Remote control: the cancer cell-intrinsic mechanisms that dictate systemic inflammation and anti-tumor immunity

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Cancer cell-intrinsic mechanisms shaping the tumor immune landscape

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Abstract

Owing to their tremendous diversity and plasticity, immune cells exert multifaceted functions in tumor-bearing hosts, ranging from anti-tumor to pro-tumor activities. Tumor immune landscapes differ greatly between and within tumor types. Only recently, studies have begun to shed light on the mechanisms that shape the variability in immune contexture between individual tumors. There is emerging evidence that genetic aberrations in cancer cells dictate the immune landscape of tumors. Here, we review the clinical observation and the mechanisms identified in genetically engineered mouse models by which common drivers of tumorigenesis modulate the tumor microenvironment. We also discuss how cancer cell-intrinsic properties can be exploited to maximize the benefit of immunomodulatory therapies. Identifying and understanding the causal relationship between cancer cell-intrinsic genetic events and the immune system provides a basis for the design of personalized immune intervention strategies for cancer patients.

Introduction

The recognition of cancer as a genetic disease is more than a century old, and stems from observations by David von Hansemann and Theodor Boveri that cancer cells display chromosomal abnormalities[1,2]. In the early 20th century, Francis Rous revealed that retroviruses could drive sarcoma formation in chickens[3]. Many decades later, in 1970, the Rous sarcoma virus was found to carry a gene called *v-Src*, the first oncogene to be identified[4,5]. Concurrently, it was discovered that not only activation, but also inactivation of so-called tumor suppressor genes (TSGs) can lead to tumorigenesis[6]. (Proto-)oncogenes and TSGs regulate essential cellular processes like cell cycle, apoptosis, migration and survival, and genetic aberrations that lead to dysregulation or loss of function of these genes can result in malignant transformation. The generation of transgenic mice carrying an activated oncogene, also called *oncomice*, in the 1980s and TSG knockout mice in the 1990s further substantiated the notion that oncogene expression or loss of TSGs in normal mammalian cells leads to cancer development[7-10]. The dependency of cancers on these dysregulated genes was demonstrated in genetically engineered mouse models (GEMMs) in which de-activation of oncogenes or re-expression of TSGs in fully established tumors led to rapid tumor regression[11-14]. These insights into the causal role of genetic aberrations in cancer initiation and progression spurred the long-held belief that tumorigenesis is entirely driven by cancer cell-intrinsic genetic traits. However, over the past couple of decades, this dogma has been challenged by new experimental evidence demonstrating that genetic aberrations alone are required, but not sufficient for a cancer to develop. Like a seed needing fertile soil for successful germination, cancer cells only survive and develop into invasive tumors in an environment that provides sufficient nutrients and oxygen, and that lacks strong cytotoxic signals. In this review, we will focus on one of the most influential cancer cell-extrinsic regulators of cancer biology, the immune system.

Similar to its physiological function, the immune system exerts multifaceted tasks in tumor-bearing hosts, with different immune cells playing different and sometimes opposing roles. The composition and function of immune cells in tumors differs greatly between, but also within cancer types. For example, of the breast cancer subtypes, triple-negative breast cancer (TNBC) presents with highest levels of tumor-infiltrating lymphocytes (TIL) and macrophages[15,16]. Striking differences in relative leukocyte composition between different tumor types were observed in a study that integrated gene expression and clinical outcome data of over 18.000 human tumors[17]. Moreover, this study revealed considerable variation in intratumoral presence of certain immune cell subsets and how these were associated with cancer-specific outcomes. For example, whereas memory $CD4^+$ T cells were associated with adverse outcome in bladder cancer patients, they correlated with favorable outcome in lung adenocarcinoma patients[17], suggesting that differences in immune profile are not only phenotypically distinct but are also of functional consequence. But what determines this substantial variation in immune contexture between different tumors? Given the surge of interest in utilizing immunomodulatory drugs for the treatment of cancer patients, it is critical to understand the underlying tumor characteristics that dictate the inter-tumor heterogeneity in immune landscapes, and to use this knowledge for rational decision-making in the clinical use of immunomodulatory strategies.

In this review, we will discuss recent insights into how cancer cell-intrinsic properties can dictate the immune landscape of tumor-bearing hosts. Specifically, we will examine which genetic aberrations correlate with immune cell composition in human tumors. Next, we

will discuss the current knowledge on oncogene- and TSG-dependent signaling pathways that underlie the differential crosstalk of cancer cells with the immune system as identified in genetically engineered mouse tumor models. Finally, we will discuss how the growing insights into these mechanisms may open new avenues for personalized immune intervention strategies for cancer patients.

Genetic makeup influencing the immune contexture of tumors – observations from the clinic

In 1863, the German pathologist Rudolf Virchow was the first to hypothesize a link between the development of tumors and the inflammatory state of their anatomical location[18]. Around the same time, William Coley, pioneer of cancer immunotherapy, demonstrated that some patients displayed tumor regression after being injected with immune stimulatory *Streptococcus pyogenes* cultures[19]. Nowadays, it is fully established that inflammation can be causally linked with human cancers, and that the immune infiltrate of human tumors contains prognostic and predictive information[17,20]. Moreover, cancer immunotherapy has revolutionized cancer treatment[21], illustrating that immune cells can be harnessed successfully to destroy tumors in a proportion of cancer patients. Recently, studies have started to explore the cancer cell characteristics – including the genetic makeup – that play a critical role in dictating the heterogeneity in immune landscape between different tumors. Studies aimed at assessing the link between the genetics of human tumors and the immune infiltrate can be roughly divided into three categories: 1) studies that have assessed the extent of the mutational load of tumors with T cell abundance, specificity and activity; 2) studies that have linked distinct molecular tumor subtypes with a certain immune landscape; 3) studies that have focused on the association between defined oncogenic driver mutations or loss of TSGs and parameters of the inflammatory tumor microenvironment. In this section, we will discuss the findings of these three different strategies to assess the impact of genetic events on the crosstalk with the immune system.

The core function of the adaptive immune system is to recognize and destroy cells expressing non-self-antigens, while not responding to self-antigens. Since cancers arise from host cells, these cancer cells, with the exception of viral-associated cancers, do not express the typical immunogenic foreign antigens as seen in infections. The recent clinical breakthrough of immune checkpoint inhibitors has fueled studies aimed at identifying the tumor antigens that are recognized by effective anti-tumor immune responses. This resulted in the hypothesis that a higher mutational load of a tumor will inevitably result in more 'foreign' peptide presentation, and consequently higher immunogenicity of the tumor. Mutations and other genomic rearrangements in cancer cells can encode for neo-antigens, antigens uniquely expressed by the tumor, that when presented by MHC molecules can potentially be recognized by the endogenous T cell repertoire[22]. Indeed, neo-antigen-specific T cells have been observed in melanoma patients[23-27] and tumor types with a relatively high mutational burden, such as melanoma, non-small cell lung cancer (NSCLC) and microsatellite-instable (MSI) tumors display increased T cell influx and have an overall better response rate to immunotherapeutics compared to tumors with a lower mutational load[28-30]. Nevertheless, there is a substantial number of patients with good response and low mutational load and *vice versa*[28,31-35]. These observations suggest that for some tumors the mutational burden of tumors can serve as a quantitative measure for T cell abundance and likelihood to respond to immune checkpoint inhibitors. However, there are clearly additional determinants of the

immune contexture in tumors besides mutational load.

Distinct molecular subtypes of human cancers can be associated with a defined immune composition and activation state in the tumor microenvironment. Several cancer types can be subtyped based on their molecular and genetic profile, thus forming separate classes within a given tumor type, often with distinct progression characteristics and treatment regimens. For example, breast tumors can be classified as Luminal A (ER/PR$^+$, HER2$^-$), Luminal B (ER/PR$^+$, HER2$^+$/$^-$), HER2-enriched (HER2$^+$) and triple-negative/basal-like (ER/PR/HER2$^-$)[36]. It has been reported that CD8$^+$ T cells preferentially infiltrate in triple negative tumors, and those patients with high intratumoral T cell abundance in show better disease-free survival[15,16,37,38]. Breast tumors that express hormone receptors or HER2 are more frequently infiltrated by FoxP3$^+$ regulatory T cells (Tregs) compared to other subtypes, suggesting dependency on these receptors in the establishment of an immunosuppressive milieu[39,40]. Accordingly, the presence of Tregs in breast tumors predicted metastatic progression and poor survival[40,41]. For other cancer types, such as colorectal cancer, glioblastoma and head and neck cancer, similar subtype-specific tumor immune infiltrates have been observed[42-45] (**Table 2.1**). These clinical observations indicate that different molecular subtypes of tumors can be characterized by distinct immune landscapes. However, due to the complex nature that underlies molecular subtypes, the exact genes and mechanisms that determine this immune heterogeneity cannot be distilled from these studies.

Table 2.1: Clinical observations on tumor subtype and genotype-immunophenotype relations. Abbreviations. CRC: colorectal cancer. HNSCC: head and neck squamous cell carcinoma. PDAC: pancreatic ductal adenocarcinoma. NA: Not assessed.

Determinant of tumor immune landscape	Cancer type	Immune cell subset	Effect on therapy/ disease outcome	References
Tumor subtype				
CMS1	CRC	↑ Cytotoxic T cells*	Overall favorable response to immune checkpoint blockade	42
Mesenchymal	Glioblastoma	↑ Immunosuppressive cells* ↑ T effector cells*	NA	43
		↑ Macrophages, neutrophils* ↓ NK cells		45
Triple-negative/basal-like	Breast cancer	↑ CD8$^+$ T cells, macrophages	High CD8$^+$ T cell abundance gives high overall survival	15,16,37,38
ER/PR/HER2$^+$		↑ Tregs	High Treg abundance gives poor overall survival	39-41
Inflamed/ mesenchymal HPV$^{+/-}$	HNSCC	↑ CD8$^+$ T cells*	NA	44
Mutated oncogenes or tumor suppressor gene				
TP53 loss or mutation	ER$^-$ & basal-like breast cancer	↓ Cytotoxic T cells	Poor survival	46
	Pan-cancer	↓ Cytotoxic T, NK cells*	NA	53
MYC, NOTCH2, FGFR1 amplification	PDAC	↓ Cytotoxic T cells*	NA	34
MYC amplification	Neuroblastoma	↓ T cells	NA	50
		↓ NK cells		51
PIK3CA, MET mutations	Pan-cancer	↑ Cytotoxic T, NK cells*	NA	53
BRAF mutations *RAS* mutations	Thyroid cancer	↑ Immunosuppressive cells* ↑ T cells*	NA	32
VHL, STK11 mutations	Pan-cancer	↓ Macrophages*	NA	53
NF1 loss	Glioblastoma	↑ Macrophages	NA	45

* Immune cell composition based on gene expression signatures.

A growing body of clinical observations indicates that defined oncogenic driver mutations and loss of TSGs in human cancers are also correlated with changes in immune composition and immunotherapy response. For example, loss of *NF1* in glioblastomas

associated with an increase in macrophages in the tumor[45]. Another study showed that loss of heterozygosity (LOH) or mutation of *TP53* in ER-negative and basal-like breast tumor tumors is associated with decreased intratumoral expression of a cytotoxic T cell signature and poor survival[46]. These studies indicate that a single TSG can be associated with the immune composition of the tumor, across different tumor subtypes, and therefore may be a dominant driving force of immune influx. Furthermore, in pancreatic ductal adenocarcinoma (PDAC), expression of genes associated with cytotoxic T cell function and immune checkpoint molecules was inversely linked with amplification of *MYC, NOTCH2* and *FGFR1*, but not with mutational load[34]. The reduced expression of cytolytic immune response markers in these *MYC-, NOTCH2-* and *FGFR1*-amplified tumors was observed across the different PDAC subtypes[34,47] and suggests that aberrant expression of oncogenic pathways also dominantly impacts the composition of the pancreatic tumor microenvironment (**Table 2.1**).

Genetic aberrations in tumors can also influence the T cell response by altering expression levels of immune checkpoint molecules by cancer cells. In a cohort of lung adenocarcinoma patients, accumulation of p53 in tumor cells, which is indicative of mutations in *TP53*, correlated with increased *PD-L1* expression, while mutant EGFR tumors were characterized by low expression of *PD-L1*[48]. In contrast, another study showed that EGFR mutated lung tumors have high levels of *PD-L1*[49], demonstrating that the role of mutant EGFR in regulating PD-L1 expression is still under debate. In metastatic neuroblastoma, amplification of *MYCN* correlated with low expression of *PD-L1* and a reduced T cell gene expression signature in the tumor compared to *MYCN*-normal tumors[50]. Moreover, *MYCN* overexpression inversely correlated with natural killer (NK) cell-activating factors such as NKG2D in primary human neuroblastoma cell lines[51]. In addition, resistance to anti-PD-1 treatment in melanoma and MSI CRC patients correlated with mutations in JAK1/2 [52]. Using human melanoma cell lines, it was shown that JAK1/2 mutations led to an impaired IFN signaling pathway-mediated PD-L1 expression, suggesting that also JAK-STAT signaling is involved in regulating immune checkpoint expression. These findings indicate that screening for expression of certain oncogenes or loss of function of specific TSGs may be exploited to improve the stratification of cancer patients for therapeutic targeting the PD-1/PD-L1 axis.

