardzumyan, D., Gutmann, D. H. & Kettenmann, H. The role of microglia and macrophages in glioma maintenance and progression. Nat. Neurosci. 19, 20–27 (2016).
30. Szulzewsky, F. et al. Glioma-Associated Microglia/Macrophages Display an Expression Profile Different from M1 and M2 Polarization and Highly Express Gpnmb and Spp1. PLoS One 10, e0116644 (2015).
31. Prins, R. M. et al. Gene expression profile correlates with T-cell infiltration and relative survival in glioblastoma patients vaccinated with dendritic cell immunotherapy. Clin. Cancer Res. 17, 1603–1615 (2011).
32. Dutoit, V. et al. Exploiting the glioblastoma peptidome to discover novel tumour-associated antigens for immunotherapy. Brain 135, 1042–1054 (2012).
33. Hilf, N. et al. Actively personalized vaccination trial for newly diagnosed glioblastoma. Nature 565, 240–245 (2019).
34. Cloughesy, T. F. et al. Neoadjuvant anti-PD-1 immunotherapy promotes a survival benefit with intratumoral and systemic immune responses in recurrent glioblastoma. Nat. Med. 1 (2019). doi:10.1038/s41591-018-0337-7
35. Zhao, J. et al. Immune and genomic correlates of response to anti-PD-1 immunotherapy in glioblastoma.

Nat. Med. 25, 462–469 (2019).
36. Schalper, K. A. et al. Neoadjuvant nivolumab modifies the tumor immune microenvironment in resectable glioblastoma. Nat. Med. 1 (2019). doi:10.1038/s41591-018-0339-5
37. Perdicchio, M. et al. Sialic acid-modified antigens impose tolerance via inhibition of T-cell proliferation and de novo induction of regulatory T cells. Proc. Natl. Acad. Sci. U. S. A. 113, (2016).
38. Crocker, P. R., Paulson, J. C. & Varki, A. Siglecs and their roles in the immune system. Nat. Rev. Immunol. 7, 255–266 (2007).
39. Pandol, S., Edderkaoui, M., Gukovsky, I., Lugea, A. & Gukovskaya, A. Desmoplasia of Pancreatic Ductal Adenocarcinoma. Clin. Gastroenterol. Hepatol. 7, S44–S47 (2009).
40. Ligorio, M. et al. Stromal Microenvironment Shapes the Intratumoral Architecture of Pancreatic Cancer. Cell 178, 160-175.e27 (2019).
41. Gore, J. & Korc, M. Pancreatic Cancer Stroma: Friend or Foe? Cancer Cell 25, 711–712 (2014).
42. Özdemir, B. C. et al. Depletion of Carcinoma-Associated Fibroblasts and Fibrosis Induces Immunosuppression and Accelerates Pancreas Cancer with Reduced Survival. Cancer Cell 25, 719–734 (2014).
43. Rhim, A. D. et al. Stromal Elements Act to Restrain, Rather Than Support, Pancreatic Ductal Adenocarcinoma. Cancer Cell 25, 735–747 (2014).
44. Peng, J. et al. Single-cell RNA-seq highlights intra-tumoral heterogeneity and malignant progression in pancreatic ductal adenocarcinoma. Cell Res. 29, 725–738 (2019).
45. Moffitt, R. A. et al. Virtual microdissection identifies distinct tumor- and stroma-specific subtypes of pancreatic ductal adenocarcinoma. Nat. Genet. 47, 1168–1178 (2015).
46. Whatcott, C. J. et al. Desmoplasia in Primary Tumors and Metastatic Lesions of Pancreatic Cancer. Clin. Cancer Res. 21, 3561–3568 (2015).
47. Tian, C. et al. Proteomic analyses of ECM during pancreatic ductal adenocarcinoma progression reveal different contributions by tumor and stromal cells. Proc. Natl. Acad. Sci. 116, 19609 LP – 19618 (2019).
48. Valkenburg, K. C., de Groot, A. E. & Pienta, K. J. Targeting the tumour stroma to improve cancer therapy. Nat. Rev. Clin. Oncol. 15, 366–381 (2018).
