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Genomic correlates of response to immune checkpoint blockade

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Abstract

Despite impressive durable responses, immune checkpoint inhibitors do not provide a long-term benefit to the majority of patients with cancer. Understanding genomic correlates of response and resistance to checkpoint blockade may enhance benefits for patients with cancer by elucidating biomarkers for patient stratification and resistance mechanisms for therapeutic targeting. Here we review emerging genomic markers of checkpoint blockade response, including those related to neoantigens, antigen presentation, DNA repair, and oncogenic pathways. Compelling evidence also points to a role for T cell functionality, checkpoint regulators, chromatin modifiers, and copy-number alterations in mediating selective response to immune checkpoint blockade. Ultimately, efforts to contextualize genomic correlates of response into the larger understanding of tumor immune biology will build a foundation for the development of novel biomarkers and therapies to overcome resistance to checkpoint blockade.

Tumors have long been known to generate varying levels of immune response, a property termed immunogenicity. Early studies revealed that certain cancers maintained high levels of tumor-infiltrating lymphocytes (TILs), associated with improved prognosis[1,2]. TILs have been shown to be dysfunctional and to express multiple co-inhibitory or checkpoint receptors, such as cytotoxic T lymphocyte antigen-4 (CTLA-4, CD152), programmed cell death-1 (PD-1, CD279), T cell immunoglobulin and mucin-domain containing-3 (TIM-3), and lymphocyte-activation gene (LAG-3)[3–5]. The insight that blockade of these checkpoints can lead to reversal of TIL dysfunction with improved cytotoxicity and proliferative capacity of these cells has changed cancer treatment paradigms (Box 1). Immune checkpoint inhibitors, including monoclonal antibodies against PD-1 and CTLA-4, have generated durable responses across many tumor types[6–13], leading to a number of Food and Drug

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Administration (FDA)-approved agents, with many others in the clinical trial pipeline. However, the majority of patients do not respond to checkpoint blockade, so predicting among patients the subset that will benefit from checkpoint inhibitors, either alone or in combination with other agents, remains a challenge.

Select tumors express high levels of the PD-1-binding ligand PD-L1, and initial trials of anti-PD-1 therapy found that PD-L1 expression, as detected by immunohistochemistry, correlated with response to therapy[6,7,14], thereby leading the FDA to approve PD-L1 companion diagnostic tests for anti-PD-1/PD-L1 therapies in some cancers[15]. Additional studies of these histological markers showed that the association with response varied over time and by tumor type, with PD-L1 positivity distinguishing responders in some settings and not others[12,16–18]. Importantly, subsequent trials have demonstrated that a sizable portion of responses occurred in patients with PD-L1-negative tumors[8,11,19–21]. These findings have prompted efforts to identify other determinants of response to checkpoint blockade, including non-genomic factors, such as the gut microbiome, environmental influences, and metabolic pathways, reviewed elsewhere[22–26].

Pivotal investigations of the cancer genome have uncovered oncogenic mutations underlying selective growth advantage, tumor suppressor inactivation, and tumorigenesis initiation, among others[27–29]. While such approaches have traditionally focused on genomic changes within tumor cells alone as a measure of responsiveness to small-molecule inhibitors or monoclonal antibodies, application of these strategies to immune checkpoint blockade requires consideration of tumor cells in conjunction with distinct immune cell populations and non-immune, non-tumor cells, such as stromal and endothelial cells. In this Review, we present a framework for emerging genomic correlates of clinical responses to immune checkpoint inhibitors (Fig. 1 and Table 1), discuss potential therapeutic applications of these findings, and outline crucial future studies needed to advance this field.

Tumor antigens and mutations

In order to avoid self-reactivity and the development of autoimmunity, the immune system discriminates self from non-self antigens. Tumors may express aberrant antigens that, in turn, may be recognized by the immune system. These include proteins normally expressed in immune-privileged sites, such as cancer/testis antigens; virally expressed proteins, as in the case of Epstein-Barr virus or human-papillomavirus-associated cancers; and proteins encoded by endogenous retroviruses, which are germline genomic elements resembling retroviruses that are transcriptionally repressed in normal cells but expressed in many tumor cells[30–32]. Furthermore, tumors contain the cumulative sum of thousands of mutations, a fraction of which alter the amino acid sequence of encoded proteins. These mutated proteins, unique to the tumor and not present in normal cells, are known as neoantigens[25,30]. Neoantigens are often specific to each tumor and are unlikely to be shared widely across tumors, even those with the same histology. If neoantigen epitopes are presented to the immune system, these epitopes generate an adaptive immune response selective for cancer cells that can be enhanced and strengthened by checkpoint blockade[30,33].