The link between the genetic makeup of tumors and their immune contexture was further strengthened by recent high-throughput next generation sequencing (NGS) studies, which allow an unbiased assessment of the genetics of tumors in parallel with high-resolution mapping of the tumor immune landscape. By correlating an RNA-based metric of immune cytolytic activity (mainly associated with T and NK cell function) with genetic data from the Cancer Genome Atlas (TCGA) dataset, it was shown that immune activity varies substantially across tumor types[53]. Consistent with the concept that a higher mutational load increases tumor immunogenicity, there was a positive correlation between adaptive immune activation gene signatures and mutational load across tumor types[53]. Interestingly, this study also revealed that expression of genes associated with cytotoxic immune activation was elevated in tumors with mutations in *PIK3CA* or *MET,* while *TP53* mutant tumors displayed low levels of these genes[53]. Additionally, mutations in *VHL* and *STK11* associated with reduced macrophage signatures[53]. In another study into genotype-immunophenotype relationships, it was found that BRAF-mutated thyroid tumors were characterized by infiltration of immunosuppressive cells, while the RAS-mutated subtype contained higher T cell influx and displayed downregulation of MHC molecules, despite comparable mutational load[32]. Accordingly, oncogenic mutations also link with response to immunotherapy. Using human datasets to predict response to

anti-CTLA-4 therapy in melanoma patients, it was demonstrated that mutations in oncogenes such as *KRAS, ATM* and *mTOR* correlated with good immunotherapy response for some tumor types[54]. These studies demonstrate that NGS studies can reveal relationships between cancer-associated genes, activation of immune cells and response to immunotherapies in a high-throughput and high-resolution manner.

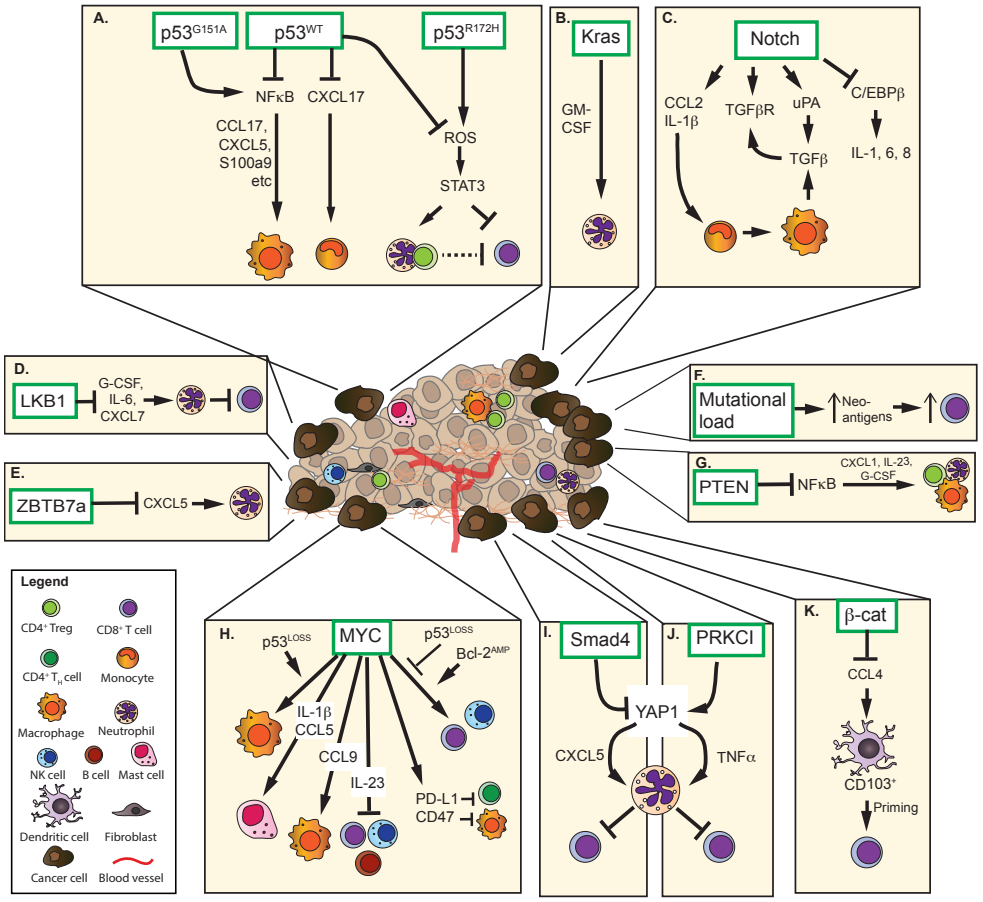
Together, these observations suggest that mutational load, tumor subtype and aberrant expression of oncogenes and TSGs highly impact the tumor microenvironment. Interestingly, for certain tumors, the tumor driver genes, mutational load and subtype are intrinsically linked, as for example aberrant expression of *BRCA1* impairs the DNA damage repair machinery and therefore has consequences for the mutational load of a tumor. However powerful, these genotype–immunophenotype studies in human cancers leave several questions open. Due to the descriptive nature of these analyses, these studies do not yield mechanistic insights into causal relationships between tumor genetics and the immune composition. From a therapeutic perspective, it is important to assess whether a causal link between tumor genetics and immune contexture exists and to elucidate the underlying molecular mechanisms, since this would open new avenues for personalized immune intervention strategies. Of note, the above described clinical studies often rely on the analysis of a small tumor biopsy at a given time point, and therefore may overlook intratumoral heterogeneity and tumor evolution. For these reasons, mechanistic studies in relevant GEMMs that mimic the development, heterogeneity and progression of human tumors in an immune-proficient setting are key to understand how cancer cell-intrinsic properties can dictate the tumor immune landscape[55]. In the next sections, we will discuss recent insights into these mechanisms and how these insights can be translated into personalized immune intervention strategies. Given the growing interest in the role of the immune system in tumorigenesis, we anticipate that more pathways will be uncovered in the years to come.

NFκB and p53: central nodes in cancer cell-mediated changes in the inflammatory microenvironment

The mechanisms by which oncogenes and TSGs orchestrate the inflammatory tumor microenvironment are now being uncovered. Specific cancer-associated genes, besides driving cancer cell-intrinsic programs, also change the secretome of cancer cells, and thereby change the immune microenvironment (**Fig. 2.1, Table 2.2**). One notable example is NFκB, a transcription factor that controls cell survival and proliferation, but also production of inflammatory cytokines. For example, NFκB signaling promoted tumor development in the *Kras$^{LSL\text{-}G12D/+}$;Trp53$^{F/F}$* lung adenocarcinoma model[56]. Interestingly, NFκB activity was increased upon loss of p53, and restoration of p53 expression reduced its activity. Cancer cell-intrinsic NFκB inactivation resulted in increased intratumoral immune cell influx and impaired lung cancer formation in *Kras$^{LSL\text{-}G12D}$;Trp53$^{F/F}$* mice[56], showing a link between loss of p53, NFκB pathway activation and an inflammatory tumor microenvironment. As one of the most frequently mutated genes in cancer[57], the tumor suppressor p53 can potentially regulate the immune infiltrate in a wide variety of tumor types, through its interactions with NFκB or otherwise. Indeed, the control of the pro-inflammatory NFκB pathway by p53 appears to be occurring across cancer types[58]. For example, in the *Pgr-cre;Cdh1$^{F/F}$;Trp53$^{F/F}$* mouse model for endometrial cancer, the combined loss of E-cadherin and p53 resulted in increased NFκB activity, which correlated with elevated cytokine expression and increased influx of macrophages, as compared to deletion of either gene alone[59]. However, in another

mouse model in which endometrial tumorigenesis is driven by loss of PTEN, loss of p53 did not alter neutrophil influx into early lesions[60], suggesting that this effect may be context dependent. Together, these and other studies show that NFκB, key regulator of immune signaling in the tumor microenvironment, is controlled by p53. In several tumor models, loss of p53 activates the NFkB pathway, stimulates the production of cytokines and other pro-inflammatory mediators from cancer cells, which through paracrine interactions modify the immune contexture.

Studies in mouse models in which chemical-induced inflammation drives malignant conversion and progression show that the NFκB-mediated inflammatory response can also be a driving force of tumorigenesis in p53-knockout models. For example, azoxymethane (AOM)-induced colonic tumorigenesis was enhanced in *Villin-cre;Trp53$^{F/F}$* mice that harbor p53 deletion in intestinal epithelial cells, as compared to mice with p53 proficient intestinal epithelial cells[61]. Mechanistic studies in these mice revealed that loss of p53 impaired the removal of pre-neoplastic transformed cells and induced NFκB-dependent cytokine production, thus driving an inflammatory tumor microenvironment[61]. Importantly, genetic ablation of IKKβ, a protein involved in NFκB activation, in cancer cells or myeloid cells,



reduced tumor proliferation and invasion, demonstrating that NFκB signaling in p53-null cancer cells or in surrounding myeloid cells plays a fundamental role in tumor progression[61].

A critical feature of p53 biology in cancer not addressed in these studies, is its wide variety of both activating and inactivating mutations, leading to very diverse and sometimes even opposing functions[62]. How one of these p53 mutations affects NFκB activation, was addressed in a gain-of-function (GOF) mutant *p53*^{G515A} mouse model that was repeatedly exposed to dextran sodium sulfate (DSS) to stimulate colitis-induced colorectal cancer (CRC)[63]. Repair of DSS-induced damaged tissue was impaired in *p53*^{G515A} mice. Combined with enhanced NFκB activity and extended inflammation, this led to an increase in colorectal tumor incidence in mice[63]. In addition, *p53*^{G515A} mutant intestinal organoids derived from these mice showed increased TNFα and CXCL1 production when compared to *p53*$^{-/-}$ cells, which could be reverted by NFκB knockdown[63]. In line with these experimental findings, expression of mutant *TP53* correlated with NFκB expression in human CRC patients[63]. These findings show that this GOF mutant p53 induces aberrant NFκB interactions, leading to different inflammatory phenotypes than observed after loss of p53. Another mutant form of p53, p53^{R172H}, has been reported to elicit similar immune phenotypes as loss of p53. *Kras*^{G12D}*;p53*^{R172H} mutant mouse pancreatic tumors drive inflammatory responses via ROS and JAK2-STAT3 activation[64]. Here, both p53^{R172H} mutant and p53-defiencient tumors displayed similar STAT3-dependent immune evasion and accelerated tumor growth, which both could be reversed by pharmacological targeting of JAK-STAT signaling[64].

Figure 2.1: Cancer cell-intrinsic signaling pathways that shape the tumor immune landscape. **A.** The p53 pathway can modulate the immune microenvironment of the tumor by regulating NFκB signaling, that is generally activated by loss or loss-of-function (LOF) mutation of p53. This results in increased cytokine production by tumor cells and recruitment and activation of immune cells, such as macrophages. In addition, by activating ROS, mutant p53 can induce JAK-STAT signaling and thereby increase macrophage, neutrophil and CD4$^+$ T cell frequencies in the tumor, while concurrently reducing CD8$^+$ T cell levels[64]. **B.** Mutant KRAS can increase GM-CSF by cancer cells, and thereby promote neutrophil recruitment to the tumor[76]. **C.** Activated Notch signaling can signal to monocytes and macrophages by driving CCL2 and IL-1β expression. Notch also drives TGFβ receptor and uPA expression, of which the latter is involved in activating macrophage-derived TGFβ, thus inducing a growth promoting signaling loop[93]. Notch can also limit the anti-tumor immune response by inhibiting C/EBPβ and thereby limiting expression of IL-1, IL-6 and IL-8 [92]. **D.** Loss of tumor suppressor gene LKB1 can drive production of G-CSF, CXCL7 and IL-6 by the tumor, which promotes neutrophil recruitment, which can block anti-tumoral cytotoxic T cells[81]. **E.** ZBTB7a blocks CXCL5 production by binding its promoter, and loss of ZBTB7a therefore can lead to CXCL5-mediated neutrophils recruitment [83]. **F.** High mutational load in tumors can increase the number of neo-antigens and thus potentially increase neo-antigen-specific T cell responses. **G.** PTEN can negatively regulate NFκB signaling. Therefore, loss of PTEN increases NFκB-mediated expression of cytokines and growth factors that drive macrophage, neutrophil and Treg accumulation in the tumor[80]. **H.** MYC can regulate macrophage recruitment, which is promoted by p53 loss[69]. Additionally, by inducing CCL5 and IL-1β, MYC can promote mast cell recruitment and activation[67,68]. MYC can also induce CCL9 and IL-23 expression, the former of which induces macrophage recruitment, while the latter limits NK, T and B cell accumulation in the tumor[72]. MYC can also inhibit CD4$^+$ T cells and macrophages by regulating PD-L1 and CD47 expression on tumor cells[71]. Lastly, the anti-tumor NK- and CD8$^+$ T cell-response to MYC amplified tumors can be counteracted by additional loss of p53 in the tumor, while amplification of Bcl-2 promotes anti-tumor immunity[70]. **I.** SMAD4 can suppress YAP1 signaling, and loss of SMAD4 in tumors therefore drives YAP1-mediated CXCL5 production, which recruits immunosuppressive neutrophils[103]. **J.** PRKCI amplification can also induce YAP signaling. Activation of YAP1 here induces TNFα-mediated recruitment and activation of immunosuppressive neutrophils[102]. **K.** Activated Wnt signaling via β-catenin can limit the priming of CD8$^+$ T cells by suppression of CCL4 production, which would otherwise activate CD103$^+$ DCs[105].