49. Lo, A. et al. Tumor-Promoting Desmoplasia Is Disrupted by Depleting FAP-Expressing Stromal Cells. Cancer Res. 75, 2800 LP – 2810 (2015).
50. Kraman, M. et al. Suppression of Antitumor Immunity by Stromal Cells Expressing Fibroblast Activation Protein{\textendash}α. Science (80-.). 330, 827–830 (2010).
51. Feig, C. et al. Targeting CXCL12 from FAP-expressing carcinoma-associated fibroblasts synergizes with anti{\ textendash}PD-L1 immunotherapy in pancreatic cancer. Proc. Natl. Acad. Sci. 110, 20212–20217 (2013).
52. Mahajan, U. M. et al. Immune Cell and Stromal Signature Associated With Progression-Free Survival of Patients With Resected Pancreatic Ductal Adenocarcinoma. Gastroenterology 155, 1625-1639.e2 (2018).
53. Sonohara, F. & Goel, A. Features of Immune Cells and the Tumor-Associated Stroma Tango as Prognostic Factors in Patients With Pancreatic Ductal Adenocarcinoma. Gastroenterology 155, 1312–1314 (2018).
54. Carstens, J. L. et al. Spatial computation of intratumoral T cells correlates with survival of patients with pancreatic cancer. Nat. Commun. 8, 15095 (2017).
55. Watt, J. & Kocher, H. M. The desmoplastic stroma of pancreatic cancer is a barrier to immune cell infiltration. Oncoimmunology 2, e26788–e26788 (2013).
56. Zilionis, R. et al. Single-Cell Transcriptomics of Human and Mouse Lung Cancers Reveals Conserved Myeloid Populations across Individuals and Species. Immunity 50, 1317-1334.e10 (2019).
57. Juric, D. et al. Convergent loss of PTEN leads to clinical resistance to a PI(3)Kα inhibitor. Nature 518, 240 (2014).
58. Thierry, A. R. et al. Circulating DNA Demonstrates Convergent Evolution and Common Resistance Mechanisms during Treatment of Colorectal Cancer. Clin. Cancer Res. 23, 4578 LP – 4591 (2017).
59. Sharma, P., Hu-Lieskovan, S., Wargo, J. A. & Ribas, A. Primary, Adaptive, and Acquired Resistance to Cancer Immunotherapy. Cell 168, 707–723 (2017).
60. Koyama, S. et al. Adaptive resistance to therapeutic PD-1 blockade is associated with upregulation of alternative immune checkpoints. Nat Commun 7, 1–9 (2016).
61. Miao, Y. et al. Adaptive Immune Resistance Emerges from Tumor-Initiating Stem Cells. Cell 177, 1172-1186. e14 (2019).
62. Paulson, K. G. et al. Acquired cancer resistance to combination immunotherapy from transcriptional loss of class I HLA. Nat. Commun. 9, 3868 (2018).
63. Galon, J. & Bruni, D. Approaches to treat immune hot, altered and cold tumours with combination immunotherapies. Nat. Rev. Drug Discov. 18, 197–218 (2019).
64. Kim, J. et al. Spatiotemporal Evolution of the Primary Glioblastoma Genome. Cancer Cell 28, 318–328 (2015).
65. Wang, J. et al. Clonal evolution of glioblastoma under therapy. Nat. Genet. 48, 768 (2016).

66. Sottoriva, A. et al. Intratumor heterogeneity in human glioblastoma reflects cancer evolutionary dynamics. Proc. Natl. Acad. Sci. 110, 4009 LP – 4014 (2013).
67. Miller, A. M. et al. Tracking tumour evolution in glioma through liquid biopsies of cerebrospinal fluid. Nature 565, 654–658 (2019).
68. Turner, K. M. et al. Extrachromosomal oncogene amplification drives tumour evolution and genetic heterogeneity. Nature 543, 122 (2017).
69. Nikolaev, S. et al. Extrachromosomal driver mutations in glioblastoma and low-grade glioma. Nat. Commun. 5, 5690 (2014).
70. Zheng, S. et al. A survey of intragenic breakpoints in glioblastoma identifies a distinct subset associated with poor survival. Genes Dev. 27, 1462–1472 (2013).