Neoantigens and tumor mutational burden.

A diversity of neoantigens may be necessary but not sufficient for clinical responses to immune checkpoint inhibitors. Mouse models of sarcoma that respond to anti-CTLA-4 and/or anti-PD-1 therapy have shown reactivation of neoantigen-specific T cells (Box 1)[34,35], and neoantigen-specific T cell reactivity has also been observed through tumor exome analysis in patients with advanced cancers who respond to checkpoint inhibitors[36,37]. The role of neoantigens in the response to immune checkpoint inhibitors has been further substantiated by studies showing that higher neoantigen load is associated with response to CTLA-4 and PD-1 blockade in patients with melanoma and non-small-cell lung cancer[38–40], as well as analyses of acquired resistance in patients with non-small-cell lung cancer demonstrating loss of neoantigens through subclone elimination and chromosomal deletions in resistant tumors[41].

As neoantigens are computationally predicted from somatic variant data, tumor mutational burden, measured as the number of nonsynonymous mutations per megabase sequenced, has therefore similarly been associated with improved responses to checkpoint blockade, initially in studies of melanoma and non-small-cell lung cancer, two of the tumor types with the highest mutation number[38,39,42]. Larger studies and phase III trials have confirmed that high tumor mutational burden correlates with enhanced checkpoint inhibitor responses and improved overall survival in certain tumor types, such as head and neck cancer[43], urothelial carcinoma[9], and lung cancer[44–48], including small-cell lung cancer[48]. However, tumor mutational burden is an incomplete correlate of checkpoint blockade response in that mutation load distributions overlap considerably between responders and nonresponders[38,39,42]. Therefore, even the most ideal receiver operating charcteristic curve cutoffs for tumor mutational burden in melanoma and lung cancer have low statistical sensitivity and specificity for predicting clinical benefit from checkpoint inhibitors[49]. Furthermore, tumor mutational burden does not correlate with response in other tumor types, including Hodgkin's lymphoma[50–51], renal cell carcinoma[52–53], and virally mediated tumors, such as polyomavirus-associated Merkel-cell carcinoma[54–56], for which other factors likely drive responses to immune checkpoint inhibitors.

While a higher mutation and neoantigen load may reflect an increased likelihood of a T cell response being generated against that tumor, this has not necessarily correlated with the identification of circulating T cells that recognize the proposed neoepitope. Mouse models of sarcoma have demonstrated that anti-CTLA-4 and anti-PD-1 therapies reactivated neoantigen-specific $CD8^+$ T cells to achieve tumor rejection[34,35], and patients with cancers that responded to checkpoint inhibitors displayed neoantigen-specific T cell reactivity[36,37,57]. In both settings, the number of identified neoantigen-specific T cells was much lower than the predicted number of neo-epitopes. Whether this reflects underlying thymic deletion of tumor-reactive T cells with partial self-reactivity or a lack of sensitivity to identify the neoantigen-specific T cell population remains to be understood.

Neoantigen prediction.

Refining neoantigen prediction algorithms for neoepitopes may improve the ability to identify patients who are most likely to respond to checkpoint blockade. Initial techniques to

identify the peptide sequences most likely to be presented by major histocompatibility complex (MHC) class I proteins relied on in silico binding assays followed by in vitro testing with T cell clones[58,59]. Subsequent efforts that were focused on large-scale mass-spectrometry approaches have led to the development of more advanced prediction algorithms[60,61]. Similar efforts to identify class II neoepitopes have lagged behind due to, at least in part, the longer amino acid sequences and less stringent binding affinity rules required for class II binding[62]. Recently, methods to detect class II epitopes have been developed using neural network analysis of data derived from proteomic experiments and single-cell T cell receptor (TCR) sequencing[63]. Improved prediction algorithms for both class I and II epitopes may enable a precision-medicine approach for neoantigen prioritization to be tested at an individual-patient level.

Neoantigen and mutation clonality.

The relative clonality of neoantigens and mutations, estimated by the portion of tumor cells harboring a mutation, has also been linked to response. In one study, clonal neoantigens, arising from founder mutations present in all or nearly all tumor cells, conferred sensitivity to PD-1 and CTLA-4 blockade in lung cancer and melanoma, while subclonal neoantigens, which arise from passenger mutations present in a subset of tumor cells, predominated in non-responders[57]. Similarly, clonal neoantigens and mutations occurred recurrently in responding tumors in a meta-analysis of 249 tumors, predominantly melanoma and non-small-cell lung cancer, that were treated with immune checkpoint blockade[53]. This correlation between neoantigen clonality and checkpoint blockade response may be explained by immu-noediting, in which immunogenic neoantigens trigger an antitumor immune response that then eliminates these neoantigens[64]. This phenomenon may thus restrict intratumor genetic heterogeneity and promote clonal dominance of nonimmunogenic neoantigens, an effect enhanced by checkpoint blockade in mouse models of cancer[64]. Neoantigen clonality may therefore identify tumors with less sophisticated immune evasion strategies than those of nonresponding tumors. Overall, neoantigen clonality needs additional validation before it can be used as a clinical biomarker.