These findings indicate that different mutations of p53 can shape the tumor microenvironment in a distinct manner. In future studies, it would be interesting to systematically dissect the differences between gain- and loss-of-function p53 mutations on NFκB interactions and the immune landscape of the tumor. Altogether, these studies demonstrate the profound role of p53-mediated regulation of key immune signaling pathways such as NFκB and STAT signaling, and its downstream effects on the tumor immune landscape.

MYC: a key controller of the immune microenvironment

The MYC oncogene is one of the most frequently amplified oncogenes in several tumor types, such as lymphoma, breast cancer and NSCLC[65]. As a transcription factor MYC regulates many essential processes in the cell. In addition, recent studies revealed that it also has a strong hold on the tumor immune landscape (**Fig. 2.1, Table 2.2**). Using the *RIP1-Tag2;TRE-Omomyc;CMV-rtTA* pancreatic ß-cell cancer mouse model, in which treatment with doxycyclin induces expression of a dominant negative MYC mutant, it was shown that inhibition of endogenous Myc in established islet tumors resulted in tumor regression, which was accompanied by a marked decrease in infiltrating macrophages and neutrophils[66]. This study illustrates that although MYC is not an oncogenic driver in this tumor model, its endogenous expression is crucial for tumor progression and has a profound effect on the inflammatory microenvironment. In another transgenic ß-cell cancer mouse model carrying a switchable form of the Myc oncoprotein in the pancreas, forced expression of Myc in ß-cells resulted in pancreatic cancer formation[67]. Importantly, Myc activation stimulated production of the potent pro-inflammatory cytokines CCL5 and IL-1β by β cells, which facilitated tumor angiogenesis and recruitment of pro-tumoral mast cells to the tumor[67,68]. These studies demonstrate that Myc can drive tumor progression at least in part through orchestrating pro-tumoral inflammatory conditions.

The effects of Myc signaling on the tumor microenvironment may not be limited to pancreatic cancer alone. In the *Eμ-tTA-TRE-Myc* mouse lymphoma model, inactivation of Myc in established tumors resulted in a marked decrease in intratumoral macrophages[69]. It would be of interest to assess whether the same MYC-controlled inflammatory mediators are involved in lymphoma and pancreatic cancer. Interestingly, upon additional loss of p19ARF, but not p53, MYC-dependent regulation of macrophage recruitment is not observed[69], suggesting that the ability of MYC to control recruitment of immune cells to tumors can be counteracted by other aberrantly expressed genes. This is also illustrated by the observation that the spontaneous anti-tumor T- and NK cell response in the *Eμ-MYC* lymphoma model could only be elicited when Bcl-2 was overexpressed, but not when p53 was deleted[70]. How p53 loss counteracts MYC activity in modulating the tumor microenvironment however remains a subject of future research.

MYC can also control the immune landscape of tumors by regulating expression of immune checkpoint molecules. In the *Eμ-tTA/tet-O-MYC* lymphoma model and cell lines with switchable *MYC* expression, MYC increased the expression of both PD-L1 and the "don't eat me" receptor CD47 on cancer cells by binding directly to their respective promoters[71]. Exogenous overexpression of PD-L1 and CD47 on cancer cells limited the $CD4^+$ T cell and macrophage recruitment to the tumor. Moreover, MYC inactivation down-regulated CD47 and PD-L1 expression and induced tumor regression, while exogenous overexpression of PD-L1 and CD47 in cancer cells enhanced disease progression[71]. Although not experimentally proven, this study suggests that MYC may facilitate tumor immune escape by induction of

immune checkpoints. Similarly, a MYC amplification-dependent T cell-poor environment has also been reported in human neuroblastomas, but in these tumors genomic amplification of N-MYC inversely correlated with *PD-L1* expression, possibly due to MYC-induced suppression of interferons and pro-inflammatory signaling pathways[50]. These studies show that MYC activation in tumors can control immune checkpoint molecules and T cell influx, but the underlying mechanisms may differ between tumor types.

Another mechanism by which MYC regulates the immune phenotype of tumors was recently demonstrated in the $Kras^{G12D}$-driven lung adenocarcinoma model. Here, conditional MYC amplification resulted in a rapid decrease of intratumoral B, T and NK cells, and an increase in macrophages[72]. Mechanistically, MYC amplification led to increased expression of IL-23 by cancer cells, which inhibited B, T and NK cell recruitment, and increased expression of CCL9, which recruited and activated macrophages in the tumor. These macrophages inhibited T cells, while also promoting angiogenesis.Interestingly, these tumors rapidly acquired dependency on MYC amplification, and MYC de-activation resulted in tumor regression in an NK cell-dependent fashion[72]. These findings suggest that targeting MYC in tumors would be an attractive therapeutic strategy to unleash anti-tumor immunity. While MYC is as of yet not directly targetable, indirect therapeutic strategies emerge. One such strategy targets the epigenetic modulators DNA methyl transferases (DNMTs) and histone deacetylases (HDACs). Combined treatment of NSCLC mouse models with DNMT and HDAC inhibitors reduced MYC expression, increased CCL5 levels, decreased macrophage influx and increased cytotoxic T cell influx and inhibited tumor growth[73].This study demonstrates that indirect targeting of MYC might prove therapeutically beneficial by limiting tumor growth and reversing immune evasion. However, this study did not formally exclude a direct effect of the epigenetic modulators on the immune system. These studies show that in addition to the key role MYC has in tumor cell-intrinsic processes, this transcription factor can exert a wide variety of functions to modulate both the innate and the adaptive immune landscape of several tumor types. While MYC is not directly targetable, insights into these mechanisms open up new ways to target MYC-regulated signaling.

Other genetic determinants of the tumor immune landscape

The effect of oncogenes and TSGs on the tumor immune landscape is not just limited to the abovementioned genes and pathways; several other genetic events and downstream immune effects have been described (**Fig. 2.1, Table 2.2**). One example is the impact of the Ras oncogene on tumor-associated myeloid cells. Mutated Ras strongly induces expression of IL-6 and IL-8 in *in vitro* models[74,75]. These Ras-controlled cytokines have been reported to facilitate myeloid cell infiltration and tumor progression[74,75]. Furthermore, $Kras^{G12D}$-induced changes in cytokine expression resulted in accumulation of $CD11b^{+}Gr1^{+}$ immunosuppressive cells in a variety of tumor models, including pancreatic and lung cancer[76-78]. Ablation of one of the $Kras^{G12D}$-induced cytokines, GM-CSF, in tumor cells impaired immunosuppressive cells from entering pancreatic tumors and consequently resulted in an increase in $CD8^{+}$ T cells[76]. These studies demonstrate the causal relationship between Ras oncogenic signaling pathways, immune-stimulatory transcription programs and immune landscape.

Table 2.2. Genetic aberrations influencing the immune landscape of tumors. Listed here are the cancer cell-intrinsic genetic aberrations that result in a change in innate and adaptive immune contexture as demonstrated in genetically engineered mouse models. Abbreviations. NA: Not assessed. SASP.: Senescence-associated secretory phenotype. CRC: Colorectal cancer. NSCLC: Non-small cell lung cancer. PDAC: Pancreatic ductal adenocarcinoma. T-ALL: T cell acute lymphoblastic leukemia. SCLC: small cell lung carcinoma.

Gene	Genetic aberration	Consequence for intratumoral immune cells	Signaling involved	Tumor type	Tumor model	Reference(s)
AKT	Loss	Macrophages ↓	AKT deletion decreases tumorigenesis by reducing pro-tumorigenic Wnt-producing macrophages in the tumor	Liver cancer	*Alb-cre;Pten$^{F/F}$* and *Alb-cre;Pten$^{F/F}$;Akt2$^{F/F}$*	122
ATR	Deletion	Macrophages ↑	NA	Melanoma	*Tyr::ER^{T2};Braf$^{V600E/+}$;Pten$^{F/F}$* and *Tyr::ER^{T2};Braf$^{V600E/+}$;Pten$^{F/F}$;ATR$^{F/F}$*	99
		B cells ↑, CD8$^+$ T cells ↓				
β-catenin	Amplification	CD8$^+$ T cells ↓	Active b-catenin inhibits CCL4, thus inhibiting CD8$^+$ T cell priming by CD103$^+$ DCs.	Melanoma	*Tyr:cre-ER;Braf$^{LSL-V600E/+}$;Pten$^{F/F}$* and *Tyr:cre-ER;Braf$^{LSL-V600E/+}$;Pten$^{F/F}$;LSL-CAT-STA*	105
CKIα	Loss	Macrophages ↓	Loss of CKIa triggers an inflammatory SASP. Subsequent loss of p53 or p21 leads to inflammation-accelerated tumorigenesis.	CRC	*Villin-cre;CKIa$^{F/F}$*, *Villin-cre;CKIa$^{F/F}$;p21$^{-/-}$* and *Villin-cre;CKIa$^{F/F}$;Trp53$^{F/F}$*	88
EGFR	Mutation	Macrophages, neutrophils ↑	NA	NSCLC	*Ccsp-rtTA;TetO-Egfr^{L858R}*	82
		CD8$^+$ T cells ↓				
		CD8$^+$ T cells ↓	EGFR pathway activates PDL1 expression in bronchial epithelial cells		*Ccsp-rtTA;TetO-EGFR^{T790M}*, *EGFR$^{T790M/L858R}$* and *EGFR$^{exon\ 19\ del/T790M}$*	49
FAK	Amplification	Macrophages, neutrophils, monocytes ↑	Potentially via STAT3 signaling.	PDAC	*p48-Cre;Kras$^{LSL-G12D/+}$*	104
		CD3$^+$ T cells ↓, Tregs ↑	Potentially due to immunosuppressive myeloid cells			
FGFR	Activation	Neutrophils ↑	FGFR drives mTOR signaling, which causes increase in G-CSF production, driving neutrophil expansion, thus promoting tumor progression	Breast cancer	*MMTV-Wnt1*, *MMTV-Wnt1-iFGFR* and *MMTV-cre;Trp53$^{F/F}$;Pten$^{F/F}$*	123
IFNAR1	Mutation	NK ↓, neutrophils ↑	Inactivating mutant of IFNAR1 promotes the establishment of an immunosuppressive microenvironment and tumor progression	CRC	AOM-DSS induced	124
		CD8$^+$ cells ↓				

Table 2.2. Genetic aberrations influencing the immune landscape of tumors (continued).

Gene	Genetic aberration	Consequence for intratumoral immune cells	Signaling involved	Tumor type	Tumor model	Reference(s)
KRAS	Mutation	Myeloid cells ↑	NA	NSCLC	*Kras*$^{LSL\text{-}G12D/+}$ and *Kras*$^{LSL\text{-}G12D/+}$; *Trp53*$^{F/F}$	82
		T cells (CD8$^+$, Treg, γδ T cells) ↑				
LKB1	Loss	Neutrophils ↑, Macrophages, CD4$^+$, CD8$^+$ T cells ↓	Loss of Lkb1 leads to an increase in CXCL7, G-CSF and IL-6, which drive neutrophil increase. Neutrophils decrease IFNg$^+$ T cells in the tumor.	NSCLC	*Kras*$^{LSL\text{-}G12D/+}$ and *Kras*$^{LSL\text{-}G12D/+}$; *Lkb1*$^{F/F}$	81
mTOR	Amplification	NK cells, macrophages ↑	mTOR regulates IL-1a levels, and IL-1a activates NFkB, thus driving SASP and immune cell recruitment	Liver cancer	Hydrodynamic tail-vein injection of *Nras*^{G12V}	90,91
		T, B cells ↑	mTOR activates tumor suppressive SASP			
MYC	Loss	Macrophages, neutrophils ↓	NA	Pancreatic cancer	*RIP1-Tag2* and *TRE-Omomyc; CMVrtTA; RIP1-Tag2*	66
	Amplification	Mast cells ↑	MYC activation drives IL-1b and CCL5 expression, leading to an influx of mast cells in the pancreatic tumor	Pancreatic cancer	*pIns-mycER*^{TAM}*;RIP7-bcl-xL*	67,68
		CD4$^+$ T cells ↓	Regulates expression of CD47 and PDL1	T-ALL	MYC T-ALL s.c. transplanted cell line, *Eμ-tTA/tet-O-MYC, LAP-tTA/tet-O-MYC*	71
		Macrophages ↑ NK ↓	MYC drives expression of CCL9, which recruits macrophages, and IL-23, which limits NK recruitment	NSCLC	*Kras*$^{LSL\text{-}G12D}$*; Rosa26-LSL-MycER*^{T2}	72
		T, B cells ↓	MYC drives expression of IL-23, which excludes T and B cells from the tumor			
NOTCH	Amplification	Macrophages ↑	NOTCH activates CCL2 and IL-1b production by tumor cells thus increasing pro-tumoral monocytes and macrophages	Breast cancer	4T1, MDA-MB-231 cell lines and *RBPJκ*^{IND}*-MMTV;MMTV-PyMT*	93
		T cells ↓	NOTCH represses CEBP/b leading to impaired clearance of senescent cells and subsequent liver tumor development	Liver cancer	Hydrodynamic tail-vein injection of *Nras*^{G12V}	92
NRAS	Mutation	Neutrophils, monocytes, NK cells, macrophages, DCs ↑	NRAS mutation induces SASP, thus recruiting immune cells and CD4$^+$ T cell-mediated clearance of tumor cells.	Liver cancer	Hydrodynamic tail-vein injection of *Nras*^{G12V} and *Nras*$^{G12V/D38A}$	125
		CD4$^+$ T cells ↑	NRAS-induced senescent cells are cleared by CD4$^+$ T cells			

Table 2.2. Genetic aberrations influencing the immune landscape of tumors (continued).