71. deCarvalho, A. C. et al. Discordant inheritance of chromosomal and extrachromosomal DNA elements contributes to dynamic disease evolution in glioblastoma. Nat. Genet. 50, 708–717 (2018).
72. Nathanson, D. A. et al. Targeted Therapy Resistance Mediated by Dynamic Regulation of Extrachromosomal Mutant EGFR DNA. Science (80-.). 343, 72 LP – 76 (2014).
73. Williams, B. P., Johnston, I. G., Covshoff, S. & Hibberd, J. M. Phenotypic landscape inference reveals multiple evolutionary paths to C4 photosynthesis. Elife 2, e00961–e00961 (2013).
74. Edwards, M. et al. Association of the OCA2 polymorphism His615Arg with melanin content in east Asian populations: further evidence of convergent evolution of skin pigmentation. PLoS Genet. 6, e1000867–e1000867 (2010).
75. Lengyel, S., Gove, A. D., Latimer, A. M., Majer, J. D. & Dunn, R. R. Convergent evolution of seed dispersal by ants, and phylogeny and biogeography in flowering plants: A global survey. Perspect. Plant Ecol. Evol. Syst. 12, 43–55 (2010).
76. Gleiss, A. C. et al. Convergent evolution in locomotory patterns of flying and swimming animals. Nat. Commun. 2, 352 (2011).
77. Parker, J. et al. Genome-wide signatures of convergent evolution in echolocating mammals. Nature 502, 228 (2013).
78. Fernald, R. D. Casting a Genetic Light on the Evolution of Eyes. Science (80-.). 313, 1914 LP – 1918 (2006).
79. Hanahan, D. & Weinberg, R. A. The Hallmarks of Cancer. Cell 100, 57–70 (2000).
80. Hanahan, D. & Weinberg, R. a. Hallmarks of cancer: The next generation. Cell 144, 646–674 (2011).
81. Wu, C.-I., Wang, H.-Y., Ling, S. & Lu, X. The Ecology and Evolution of Cancer: The Ultra-Microevolutionary Process. Annu. Rev. Genet. 50, 347–369 (2016).
82. McGranahan, N. & Swanton, C. Clonal Heterogeneity and Tumor Evolution: Past, Present, and the Future. Cell 168, 613–628 (2017).
83. Fisher, R. et al. Development of synchronous VHL syndrome tumors reveals contingencies and constraints to tumor evolution. Genome Biol. 15, 433 (2014).
84. Chen, H. & He, X. The Convergent Cancer Evolution toward a Single Cellular Destination. Mol. Biol. Evol. 33, 4–12 (2015).
85. Fortunato, A. et al. Natural Selection in Cancer Biology: From Molecular Snowflakes to Trait Hallmarks. Cold Spring Harb. Perspect. Med. 7, a029652 (2017).
86. Ma, P. et al. Simultaneous evolutionary expansion and constraint of genomic heterogeneity in multifocal lung cancer. Nat. Commun. 8, 823 (2017).
87. Venkatesan, S. & Swanton, C. Tumor Evolutionary Principles: How Intratumor Heterogeneity Influences Cancer Treatment and Outcome. Am. Soc. Clin. Oncol. Educ. B. e141–e149 (2016). doi:10.1200/EDBK_158930
88. Lässig, M., Mustonen, V. & Walczak, A. M. Predicting evolution. Nat. Ecol. Evol. 1, 77 (2017).
89. Sottoriva, A. et al. A Big Bang model of human colorectal tumor growth. Nat. Genet. 47, 209 (2015).
90. Williams, M. J., Werner, B., Barnes, C. P., Graham, T. A. & Sottoriva, A. Identification of neutral tumor evolution across cancer types. Nat. Genet. 48, 238 (2016).
91. Chen, D. S. & Mellman, I. Elements of cancer immunity and the cancer–immune set point. Nature 541, 321 (2017).
92. Dienstmann, R. et al. Consensus molecular subtypes and the evolution of precision medicine in colorectal cancer. Nat. Rev. Cancer 17, 79 (2017).
93. Bornhöfft, K. F., Goldammer, T., Rebl, A. & Galuska, S. P. Siglecs: A journey through the evolution of sialic acid-binding immunoglobulin-type lectins. Dev. Comp. Immunol. 86, 219–231 (2018).

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