Tumor antigen immunogenicity.

Furthermore, the similarity of neoantigens and other tumor antigens to non-self antigens, such as those expressed by viruses and other pathogens, may enhance immune recognition and checkpoint blockade response. Recent efforts have focused on quantifying the foreignness of neoantigens by comparing the neoantigen peptide sequences with those of immune epitopes that have been experimentally validated as targets of adaptive immune responses[65,66]. In one neoantigen fitness model, tumors with more immunogenic neoantigens, which had greater homology with infectious-disease-derived peptides, were defined as having lower fitness[65,66]. Patients with low-fitness melanoma and lung cancer tumors had longer survival after CTLA-4 and PD-1 blockade than patients with high-fitness tumors[65]. A similar concept may explain the improved responses to checkpoint blockade that were observed in virus-associated cancers, which have more immunogenic non-self antigens[31,54]. Likewise, tumors with aberrant expression of germline endogenous retroviruses, which are transcriptionally repressed in normal cells, have demonstrated greater immune cytolytic activity and more frequent responses to checkpoint blockade[32,67].

Altogether, the foreignness of tumor antigens may prove to be a stronger driver of checkpoint inhibitor response than the quantity of neoantigens.

Cancer/testis antigens.

Cancer/testis antigens, whose normal expression is restricted to developing reproductive tissues[30,33], represent an alternative class of tumor antigens in that they consist of nonmutated self peptides that induce incomplete T cell tolerance and thus promote tumor immune evasion. Cancer/testis antigens are commonly expressed in many tumor types and have recently been implicated in resistance to immune checkpoint blockade. In metastatic melanoma, anti-CTLA-4 non-responders, but not anti-PD-1 non-responders harbored upregulated gene expression of a subcluster of melanoma-associated antigen (MAGE)-A cancer-germline antigens[68]. Notably, MAGE-A protein expression correlated with decreased expression of autophagy proteins, indicating that MAGE-A proteins may suppress autophagy to prevent T cell priming and avert immune recognition[68]. Whether other cancer/testis antigens similarly trigger immune tolerance and checkpoint blockade resistance remains to be determined.

Antigen presentation

HLA pathway alterations.

Anti-tumor immunity requires T cell recognition of the tumor epitope when presented on the MHC, a process known as antigen presentation. Human leukocyte antigen (HLA) genes *HLA-A, HLA-B*, and *HLA-C* encode the MHC class I proteins that present intracellular peptides on the cell surface to TCRs and require β−2 microglobulin (B2M) for stabilization on the cell surface (Fig. 2a)[69]. While B2M loss was initially observed in tumor immune escape after adoptive T cell therapies[70], *B2M* mutations and loss of heterozygosity have more recently been demonstrated to be innate and acquired resistance mechanisms to CTLA-4 and PD-1 inhibitors in cohorts of patients with melanoma[71], as well as in one or two patients who had melanoma, gastrointestinal cancer, or lung cancer[37,72,73]. These clinical observations are backed by preclinical experiments in which CRISPR-mediated knockout of *B2m* conferred resistance to PD-1 blockade in mouse models[7376]. The heterogeneity of alterations in *B2M* and tumor types in which they are found suggest that this mechanism of resistance may prove to be more widespread as more studies are performed.

Similarly, mutations within HLA genes, loss of diversity of HLA alleles, or alterations in HLA expression may diminish responses to checkpoint blockade. The extreme polymorphism of HLA genes has led to the concept that broader HLA diversity confers a survival advantage by promoting recognition by T cells of larger numbers of microbial and tumor antigens[77,78]. An individual's HLA repertoire may exert a negative selection pressure on tumor mutations in that patient-specific MHC I and MHC II genotypes correlate with tumor mutations that produce neoantigens least likely to be presented, thus indicating immune evasion[79,80]. Likewise, the presence of HLA mutations directly correlated with neoantigen frequency and TIL quantity in colorectal tumors, suggesting that positive selection was occurring for HLA mutations in tumors under immune attack[81]. HLA loss of

heterozygosity was similarly associated with a high burden of subclonal neoantigens that were predicted to bind to the lost HLA alleles, implying that there was enhanced immune evasion after the loss of heterozygosity event[57].