Gene	Genetic aberration	Consequence for intratumoral immune cells	Signaling involved	Tumor type	*Tumor model*	Reference(s)
p53	Mutation	Myeloid cells ↑	Mutant p53 activates NFkB and thus drives cytokine production and inflammation-associated tumor progression	CRC	DSS-induced	63
	Loss	Neutrophils, macrophages ↑	Potentially via dysregulation of NFkB	Lung cancer	*Kras*$^{LSL-G12D/+}$ and *Kras*$^{LSL-G12D/+}$; *Trp53*$^{F/F}$	56
		Macrophages ↑	Loss of p53 leads to an impaired intestinal epithelial barrier, thus triggering intestinal microflora-mediated immune activation via NFkB.	CRC	*Villin-creER*^{T2}; *Trp53*$^{F/F}$ and AOM-induced	61
		Macrophages, monocytes, neutrophils ↑	STAT3-mediated establishment of an immunosuppressive microenvironment	PDAC	*Ptfa1-cre;* *Kras*$^{LSL-G12D/+}$, *Ptfa1-cre;* *Kras*$^{LSL-G12D/+}$;*p53*$^{F/F}$ and *Ptfa1-cre;* *Kras*$^{LSL-G12D/+}$; *p53*$^{R172H/+}$	64
		Monocytes ↑	p53 transcriptionally regulates CXCL17, and loss of p53 leads to an increase of CXCL17, thus recruiting monocytes to the tumor	Prostate cancer	*Pb-cre;Pten* $^{F/F}$; *Trp53*$^{F/F}$	83
PRKCι	Amplification	NK cells ↓, CD11b^{+}Gr1^{+} cells ↑	PRKCI activates YAP1, inducing TNFa to promote an immunosuppressive microenvironment	High-grade serous ovarian carcinoma	*Pax8-cre;* *tetO*$^{LSL-PRKCI}$;*Pten*$^{F/F}$; *Trp53*$^{F/F}$ with inducible loss of PRKCI and cell lines derived from these tumors	102
		CD8^{+} T cells↓	PRKCI amplification induces immunosuppressive neutrophils, thus reducing CD8^{+} T cells			
PTEN	Loss	CD11b^{+}Gr1^{+} cells ↑	PTEN loss activates NFkB and thereby expression of CXCL1, G-CSF, IL-23	PDAC	*p48-Cre;* *Kras*$^{LSL-G12D}$;*Pten*$^{F/+}$	80
		CD8^{+} T cells ↓	Loss of PTEN promotes resistance to T cell killing by inhibiting autophagy	Melanoma	Cell line inoculation models and *Tyr:CreER;Pten*$^{F/F}$; *Braf* $^{V600E/+}$	107
RAS	Mutation	CD11b^{+}Gr1^{+} cells ↑	Via GM-CSF production by tumor cells	PDAC	*Kras*^{G12D} inoculation model	74,76
RB	Loss	Macrophages ↓	NA	SCLC	*Rb1*$^{F/F}$;*Trp53*$^{F/F}$	82
SMAD4	Loss	Neutrophils ↑	SMAD4 loss increases YAP1-mediated CXCL5 expression, thus driving immunosuppressive neutrophils.	Prostate cancer	*Pb-cre;* *Pten*$^{F/F}$;*Trp53*$^{F/F}$	103
		CD8^{+} T cells, Tregs ↓	PRKCI amplification induces immunosuppressive neutrophils, thus reducing CD8^{+} T cells and Tregs			
ZBTB7a	Loss	Neutrophils ↑	p53 transcriptionally regulates SOX-9, and loss of p53 leads to an increase of SOX-9, which in turn activates CXCL5, thus recruiting neutrophils to the tumor	Prostate cancer	*Pb-cre;* *Pten* $^{F/F}$;*Trp53*$^{F/F}$	83

Another study revealed a role for adherence junction protein α-catenin in inflammatory signaling. In the *K14-Cre;α-catenin$^{F/F}$* mouse model for skin squamous cell carcinoma (SCC), loss of α-catenin activates NFκB and its downstream inflammatory target genes, such as IL-1β and IL-6, and stimulates SCC, thus again linking tumor-initiating oncogenic events with NFκB-mediated immune signaling[79]. Likewise, by comparing the *Pdx1-cre; Kras$^{LSL-G12D}$* and the *Pdx1-cre;Kras$^{LSL-G12D}$;Pten$^{+/F}$* mouse models for pancreatic cancer, it was demonstrated that loss of *Pten* resulted in increased activation of the NFκB pathway, driving expression of several immune regulators by cancer cells, such as G-CSF, IL-23 and CXCL1 [80]. *Pten* loss and the downstream NFκB activation not only accelerated tumor progression, but also influenced the frequency of intratumoral neutrophils, monocytes and Tregs[80]. Another study showed a profound role for the *STK11/LKB1* tumor suppressor in NSCLC. Comparing *Kras$^{G12D/+}$* with *Kras$^{G12D/+}$;Lbk1$^{-/-}$* mice, it was found that loss of *Lkb1* resulted in increased IL-6 production, which resulted in higher intratumoral and systemic immunosuppressive neutrophil levels[81]. Indeed, blockade of IL-6 resulted in increased levels cytotoxic CD8$^+$ T cells and tumor control[81]. Although not all of these studies elucidated the functional consequence of the altered immune landscape on tumor growth, they demonstrate that a wide variety of cancer-driving mutations can dictate the composition of the tumor microenvironment.

Collectively, studies pertaining to cancer cell intrinsic pathways and immune contexture are gaining ground and have identified various cancer-driving genes that orchestrate diverse immune landscapes in the tumor. Thus far, many of these studies have been relatively biased and focused on a single genetic pathway in a single mouse tumor model. A more systematic assessment of immune cell populations in relation to tumor genotypes was recently performed in two studies. One compared four independent lung cancer GEMMs: *Ccsp-rtTA;TetO-Egfr^{L858R}*, *Rb1$^{F/F}$;Trp53$^{F/F}$*, *Kras$^{LSL-G12D/+}$* and *Kras$^{LSL-G12D/+;}$Trp53$^{F/F}$* models, representing molecularly distinct human SCLC and NSCLC subtypes[82]. This approach revealed key differences in immune cell content between the different tumor genotypes, such as that *Egfr^{L858R}*-driven tumors showed lower frequencies and activation of CD8$^+$ T cells compared to *Kras*-driven tumors, whereas NK cells in *Kras*-driven tumors, but not EGFR mutants, show downregulation of activation markers[82]. A second study compared the *Pb-cre;Pten$^{F/F}$;Zbtb7a$^{F/F}$, Pb-cre;Pten$^{F/F}$;Trp53$^{F/F}$* and *Pb-cre;Pten$^{F/F}$; Pml$^{F/F}$* prostate cancer models and observed profound differences in composition of the tumor microenvironment[83]. Mechanistic studies revealed distinct chemokine production by tumors controlled by loss of Zbtb7a, p53 or Pml and blockade of the respective signaling pathways impaired innate immune cell recruitment and tumor progression. These studies demonstrate the powerful potential of GEMMs in identifying the complex mechanisms that control the tumor microenvironment and potential for immunomodulatory therapeutic intervention based on genetic aberrations in the tumor. With the rapid developments in mouse model-generating techniques[84], future systematic approaches in GEMMs may increasingly reveal causal genotype-immunophenotype relationships, and its impact on tumor progression.

The role of oncogene-induced senescence in promoting an inflammatory tumor microenvironment

A cancer cell-intrinsic pathway in which many of the above-mentioned cancer-driving genes are involved and that strongly influences the intratumoral immune landscape is cellular senescence. In a process called oncogene-induced senescence (OIS), precancerous cells undergo cell cycle arrest upon activation of oncogenic signaling. Cellular senescence is a

physiological program that can be activated in response to cellular stress and aging, leading to an essentially irreversible cell proliferation arrest[85]. Senescent cells can persist and actively secrete cytokines and other inflammatory and growth-promoting factors, a process called the senescence-associated secretory phenotype (SASP)[86]. Through their SASP, senescent cells can exert a significant, and sometimes opposing, impact on the immune landscape of the tumor. SASP can lead to immune-mediated clearance of pre-malignant cells, or via stimulation of chronic inflammation promote tumor progression. Below we discuss how oncogenes and TSGs, via SASP activation, shape the inflammatory microenvironment.

Several oncogenes and TSGs have been linked with SASP activation (**Fig. 2.2**). The p53 pathway plays an important role in the induction of OIS. This was demonstrated by the induction of senescence and tumor clearance upon doxycyclin-mediated activation of p53 in a *Hras^{G12V};TRE.shp53* inoculation model for liver cancer[87]. Activation of p53 did not lead to tumor cell death in a cell-autonomous manner, but rather neutrophils, macrophages and NK cells were recruited to these tumors by activated SASP and removed the senescent cells[87]. Indeed, maintenance of WT p53 was a prerequisite of senescence induction, as also observed in other tumor models[63,88]. Since NFκB is a key transcription factor in SASP activation[89], the regulation of NFκB by the p53 pathway might play an important role in SASP regulation. In colorectal tumor models, WNT signaling can also regulate SASP induction. *Villin-creER^{T2};CKIα$^{F/F}$* mice, which display hyper-activated WNT signaling due to loss of *CKIα*, exhibit growth arrest of colorectal tumors and induction of senescence, paired with an inflammatory response[88]. SASP is maintained upon additional p53 deletion in this model, however, it dissociates from growth arrest while the inflammatory response continues, resulting in inflammation-accelerated tumorigenesis[88]. These findings illustrate that depending on the genetic makeup of cancer cells, the senescence-associated inflammatory response can result in two opposing outcomes: tumor inhibition or tumor promotion. In addition to p53 and WNT, mTOR signaling was shown to induce SASP in CRC and prostate cancer cells *in vitro*[90]. mTOR inhibition by rapamycin decreased mTOR-induced SASP and decreased influx of macrophages, T, B and NK cells into inoculated Nras^{G12V} mutant liver tumors[91]. These studies suggest that targeted therapies, such as rapamycin, may reduce tumor-induced inflammation, but potentially also reduce senescent tumor cell clearance by infiltrating immune cells, thus demonstrating the complexity of targeting SASP. Nonetheless, these studies reveal the essential role of oncogenes and TSGs in SASP induction and the potential of targeting these genes to revert tumor-promoting SASP.

The composition of SASP mediators secreted by senescence cells is dynamic and experimental evidence points towards NOTCH1 as one of the master regulators controlling this SASP diversity. In Nras^{G12V} mutant tumor models, Nras^{G12V}-induced senescence was accompanied by fluctuations in endogenous Notch expression levels[92]. Ectopic expression of active Notch in an Nras^{G12V}-dependent oncogene-induced senescence liver model increased cancer progression in a non-cell autonomous fashion [92]. In this model, Notch levels determined the composition of the SASP and subsequent immune function. Notch inhibited lymphocyte-mediated clearance of senescent cells through repression of C/EBPβ. Reversely, inhibition of Notch during senescence led to an increase of lymphocyte-mediated senescent cell clearance[92]. This Notch-dependent cytokine production and shaping of the immune phenotype of tumors was also demonstrated in breast cancer, where tumor-intrinsic Notch signaling increased monocyte and macrophage accumulation by increasing expression of IL-1β and CCL2 [93]. These studies demonstrate that immune cell influx can be strongly

influenced by SASP, but also that the activity of cancer cell-intrinsic genes play important roles in determining the spectrum of inflammatory mediators produced within the tumor. Indeed, in the *Ptf1a-cre;Kras*$^{LSL\text{-}G12D/+}$ mouse model for pancreatic cancer, genetic deletion of *RelA*, the gene that encodes the NFκB subunit p65, abrogated senescence and SASP, thus enhancing progression of pancreatic tumors[94]. While reducing SASP, *RelA* deletion led to a marked increase in immunosuppressive cells and decreased T cell activation in the pancreata of these mice[94]. Therefore, in these tumors, the cancer–immune cell crosstalk is not limited to SASP.

The infiltrating immune cells can also impact senescence itself. In *Pten*-induced senescent prostate tumors, CD11b^{+}Gr-1^{+} cells can actively counteract SASP by producing IL-1 receptor antagonist[95]. Additionally, senescence programs in tumor-associated stromal cells also impact tumorigenesis through modulation of immune responses. In a carbon tetrachloride (CCl_4)-induced liver fibrosis model, p53 activity in hepatic stellate cells (HSCs) limits fibrosis and cirrhosis, and reduced liver tumorigenesis in mice treated with CCl_4 and diethylnitrosamine (DEN)[96]. Here, wild-type p53 cooperated with NFκB to induce senescence and SASP in HSCs, which induced a tumor-inhibiting phenotype in macrophages. Loss of p53 in stromal HSCs changed their secretome, induced the polarization of macrophages towards a tumor-promoting phenotype and accelerated inflammation-induced hepatocellular carcinoma[96], indicating that also stromal cell intrinsic p53 controls tumorigenesis via modulation of the immune system.

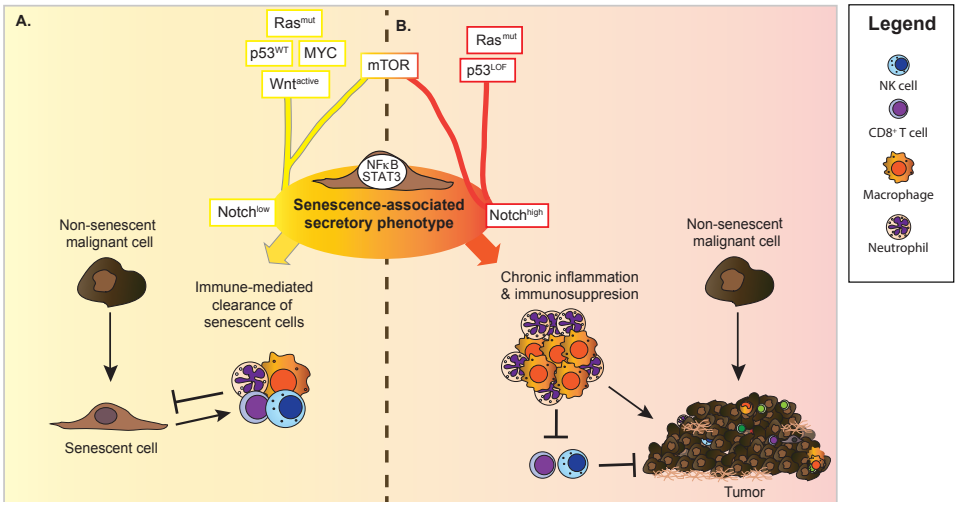


Figure 2.2: Relationship between genetic events in cancer cells, the dynamic aspects of SASP and the immune system. A. OIS in combination with WT p53, activated MYC, low Notch signaling, active Wnt signaling, activated RAS, or active mTOR signaling induces a senescence-associated secretory phenotype (SASP) that leads to the recruitment and activation of macrophages, neutrophils, NK cells and CD8^{+} T cells that clear senescent cells and thus limit tumorigenesis. **B.** Loss or loss-of-function mutations in p53, or activated RAS, Notch or mTOR signaling can lead to an alternative SASP that also attributes to a chronic inflammatory state that establishes an immunosuppressive tumor microenvironment. Immunosuppressive macrophages and neutrophils limit NK and CD8^{+} T cell-mediated anti-tumor response and thus promote tumorigenesis. NFκB and STAT3 signaling in senescent cells is key in SASP induction.

Collectively, depending on the tumor type and oncogenic wiring, the activated SASP-related genes and downstream inflammatory profile may differ, resulting in a wide spectrum of immune responses that range from tumor-promoting chronic inflammatory responses to immune-mediated clearance of cancer cells (**Fig. 2.2**). Deeper mechanistic insights into the causal relationship between genetic events in cancer cells and the dynamic aspects of SASP may open new avenues for therapeutic intervention. Indeed, this is exemplified by a study showing that the efficacy of docetaxel could be enhanced by pharmacologically targeting *Pten*-loss-induced SASP in a transgenic prostate tumor model[97]. Important to note however, is that senescent cells are not the only cells actively secreting inflammatory mediators in the tumor, and the cytokine milieu and its net effect on the immune landscape is not only determined by SASP. Therefore, it is of key importance to delineate how the tumor-promoting aspects of SASP can be reverted, while enhancing the tumor-limiting aspects.

Mechanisms of cancer cell-intrinsic regulation of parameters of the cancer immunity cycle and immune checkpoint blockade response

As discussed above, the mutational load of tumors is one of the determinants linked with responsiveness to immune checkpoint inhibition. The expectation is that many other parameters, including the activation of certain oncogenes or inactivation of TSGs, are associated with therapeutic benefit as well, and that they may differ per tumor (sub)type. As of yet, preclinical studies focused on unlocking the relationship between tumor genetics and response to immunotherapy are still relatively limited, however, the concept is emerging that genetic events in cancer cells dictate various aspects of the tumor-immunity cycle[98], such as activation of immunosuppressive myeloid cells, induction of immune checkpoint molecule expression, regulation of DC activation and T cell priming, and induction of tumor resistance to T cell attack.

One such genetic event is mutation in the serine/threonine-protein kinase ATR. ATR is a DNA damage sensor and is frequently mutated in melanoma. It has been reported to influence important parameters of immunotherapy response, such as intratumoral T cell influx and expression of immune checkpoints. Transgenic expression of an ATR LOF-mutant in the *Tyr::CreERT2; Braf^{V600E};Pten$^{F/F}$* model for melanoma diminished T cell influx in the tumor, while increasing B cells and macrophages[99]. This was associated with an increase in expression of *Arginase 1*, *CD206* and *PD-L1* in the tumor, suggesting a more T cell suppressed environment. Cyclin-dependent kinases (CDKs) – essential regulators of the cell cycle – have also been shown to be involved in immune checkpoint regulation. In medulloblastoma (MB) cell line inoculation models, the anti-tumor function of CD4$^+$ T cells depends on disruption of CDK5 in MB cells[100]. In this model, CDK5 is required for PD-L1 expression by MB cells, as CDK5 is a repressor of IRF2 and IRF2BP2, that both regulate IFN-γ-mediated PD-L1 expression[100]. Additionally, it was recently shown that the activating Ras^{G12V} mutation can cause stabilization of *PD-L1* mRNA via activation of MEK[101]. However, the functional relevance of these changes for immunotherapy and disease progression in relation to ATR, CDK5 and RAS remains unaddressed in these studies.

Another mechanism by which tumor cells may regulate immunotherapy response is via establishment of an immunosuppressive microenvironment. Overexpression of PRCKI, a protein kinase, is frequently observed in a variety of cancer types, including high-grade serous ovarian carcinoma[102]. Upon conditional overexpression of PRKCI in the *Pax8-rtta;TetO-Cre;Trp53$^{F/F}$;Pten$^{F/F}$* mouse model for ovarian cancer, tumors up-regulate TNFα, as a result of

which tumors were strongly infiltrated by immunosuppressive neutrophils, thus decreasing $CD8^+$ T cell influx[102]. This TNFα-mediated neutrophil recruitment was dependent on PRKCI-induced YAP1 – a key transcriptional regulator and oncogene – signaling in cancer cells[102]. Likewise, by comparing *Pb-cre4;Pten$^{F/F}$* with *Pb-cre4;Pten$^{F/F}$;Smad4$^{F/F}$* prostate cancer mouse models, a strong YAP1-dependent influx of neutrophils was observed upon cancer cell-intrinsic Smad4 loss[103]. Here, Smad4 loss caused YAP1-mediated upregulation of CXCL5 in tumor cells. This in turn recruited $CXCR2^+$ neutrophils, which suppressed the $CD8^+$ T cell response to the tumor[103]. These studies show that Smad4 and PRCKI both function as inducers of immunosuppression via cancer cell-intrinsic YAP signaling, and that YAP inhibitors – which are currently in preclinical development – may prove beneficial to alleviate T cell suppression. Collectively, these studies show that oncogenic pathway activation can significantly impact on parameters of the cancer-immunity cycle. However, the functional consequences of these genetic changes on immunotherapy response have not been addressed in these studies. Focal Adhesion Kinase (FAK) activity in cancer cells has also been identified as an important regulator of immunosuppression in the tumor microenvironment, and its impact on immunotherapy efficacy has been addressed experimentally. FAK amplification was observed in the *p48-Cre;Kras$^{LSL-G12D}$;Trp53$^{F/+}$* model for PDAC, and therapeutic targeting of FAK improved survival by alleviating the immunosuppressive microenvironment, mainly by reducing macrophages, monocytes and neutrophils in the tumor[104]. This held true for cancer cell-specific ablation of FAK, indicating that immune cell changes occur via FAK targeting in cancer cells. Importantly, inhibition of FAK synergized with anti-CTLA-4/anti-PD-1 combination immunotherapy[104], indicating that interference with this cancer cell-intrinsic signaling pathway renders tumors sensitive to immunotherapy.

DC activation and T cell priming can also be influenced by cancer cell-intrinsic signaling pathways. Using the *Braf^{V600E};Pten$^{-/-}$;CAT-STA* mouse model for melanoma, which expresses constitutively active ß-catenin, it was revealed that ß-catenin signaling prevented expression of CCL4 by cancer cells, resulting in suppression of recruitment of $CD103^+$ DCs and impaired priming and intratumoral accumulation of T cells[105]. As a consequence, ß-catenin-active tumors failed to respond to anti-CTLA-4/anti-PD-1 treatment. In line with these data, active WNT/ß-catenin signaling in human metastatic melanomas correlated with absence of a T cell gene expression signature[105]. This study highlights the importance of cancer cell-intrinsic WNT/ß-catenin signaling in immune evasion of tumors, and suggests that targeting the WNT pathway may improve the therapeutic benefit of immune checkpoint inhibition in tumors with active β-catenin signaling.

Some oncogenes and TSGs have been demonstrated to regulate immune checkpoint molecule expression in a cell-autonomous fashion, and thus influence response to immunotherapy. In EGFR-driven lung cancer mouse models, EGFR mutation caused rapid induction of an immunosuppressive tumor microenvironment[49]. The EGFR mutant lung tumors displayed increased expression of immune checkpoint molecules such as PD-1 and PD-L1, which led to an increased sensitivity to anti-PD-1 monotherapy in these tumor-bearing mice. In line with these pre-clinical findings, EGFR pathway activating mutations in human lung tumors, and not the other prevalent driver mutation $KRAS^{G12V}$, correlated with PD-L1 expression[49]. Intriguingly, another study reported KRAS mutant lung tumors in patients treated with anti-PD-1 to have higher PD-L1 levels relative to EGFR mutated tumors[106], potentially mediated by KRAS-induced stabilization of PD-L1 [101]. The different levels of PD-L1 regulation by mutated oncogenes and the underlying mechanisms will therefore be an

important topic of future research.

Similarly, PTEN status is implicated in immunotherapy response due to its ability to render cancer cells resistant to T cell attack. In a cohort of melanoma patients, PTEN loss correlated with low TIL influx and poor response to anti-PD-1 therapy[107]. Using xenograft mouse models for melanoma, it was shown that PTEN loss in cancer cells reduced T cell influx, and resulted in reduced autophagy, leading to resistance to T cell-mediated killing[107]. Treating PTEN-null tumors with an PI3Kβ inhibitor, thus reducing the dysregulated AKT activity in these tumors, improved response to anti-PD-1 therapy, highlighting a potential therapeutic approach for PTEN-null melanoma in controlling resistance to anti-PD-1 therapy.

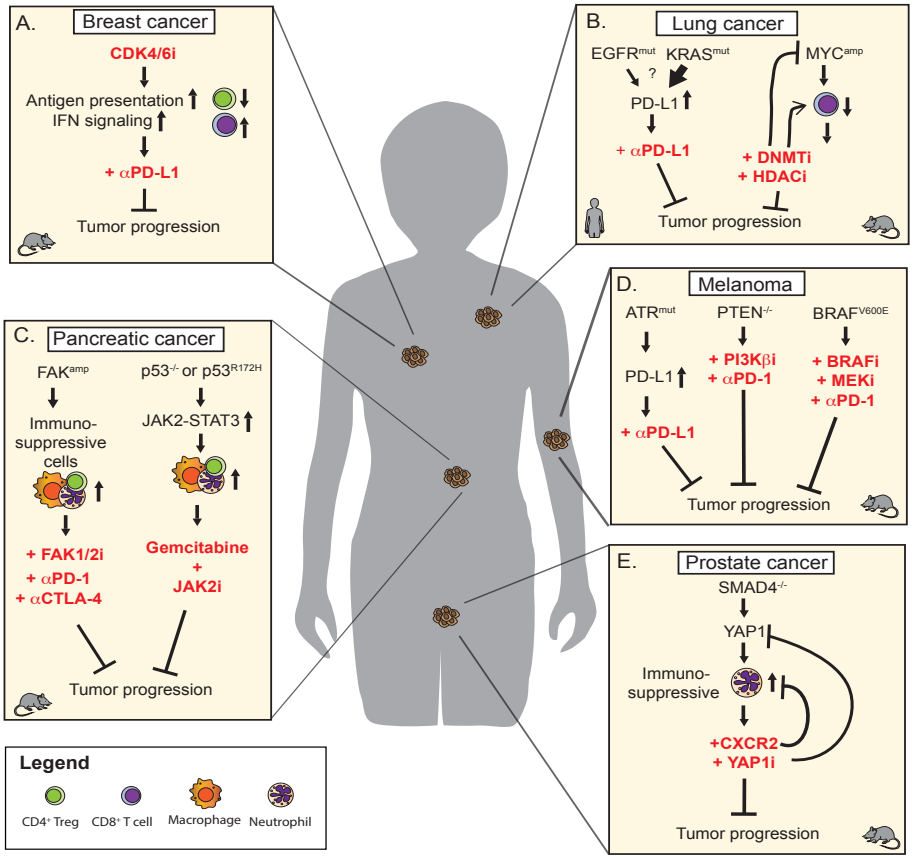
Altogether, these studies show that aberrant signaling pathways in cancer cells can impact the anti-cancer immune response and the response to immune checkpoint inhibition (**Fig. 2.3**). One aspect that needs to be taken into account when using GEMMs to model human cancers with high mutational load, is that the mutational load in transgenic mice may not correspond to that of the human tumors, due to the strong driver mutations engineered in these mice. This could be overcome by for example exposing early melanoma lesions to UV irradiation, or early lung lesions to carcinogens. The drawback however, is that this may not result in clonal antigens and the mutational spectrum may be highly variable from one mouse to the next. Alternatively, transgenic models that are prone to generate high mutational load tumors can be used, such as those with mutations in DNA repair machinery, or mutations can be engineered in a tissue-specific manner. This would allow for physiological modelling and therefore correct assessment of pre-clinical immunotherapeutic strategies in an immunocompetent setting.

Targeting genetic pathways to unleash anti-tumor immunity

One major theme that emerges from the aforementioned studies is that many targeted therapies, specific for hyperactive signaling pathways, are likely to also exert a major impact on the immune contexture of tumors. Most targeted drugs initially induce very strong anti-cancer effects in patients, however, the rate of durable clinical responses is disappointingly

Figure 2.3: How to exploit the genetic makeup of individual tumors to allow for patient-specific immune-based therapeutic interventions. Maximizing therapeutic efficacy by rational selection of targeted drugs and immunomodulatory compounds based on the genetics of the tumor. Examples depicted here are based on pre-clinical intervention studies, with therapeutic modalities highlighted in red. For every example a mouse or human symbol is used to depict what is based on clinical or pre-clinical evidence. **A.** In breast cancer, CDK4/6 inhibition increases antigen presentation, interferon signaling and $CD8^+$ T cell levels, while decreasing Tregs in the tumor. Combined with anti-PD-L1 treatment, this leads to a marked tumor regression[113]. **B.** In EGFR mutant lung cancer, PD-L1 has been described to be up-regulated, increasing the sensitivity to anti-PD-L1 therapy[49]. KRAS mutation in lung cancer can also drive PD-L1 expression, to a higher extent than EGFR mutation[106]. In MYC-driven lung tumors, combined inhibitors against HDAC and DNMT both target MYC and $CD8^+$ T cells, thus limiting tumor growth[73]. **C.** Pancreatic tumors with FAK amplification show an accumulation of immunosuppressive cells in the tumor. FAK1/2 inhibitors alleviate this, and combined with anti-PD-1 and anti-CTLA-4 treatment limit tumor progression[104]. Pancreatic tumors with p53 loss or mutation establish an immunosuppressive microenvironment by JAK-STAT signaling. Targeting JAK2 in combination with gemcitabine reduces tumor burden[64]. **D.** In melanoma, ATR loss-of-function mutation increases PD-L1 and thereby potentially sensitizes these tumors to anti-PD-L1 treatment. In PTEN-null melanomas, the resulting activated AKT signaling can be reduced by PI3Kβ inhibitors, which in combination with anti-PD-1 limits tumor growth[107]. Combining MEK and BRAF inhibitors in $BRAF^{V600E}$ mutant melanoma also synergize with anti-PD-1 treatment[112]. **E.** In prostate tumors with loss of SMAD4, YAP1-mediated immunosuppressive neutrophil recruitment can be counteracted by YAP1 inhibitors or anti-CXCR2 treatment[103].

low[108]. Given the previously unrecognized impact of these targeted drugs on the immune landscape of tumors, the question arises whether we can rationally induce a favorable immune environment in tumors or even sensitize tumors to immunomodulatory drugs by selective usage of targeted therapy. In this regard, we can learn from the growing number of pre-clinical studies that have addressed the impact of targeted drugs on the immune microenvironment of tumors and their response to immunotherapy. For example, as described above, BRAF-mutant thyroid tumors are characterized by infiltration of immunosuppressive cells[32], raising the question whether inhibition of mutant BRAF in thyroid cancer would induce a more favorable immune contexture. Indeed, combined targeting of $BRAF^{V600E}$ and SRC increased influx of $CD8^+$ T cells, B cells and macrophages and reduced tumor growth in an orthotopic inoculation model for anaplastic thyroid cancer[109]. Also in patients with $BRAF^{V600E}$ mutated metastatic melanoma, BRAF inhibition with vemurafenib enhanced melanoma antigen presentation by cancer cells, increased cytotoxic T cell influx, and decreased immunosuppression[110]. This is in line with findings in $BRAF^{V600E}$ melanoma mouse models in which BRAF inhibition improved adoptive T cell therapy[111] and BRAF inhibition combined with MEK inhibition synergized with anti-PD-1 treatment[112]. These studies indicate that therapeutic targeting of cancer cell-intrinsic oncogenic driver mutations can be exploited to induce a favorable immune environment, and thus sensitize tumors to cancer immunotherapy.



Other targeted therapies have also been reported to exert strong effects on the cancer-immune cell crosstalk. For example, CDK4/6 inhibitors were originally designed to selectively inhibit cell cycle progression, but emerging experimental evidence reveals that part of the therapeutic benefit of these inhibitors lies in their anti-tumor immunity promoting capacity. In the *MMTV-rtTA/tetO-HER2* mouse model for breast cancer, treatment with the CDK4/6 inhibitor abemaciclib leads to tumor regression by inducing anti-tumor immunity[113]. *In vitro* studies revealed that CDK4/6 inhibition increased antigen presentation and production of type III interferons by cancer cells, which induced $CD8^+$ T cell proliferation and activation[113]. Simultaneously, CDK4/6 inhibition reduced systemic and intra-tumoral regulatory T cell numbers, which occurred independent of the presence of a tumor. Both the effect of the CDK4/6 inhibitor on antigen presentation by cancer cells and the impact on regulatory T cells was dependent on inhibition of the RB-E2F-DNMT1 axis[113]. Importantly, by modulating the immune microenvironment, anti-CDK4/6 treatment improved response to anti-PDL1 in *MMTV-rtTA/tetO-HER2* mice[113]. Also, in an *in vitro* small molecule screen, CDK4/6 inhibitors were identified to directly enhance T cell activity. Mechanistically, CDK4/6 inhibition resulted in de-repression of NFAT activity in T cells, resulting in increased T cell accumulation in lung tumors of $Kras^{LSL\text{-}G12D};Trp53^{F/F}$ mice, which synergized with immune checkpoint inhibition[114]. These two studies illustrate that the CDK4/6 inhibitors originally developed to induce cell cycle arrest in cancer cells work in part by overcoming tumor immune evasion, which is a result of combined targeting of cancer cell-intrinsic pathways changing parameters of the cancer-immunity cycle, and direct targeting of T cells.

Targeted therapies have also been reported to affect the abundance and function of myeloid cells in tumor-bearing hosts, since the signaling pathways targeted by these drugs also play functional roles in the immune system[115]. For example, neutrophils in the *Hgf-*$Cdk4^{R24C}$ model for melanoma and cell line inoculation models impair the anti-tumor $CD8^+$ T cell response[116]. In this study, cMET inhibition enhanced the efficacy of adoptive cell transfer and immune checkpoint therapies by direct targeting of immunosuppressive neutrophils that express the cMET receptor[116]. However, targeting cMET-expressing neutrophils in another study promotes tumor progression[117], highlighting the complex model-dependent and dual role of neutrophils in cancer biology[118]. Likewise, it has been reported that the depletion of immunosuppressive $CD11b^+Gr1^+$ cells as a bystander effect of other targeted therapies, for example by ITK/BTK-inhibitor ibrutinib, benefits the response to immunotherapies in cell line inoculation models for breast cancer and melanoma[119,120]. Ibrutinib can also reprogram macrophages, relieve immunosuppression and facilitate $CD8^+$ cytotoxicity in PDAC-bearing mice[121]. These studies highlight that targeted drugs can impact the immune contexture of tumors via their working mechanism on cancer cells, which indirectly changes the immune landscape, and via their direct effect on immune cells. Insights into the complexity of the combined effect of these targeted drugs on the cancer cells and tumor microenvironment will help us to maximize the therapeutic benefit of targeted drugs in combination with immunomodulatory strategies (**Fig. 2.3**).

Conclusions and future directions

From the studies discussed in this review it has become clear that activation of oncogenes or loss of TSGs not only exert an intrinsic influence on the fate of cancer cells, but can have profound effects on tumor-host interactions. Commonly mutated genes that lie at the basis of tumorigenesis can actively participate in recruitment, activation or dampening of the immune

system. This could in part explain the heterogeneity between and within tumor types in immune infiltration and activation. From a clinical perspective, these insights will help identify patients that would or would not benefit from immunomodulation. Moreover, identifying the mechanisms underlying the causal relationship between the genetic makeup of tumors and their immune landscape may identify novel targets for anti-cancer immunomodulatory therapies. The studies presented here likely only reveal the tip of the iceberg. Most studies focus on one particular oncogene or TSG, and the majority of research is concentrated on the primary tumor. This leaves the effect on the systemic immune milieu and metastasis largely unaddressed. With increasingly sophisticated methodologies to generate mouse models that closely mimic the genetics and biology of human cancer and approaches to analyze tumors in depth, it will be possible to screen for a multitude of genetic and epigenetic alterations and their effect on the immune system. *In vivo* genetic manipulation will be key to delineate the spatiotemporal regulation of the tumor immune landscape, both in the primary as well as the metastatic lesion. This knowledge will help maximize the potential of immunomodulatory therapeutics for cancer patients and provide rationale for personalized combination therapies based on the genetic profile of tumors.

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References

1 von Hansemann, D. Ueber asymmetrische Zelltheilung in Epithelkrebsen und deren biologische Bedeutung. *Virchows Arch. Path. Anat.* **119**, 299–326, doi:https://doi.org/10.1007/BF01882039 (1890).

2 Boveri, T. Zur Frage der Entstehung Maligner Tumoren. *Gustav Fischer, 1914*, 1–64 (1914).

3 Rous, P. A Sarcoma of the Fowl Transmissible by an Agent Separable from the Tumor Cells. *J. Exp. Med.* **13**, 397-411 (1911).

4 Duesberg, P. H. & Vogt, P. K. Differences between the ribonucleic acids of transforming and nontransforming avian tumor viruses. *Proc. Natl. Acad. Sci. U. S. A.* **67**, 1673-1680 (1970).

5 Stehelin, D., Varmus, H. E., Bishop, J. M. & Vogt, P. K. DNA related to the transforming gene(s) of avian sarcoma viruses is present in normal avian DNA. *Nature* **260**, 170–173, doi:doi:10.1038/260170a0 (1976).

6 Knudson, A. G., Jr. Mutation and cancer: statistical study of retinoblastoma. *Proc. Natl. Acad. Sci. U. S. A.* **68**, 820-823 (1971).

7 Adams, J. M. *et al.* The c-myc oncogene driven by immunoglobulin enhancers induces lymphoid malignancy in transgenic mice. *Nature* **318**, 533-538 (1985).

8 Stewart, T. A., Pattengale, P. K. & Leder, P. Spontaneous mammary adenocarcinomas in transgenic mice that carry and express MTV/myc fusion genes. *Cell* **38**, 627-637 (1984).

9 Donehower, L. A. *et al.* Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours. *Nature* **356**, 215-221, doi:10.1038/356215a0 (1992).

10 Hanahan, D., Wagner, E. F. & Palmiter, R. D. The origins of oncomice: a history of the first transgenic mice genetically engineered to develop cancer. *Genes Dev.* **21**, 2258-2270, doi:10.1101/gad.1583307 (2007).

11 Fisher, G. H. *et al.* Induction and apoptotic regression of lung adenocarcinomas by regulation of a K-Ras transgene in the presence and absence of tumor suppressor genes. *Genes Dev.* **15**, 3249-3262, doi:10.1101/gad.947701 (2001).

12 Jain, M. *et al.* Sustained loss of a neoplastic phenotype by brief inactivation of MYC. *Science* **297**, 102-104, doi:10.1126/science.1071489 (2002).

13 Moody, S. E. *et al.* Conditional activation of Neu in the mammary epithelium of transgenic mice results in reversible pulmonary metastasis. *Cancer Cell* **2**, 451-461 (2002).

14 Ventura, A. *et al.* Restoration of p53 function leads to tumour regression in vivo. *Nature* **445**, 661-665, doi:10.1038/nature05541 (2007).

15 Stanton, S. E., Adams, S. & Disis, M. L. Variation in the Incidence and Magnitude of Tumor-Infiltrating Lymphocytes in Breast Cancer Subtypes: A Systematic Review. *JAMA Oncol.* **2**, 1354-1360, doi:10.1001/jamaoncol.2016.1061 (2016).

16 Medrek, C., Ponten, F., Jirstrom, K. & Leandersson, K. The presence of tumor associated macrophages in tumor stroma as a prognostic marker for breast cancer patients. *BMC Cancer* **12**, 306, doi:10.1186/1471-2407-12-306 (2012).

17 Gentles, A. J. *et al.* The prognostic landscape of genes and infiltrating immune cells across human cancers. *Nat. Med.* **21**, 938-945, doi:10.1038/nm.3909 (2015).

18 Balkwill, F. & Mantovani, A. Inflammation and cancer: back to Virchow? *Lancet* **357**, 539-545 (2001).

19 Coley, W. B. The treatment of malignant tumors by repeated inoculations of erysipelas: with a report of ten original cases. *Am. J. Med. Sci.* **105**, 487-511 (1893).

20 Diakos, C. I., Charles, K. A., McMillan, D. C. & Clarke, S. J. Cancer-related inflammation and treatment effectiveness. *Lancet Oncol.* **15**, e493-503, doi:10.1016/S1470-2045(14)70263-3 (2014).

21 Yang, Y. Cancer immunotherapy: harnessing the immune system to battle cancer. *J Clin. Invest.* **125**, 3335-3337, doi:10.1172/JCI83871 (2015).

22 Schumacher, T. N. & Schreiber, R. D. Neoantigens in cancer immunotherapy. *Science* **348**, 69-74, doi:10.1126/science.aaa4971 (2015).

23 Lennerz, V. *et al.* The response of autologous T cells to a human melanoma is dominated by mutated neoantigens. *Proc. Natl. Acad. Sci. U. S. A.* **102**, 16013-16018, doi:10.1073/pnas.0500090102 (2005).

24 van Rooij, N. *et al.* Tumor exome analysis reveals neoantigen-specific T-cell reactivity in an ipilimumab-responsive melanoma. *J Clin. Oncol.* **31**, e439-442, doi:10.1200/JCO.2012.47.7521

(2013).
25 Robbins, P. F. *et al.* Mining exomic sequencing data to identify mutated antigens recognized by adoptively transferred tumor-reactive T cells. *Nat. Med.* **19**, 747-752, doi:10.1038/nm.3161 (2013).
26 Linnemann, C. *et al.* High-throughput epitope discovery reveals frequent recognition of neo-antigens by CD4+ T cells in human melanoma. *Nat. Med.* **21**, 81-85, doi:10.1038/nm.3773 (2015).
27 Wölfel, T. *et al.* A p16INK4a-insensitive CDK4 mutant targeted by cytolytic T lymphocytes in a human melanoma. *Science* **269**, 1281-1284 (1995).
28 Rizvi, N. A. *et al.* Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. *Science* **348**, 124-128, doi:10.1126/science.aaa1348 (2015).
29 Van Allen, E. M. *et al.* Genomic correlates of response to CTLA-4 blockade in metastatic melanoma. *Science* **350**, 207-211, doi:10.1126/science.aad0095 (2015).
30 Le, D. T. *et al.* PD-1 Blockade in Tumors with Mismatch-Repair Deficiency. *N. Engl. J. Med.* **372**, 2509-2520, doi:10.1056/NEJMoa1500596 (2015).
31 Robinson, D. R. *et al.* Integrative clinical genomics of metastatic cancer. *Nature* **548**, 297-303, doi:10.1038/nature23306 (2017).
32 Charoentong, P. *et al.* Pan-cancer Immunogenomic Analyses Reveal Genotype-Immunophenotype Relationships and Predictors of Response to Checkpoint Blockade. *Cell Rep.* **18**, 248-262, doi:10.1016/j.celrep.2016.12.019 (2017).
33 Hugo, W. *et al.* Genomic and Transcriptomic Features of Response to Anti-PD-1 Therapy in Metastatic Melanoma. *Cell* **165**, 35-44, doi:10.1016/j.cell.2016.02.065 (2016).
34 Balli, D., Rech, A. J., Stanger, B. Z. & Vonderheide, R. H. Immune Cytolytic Activity Stratifies Molecular Subsets of Human Pancreatic Cancer. *Clin. Cancer Res.* **23**, 3129-3138, doi:10.1158/1078-0432.CCR-16-2128 (2017).
35 Spranger, S. *et al.* Density of immunogenic antigens does not explain the presence or absence of the T-cell-inflamed tumor microenvironment in melanoma. *Proc. Natl. Acad. Sci. U. S. A.* **113**, E7759-E7768, doi:10.1073/pnas.1609376113 (2016).
36 Parker, J. S. *et al.* Supervised risk predictor of breast cancer based on intrinsic subtypes. *J. Clin. Oncol.* **27**, 1160-1167, doi:10.1200/JCO.2008.18.1370 (2009).
37 Chen, Z. *et al.* Intratumoral CD8(+) cytotoxic lymphocyte is a favorable prognostic marker in node-negative breast cancer. *PLoS One* **9**, e95475, doi:10.1371/journal.pone.0095475 (2014).
38 Savas, P. *et al.* Clinical relevance of host immunity in breast cancer: from TILs to the clinic. *Nat. Rev. Clin. Oncol.* **13**, 228-241, doi:10.1038/nrclinonc.2015.215 (2016).
39 Decker, T. *et al.* 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, doi:10.1007/s00432-012-1258-3 (2012).
40 Jiang, D., Gao, Z., Cai, Z., Wang, M. & He, J. Clinicopathological and prognostic significance of FOXP3+ tumor infiltrating lymphocytes in patients with breast cancer: a meta-analysis. *BMC Cancer* **15**, 727, doi:10.1186/s12885-015-1742-7 (2015).
41 Liu, F. *et al.* CD8(+) cytotoxic T cell and FOXP3(+) regulatory T cell infiltration in relation to breast cancer survival and molecular subtypes. *Breast Cancer Res. Treat.* **130**, 645-655, doi:10.1007/s10549-011-1647-3 (2011).
42 Becht, E. *et al.* Immune and Stromal Classification of Colorectal Cancer Is Associated with Molecular Subtypes and Relevant for Precision Immunotherapy. *Clin. Cancer Res.* **22**, 4057-4066, doi:10.1158/1078-0432.CCR-15-2879 (2016).
43 Doucette, T. *et al.* Immune heterogeneity of glioblastoma subtypes: extrapolation from the cancer genome atlas. *Cancer Immunol. Res.* **1**, 112-122, doi:10.1158/2326-6066.CIR-13-0028 (2013).
44 Keck, M. K. *et al.* Integrative analysis of head and neck cancer identifies two biologically distinct HPV and three non-HPV subtypes. *Clin. Cancer Res.* **21**, 870-881, doi:10.1158/1078-0432.CCR-14-2481 (2015).
45 Wang, Q. *et al.* Tumor Evolution of Glioma-Intrinsic Gene Expression Subtypes Associates with Immunological Changes in the Microenvironment. *Cancer Cell* **32**, 42-56 e46, doi:10.1016/j.ccell.2017.06.003 (2017).
46 Quigley, D. *et al.* Lymphocyte Invasion in IC10/Basal-Like Breast Tumors Is Associated with

Wild-Type TP53. *Mol. Cancer Res.* **13**, 493-501, doi:10.1158/1541-7786.MCR-14-0387 (2015).
47 Bailey, P. *et al.* Genomic analyses identify molecular subtypes of pancreatic cancer. *Nature* **531**, 47-52, doi:10.1038/nature16965 (2016).
48 Cha, Y. J., Kim, H. R., Lee, C. Y., Cho, B. C. & Shim, H. S. Clinicopathological and prognostic significance of programmed cell death ligand-1 expression in lung adenocarcinoma and its relationship with p53 status. *Lung Cancer* **97**, 73-80, doi:10.1016/j.lungcan.2016.05.001 (2016).
49 Akbay, E. A. *et al.* Activation of the PD-1 pathway contributes to immune escape in EGFR-driven lung tumors. *Cancer Discov.* **3**, 1355-1363, doi:10.1158/2159-8290.CD-13-0310 (2013).
50 Layer, J. P. *et al.* Amplification of N-Myc is associated with a T-cell-poor microenvironment in metastatic neuroblastoma restraining interferon pathway activity and chemokine expression. *Oncoimmunology* **6**, e1320626, doi:10.1080/2162402X.2017.1320626 (2017).
51 Brandetti, E. *et al.* MYCN is an immunosuppressive oncogene dampening the expression of ligands for NK-cell-activating receptors in human high-risk neuroblastoma. *Oncoimmunology* **6**, e1316439, doi:10.1080/2162402X.2017.1316439 (2017).
52 Shin, D. S. *et al.* Primary Resistance to PD-1 Blockade Mediated by JAK1/2 Mutations. *Cancer Discov.* **7**, 188-201, doi:10.1158/2159-8290.CD-16-1223 (2017).
53 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, doi:10.1016/j.cell.2014.12.033 (2015).
54 Ock, C. Y. *et al.* Genomic landscape associated with potential response to anti-CTLA-4 treatment in cancers. *Nat. Commun.* **8**, 1050, doi:10.1038/s41467-017-01018-0 (2017).
55 Kersten, K., de Visser, K. E., van Miltenburg, M. H. & Jonkers, J. Genetically engineered mouse models in oncology research and cancer medicine. *EMBO Mol. Med.* **9**, 137–153, doi:10.15252/emmm.201606857 (2016).
56 Meylan, E. *et al.* Requirement for NF-kappaB signalling in a mouse model of lung adenocarcinoma. *Nature* **462**, 104-107, doi:10.1038/nature08462 (2009).
57 Kastenhuber, E. R. & Lowe, S. W. Putting p53 in Context. *Cell* **170**, 1062-1078, doi:10.1016/j.cell.2017.08.028 (2017).
58 Cooks, T., Harris, C. C. & Oren, M. Caught in the cross fire: p53 in inflammation. *Carcinogenesis* **35**, 1680-1690, doi:10.1093/carcin/bgu134 (2014).
59 Stodden, G. R. *et al.* Loss of Cdh1 and Trp53 in the uterus induces chronic inflammation with modification of tumor microenvironment. *Oncogene* **34**, 2471-2482, doi:10.1038/onc.2014.193 (2015).
60 Blaisdell, A. *et al.* Neutrophils Oppose Uterine Epithelial Carcinogenesis via Debridement of Hypoxic Tumor Cells. *Cancer Cell* **28**, 785-799, doi:10.1016/j.ccell.2015.11.005 (2015).
61 Schwitalla, S. *et al.* Loss of p53 in enterocytes generates an inflammatory microenvironment enabling invasion and lymph node metastasis of carcinogen-induced colorectal tumors. *Cancer Cell* **23**, 93-106, doi:10.1016/j.ccr.2012.11.014 (2013).
62 Muller, P. A. & Vousden, K. H. Mutant p53 in cancer: new functions and therapeutic opportunities. *Cancer Cell* **25**, 304-317, doi:10.1016/j.ccr.2014.01.021 (2014).
63 Cooks, T. *et al.* Mutant p53 prolongs NF-kappaB activation and promotes chronic inflammation and inflammation-associated colorectal cancer. *Cancer Cell* **23**, 634-646, doi:10.1016/j.ccr.2013.03.022 (2013).
64 Wormann, S. M. *et al.* Loss of P53 Function Activates JAK2-STAT3 Signaling to Promote Pancreatic Tumor Growth, Stroma Modification, and Gemcitabine Resistance in Mice and is Associated With Patient Survival. *Gastroenterology* **151**, 180-193, doi:10.1053/j.gastro.2016.03.010 (2016).
65 Beroukhim, R. *et al.* The landscape of somatic copy-number alteration across human cancers. *Nature* **463**, 899-905, doi:10.1038/nature08822 (2010).
66 Sodir, N. M. *et al.* Endogenous Myc maintains the tumor microenvironment. *Genes Dev.* **25**, 907-916, doi:10.1101/gad.2038411 (2011).
67 Shchors, K. *et al.* The Myc-dependent angiogenic switch in tumors is mediated by interleukin 1beta. *Genes Dev.* **20**, 2527-2538, doi:10.1101/gad.1455706 (2006).
68 Soucek, L. *et al.* Mast cells are required for angiogenesis and macroscopic expansion of Myc-induced pancreatic islet tumors. *Nat. Med.* **13**, 1211-1218, doi:10.1038/nm1649 (2007).
69 Yetil, A. *et al.* p19ARF is a critical mediator of both cellular senescence and an innate immune

response associated with MYC inactivation in mouse model of acute leukemia. *Oncotarget* **6**, 3563-3577, doi:10.18632/oncotarget.2969 (2015).
70 Schuster, C. *et al.* The cooperating mutation or "second hit" determines the immunologic visibility toward MYC-induced murine lymphomas. *Blood* **118**, 4635-4645, doi:10.1182/blood-2010-10-313098 (2011).
71 Casey, S. C. *et al.* MYC regulates the antitumor immune response through CD47 and PD-L1. *Science* **352**, 227-231, doi:10.1126/science.aac9935 (2016).
72 Kortlever, R. M. *et al.* Myc Cooperates with Ras by Programming Inflammation and Immune Suppression. *Cell* **171**, 1301-1315, doi:10.1016/j.cell.2017.11.013 (2017).
73 Topper, M. J. *et al.* Epigenetic Therapy Ties MYC Depletion to Reversing Immune Evasion and Treating Lung Cancer. *Cell* **171**, 1284-1300 e1221, doi:10.1016/j.cell.2017.10.022 (2017).
74 Ancrile, B., Lim, K. H. & Counter, C. M. Oncogenic Ras-induced secretion of IL6 is required for tumorigenesis. *Genes Dev.* **21**, 1714-1719, doi:10.1101/gad.1549407 (2007).
75 Sparmann, A. & Bar-Sagi, D. Ras-induced interleukin-8 expression plays a critical role in tumor growth and angiogenesis. *Cancer Cell* **6**, 447-458 (2004).
76 Pylayeva-Gupta, Y., Lee, K. E., Hajdu, C. H., Miller, G. & Bar-Sagi, D. Oncogenic Kras-induced GM-CSF production promotes the development of pancreatic neoplasia. *Cancer Cell* **21**, 836-847, doi:10.1016/j.ccr.2012.04.024 (2012).
77 Wislez, M. *et al.* High expression of ligands for chemokine receptor CXCR2 in alveolar epithelial neoplasia induced by oncogenic kras. *Cancer Res.* **66**, 4198-4207, doi:10.1158/0008-5472.CAN-05-3842 (2006).
78 Ji, H. *et al.* K-ras activation generates an inflammatory response in lung tumors. *Oncogene* **25**, 2105-2112, doi:10.1038/sj.onc.1209237 (2006).
79 Kobielak, A. & Fuchs, E. Links between alpha-catenin, NF-kappaB, and squamous cell carcinoma in skin. *Proc. Natl. Acad. Sci. U. S. A.* **103**, 2322-2327, doi:10.1073/pnas.0510422103 (2006).
80 Ying, H. *et al.* PTEN is a major tumor suppressor in pancreatic ductal adenocarcinoma and regulates an NF-kappaB-cytokine network. *Cancer Discov.* **1**, 158-169, doi:10.1158/2159-8290.CD-11-0031 (2011).
81 Koyama, S. *et al.* STK11/LKB1 Deficiency Promotes Neutrophil Recruitment and Proinflammatory Cytokine Production to Suppress T-cell Activity in the Lung Tumor Microenvironment. *Cancer Res.* **76**, 999-1008, doi:10.1158/0008-5472.CAN-15-1439 (2016).
82 Busch, S. E. *et al.* Lung Cancer Subtypes Generate Unique Immune Responses. *J. Immunol.* **197**, 4493-4503, doi:10.4049/jimmunol.1600576 (2016).
83 Bezzi, M. *et al.* Diverse genetic-driven immune landscapes dictate tumor progression through distinct mechanisms. *Nat. Med.* **24**, 165–175, doi:10.1038/nm.4463 (2018).
84 Huijbers, I. J. Generating Genetically Modified Mice: A Decision Guide. *Methods Mol. Biol.* **1642**, 1-19, doi:10.1007/978-1-4939-7169-5_1 (2017).
85 Munoz-Espin, D. & Serrano, M. Cellular senescence: from physiology to pathology. *Nat. Rev. Mol. Cell Biol.* **15**, 482-496, doi:10.1038/nrm3823 (2014).
86 Perez-Mancera, P. A., Young, A. R. & Narita, M. Inside and out: the activities of senescence in cancer. *Nat. Rev. Cancer* **14**, 547-558, doi:10.1038/nrc3773 (2014).
87 Xue, W. *et al.* Senescence and tumour clearance is triggered by p53 restoration in murine liver carcinomas. *Nature* **445**, 656-660, doi:10.1038/nature05529 (2007).
88 Pribluda, A. *et al.* A senescence-inflammatory switch from cancer-inhibitory to cancer-promoting mechanism. *Cancer Cell* **24**, 242-256, doi:10.1016/j.ccr.2013.06.005 (2013).
89 Chien, Y. *et al.* Control of the senescence-associated secretory phenotype by NF-kappaB promotes senescence and enhances chemosensitivity. *Genes Dev.* **25**, 2125-2136, doi:10.1101/gad.17276711 (2011).
90 Laberge, R. M. *et al.* MTOR regulates the pro-tumorigenic senescence-associated secretory phenotype by promoting IL1A translation. *Nat. Cell Biol.* **17**, 1049-1061, doi:10.1038/ncb3195 (2015).
91 Herranz, N. *et al.* mTOR regulates MAPKAPK2 translation to control the senescence-associated secretory phenotype. *Nat. Cell Biol.* **17**, 1205-1217, doi:10.1038/ncb3225 (2015).
92 Hoare, M. *et al.* NOTCH1 mediates a switch between two distinct secretomes during senescence. *Nat. Cell Biol.* **18**, 979-992, doi:10.1038/ncb3397 (2016).
93 Shen, Q. *et al.* Notch Shapes the Innate Immunophenotype in Breast Cancer. *Cancer Discov.*

7, 1320-1335, doi:10.1158/2159-8290.CD-17-0037 (2017).
94 Lesina, M. *et al.* RelA regulates CXCL1/CXCR2-dependent oncogene-induced senescence in murine Kras-driven pancreatic carcinogenesis. *J. Clin. Invest.* **126**, 2919-2932, doi:10.1172/JCI86477 (2016).
95 Di Mitri, D. *et al.* Tumour-infiltrating Gr-1+ myeloid cells antagonize senescence in cancer. *Nature* **515**, 134-137, doi:10.1038/nature13638 (2014).
96 Lujambio, A. *et al.* Non-cell-autonomous tumor suppression by p53. *Cell* **153**, 449-460, doi:10.1016/j.cell.2013.03.020 (2013).
97 Toso, A. *et al.* Enhancing chemotherapy efficacy in Pten-deficient prostate tumors by activating the senescence-associated antitumor immunity. *Cell Rep.* **9**, 75-89, doi:10.1016/j.celrep.2014.08.044 (2014).
98 Chen, D. S. & Mellman, I. Oncology meets immunology: the cancer-immunity cycle. *Immunity* **39**, 1-10, doi:10.1016/j.immuni.2013.07.012 (2013).
99 Chen, C. F. *et al.* ATR Mutations Promote the Growth of Melanoma Tumors by Modulating the Immune Microenvironment. *Cell Rep.* **18**, 2331-2342, doi:10.1016/j.celrep.2017.02.040 (2017).
100 Dorand, R. D. *et al.* Cdk5 disruption attenuates tumor PD-L1 expression and promotes antitumor immunity. *Science* **353**, 399-403, doi:10.1126/science.aae0477 (2016).
101 Coelho, M. A. *et al.* Oncogenic RAS Signaling Promotes Tumor Immunoresistance by Stabilizing PD-L1 mRNA. *Immunity* **47**, 1083–1099, doi:10.1016/j.immuni.2017.11.016 (2017).
102 Sarkar, S. *et al.* PRKCI promotes immune suppression in ovarian cancer. *Genes Dev.* **31**, 1109-1121, doi:10.1101/gad.296640.117 (2017).
103 Wang, G. *et al.* Targeting YAP-Dependent MDSC Infiltration Impairs Tumor Progression. *Cancer Discov.* **6**, 80-95, doi:10.1158/2159-8290.CD-15-0224 (2016).
104 Jiang, H. *et al.* Targeting focal adhesion kinase renders pancreatic cancers responsive to checkpoint immunotherapy. *Nat. Med.* **22**, 851-860, doi:10.1038/nm.4123 (2016).
105 Spranger, S., Bao, R. & Gajewski, T. F. Melanoma-intrinsic beta-catenin signalling prevents anti-tumour immunity. *Nature* **523**, 231-235, doi:10.1038/nature14404 (2015).
106 Garon, E. B. *et al.* Pembrolizumab for the treatment of non-small-cell lung cancer. *N. Engl. J. Med.* **372**, 2018-2028, doi:10.1056/NEJMoa1501824 (2015).
107 Peng, W. *et al.* Loss of PTEN Promotes Resistance to T Cell-Mediated Immunotherapy. *Cancer Discov.* **6**, 202-216, doi:10.1158/2159-8290.CD-15-0283 (2016).
108 Groenendijk, F. H. & Bernards, R. Drug resistance to targeted therapies: deja vu all over again. *Mol. Oncol.* **8**, 1067-1083, doi:10.1016/j.molonc.2014.05.004 (2014).
109 Vanden Borre, P. *et al.* Combined BRAF(V600E)- and SRC-inhibition induces apoptosis, evokes an immune response and reduces tumor growth in an immunocompetent orthotopic mouse model of anaplastic thyroid cancer. *Oncotarget* **5**, 3996-4010, doi:10.18632/oncotarget.2130 (2014).
110 Frederick, D. T. *et al.* BRAF inhibition is associated with enhanced melanoma antigen expression and a more favorable tumor microenvironment in patients with metastatic melanoma. *Clin. Cancer Res.* **19**, 1225-1231, doi:10.1158/1078-0432.CCR-12-1630 (2013).
111 Koya, R. C. *et al.* BRAF inhibitor vemurafenib improves the antitumor activity of adoptive cell immunotherapy. *Cancer Res.* **72**, 3928-3937, doi:10.1158/0008-5472.CAN-11-2837 (2012).
112 Hu-Lieskovan, S. *et al.* Improved antitumor activity of immunotherapy with BRAF and MEK inhibitors in BRAF(V600E) melanoma. *Sci. Transl. Med.* **7**, 279ra241, doi:10.1126/scitranslmed.aaa4691 (2015).
113 Goel, S. *et al.* CDK4/6 inhibition triggers anti-tumour immunity. *Nature* **548**, 471-475, doi:10.1038/nature23465 (2017).
114 Deng, J. *et al.* CDK4/6 Inhibition Augments Anti-Tumor Immunity by Enhancing T Cell Activation. *Cancer Discov.* **8**, 217-230, doi:10.1158/2159-8290.CD-17-0915 (2017).
115 Munoz-Fontela, C., Mandinova, A., Aaronson, S. A. & Lee, S. W. Emerging roles of p53 and other tumour-suppressor genes in immune regulation. *Nat. Rev. Immunol.* **16**, 741-750, doi:10.1038/nri.2016.99 (2016).
116 Glodde, N. *et al.* Reactive Neutrophil Responses Dependent on the Receptor Tyrosine Kinase c-MET Limit Cancer Immunotherapy. *Immunity* **47**, 789-802 e789, doi:10.1016/j.immuni.2017.09.012 (2017).
117 Finisguerra, V. *et al.* MET is required for the recruitment of anti-tumoural neutrophils. *Nature* **522**, 349-353, doi:10.1038/nature14407 (2015).

118 Coffelt, S. B., Wellenstein, M. D. & de Visser, K. E. Neutrophils in cancer: neutral no more. *Nat. Rev. Cancer* **16**, 431–446, doi:10.1038/nrc.2016.52 (2016).
119 Sagiv-Barfi, I. *et al*. Therapeutic antitumor immunity by checkpoint blockade is enhanced by ibrutinib, an inhibitor of both BTK and ITK. *Proc. Natl. Acad. Sci. U. S. A.* **112**, 966-972, doi:10.1073/pnas.1500712112 (2015).
120 Stiff, A. *et al.* Myeloid-Derived Suppressor Cells Express Bruton's Tyrosine Kinase and Can Be Depleted in Tumor-Bearing Hosts by Ibrutinib Treatment. *Cancer Res.* **76**, 2125-2136, doi:10.1158/0008-5472.CAN-15-1490 (2016).
121 Gunderson, A. J. *et al.* Bruton Tyrosine Kinase-Dependent Immune Cell Cross-talk Drives Pancreas Cancer. *Cancer Discov.* **6**, 270-285, doi:10.1158/2159-8290.CD-15-0827 (2016).
122 Debebe, A. *et al.* Wnt/beta-catenin activation and macrophage induction during liver cancer development following steatosis. *Oncogene* **36**, 6020-6029, doi:10.1038/onc.2017.207 (2017).
123 Welte, T. *et al.* Oncogenic mTOR signalling recruits myeloid-derived suppressor cells to promote tumour initiation. *Nat. Cell Biol.* **18**, 632-644, doi:10.1038/ncb3355 (2016).
124 Katlinski, K. V. *et al.* Inactivation of Interferon Receptor Promotes the Establishment of Immune Privileged Tumor Microenvironment. *Cancer Cell* **31**, 194-207, doi:10.1016/j.ccell.2017.01.004 (2017).
125 Kang, T. W. *et al.* Senescence surveillance of pre-malignant hepatocytes limits liver cancer development. *Nature* **479**, 547-551, doi:10.1038/nature10599 (2011).