In the clinic, broader HLA diversity enhanced immune recognition of tumor neoantigens and improved overall survival in individuals with cancer treated with immune checkpoint inhibitors[82]. Similarly, higher expression of *MEX3B*, a gene encoding an RNA-binding protein that downregulates *HLA-A* expression (Fig. 2a), was associated with primary resistance to anti-PD-1 therapy in mel-anoma[83]. Finally, in patients with melanoma who were treated with immune checkpoint inhibitors, loss of MHC class I protein expression on tumor cells correlated with transcriptional downregulation of *HLA-A, HLA-B, HLA-C*, and *B2M* and primary resistance to anti-CTLA-4 therapy, while MHC class II protein expression predicted response to anti-PD-1 therapy, again highlighting the importance of HLA expression in checkpoint blockade response[84].

Interferon-γ pathway alterations.

Expression levels of HLA molecules and response to checkpoint blockade may in part be driven by signaling through the interferon (IFN)-γ receptor. As shown in Fig. 2b, IFN-γ, released by activated T cells upon recognizing neoantigens, binds IFN-γ receptors on tumors, which triggers Janus kinase 1 (JAK1) 1 and JAK2 and signal transducers and activators of transcription (STAT) signaling that activates IFN-related genes, including interferon regulatory factor 1 *(IRF1)*, which induces the transcription of other genes to increase the surface expression of PD-L1 and MHC molecules. Gene expression from 220 anti-PD-1-treated patients with nine cancer types revealed a T-cell-inflamed signature that included upregulated IFN-γ response that was necessary but not always sufficient for clinical benefit from therapy[85]. Similarly, enriched expression of genes related to antigen presentation and the IFN-γ pathway was an early on-treatment signature of melanoma tumor response to anti-PD-1 therapy after prior progression on anti-CTLA-4 therapy[86].

In addition to overall expression, copy-number alterations (CNAs) and mutations within the IFN-γ pathway have been identified as a mechanism of checkpoint inhibitor resistance. Of 12 melanoma tumors with primary resistance to anti-CTLA-4 therapy, 75% harbored genomic defects in the IFN-γ pathway, including copy-number loss of interferon-γ receptor 1 *(IFNGR1), IFNGR2, IRF1*, and *JAK2*, as well as amplification of IFN-γ pathway inhibitors, such as suppressor of cytokine signaling 1 *(SOCS1)* and protein inhibitor of activated STAT4 *(PIAS4)* (Fig. 2b)[87]. Likewise, significant enrichment of IFN-γ CNAs occurred in non-responding compared with responding tumors in a meta-analysis of 249 checkpoint-inhibitor-treated tumors[53]. Collectively, these results highlight the importance of antigen presentation and IFN-γ pathway genes in response to immune checkpoint blockade.

Mutations in the JAK-STAT pathway have similarly been identified in primary and acquired resistance to immune checkpoint inhibitors, albeit at a lower frequency than IFN-γ expression changes. JAK1 or JAK2 mutations have been reported in small numbers of patients with metastatic melanoma and MMR-deficient colon cancer that demonstrated primary or acquired resistance to anti-PD-1 therapy[72,88,89]. Although JAK1 and jAk2 are canonically thought to act downstream of the IFN-γ receptor, other JAK-STAT pathway

components may be involved in PD-L1 induction, as demonstrated by genomic profiling of a lung adenocarcinoma tumor responding to anti-PD-L1 therapy, which revealed activating germline and somatic *JAK3* mutations that were associated with increased PD-L1 expression in response to IFN-γ (ref.[90]). These clinical observations prompted preclinical CRISPR-Cas9 screens that aimed to identify regulators of the IFN-γ pathway[74–76], which require validation in patients. These findings suggest that the full spectrum of factors mediating IFN-γ resistance is not fully understood and highlight this as an area to investigate for therapeutic targets to boost checkpoint blockade response.

PDL1 amplification and regulation

PDL1 amplification.

Amplification of the chromosome 9p24.1 locus, the region containing *PDL1, PDL2*, and *JAK2*, was first linked to increased expression of PD-L1 on the Reed-Sternberg cells of Hodgkin's lymphoma[91]. Augmented JAK-STAT signaling and PD-L1 overexpression may contribute to impressive anti-PD-1 response rates of 69–87% in relapsed or refractory classic Hodgkin's lymphoma[50,92,93]. PD-L1 overexpression has also been correlated with CNAs in 9p24.1 in primary central nervous system B cell lymphoma and primary testicular B cell lymphoma, with corresponding high responses to anti-PD-1 therapy[94]. Conversely, PD-L1 overexpression in T cell non-Hodgkin lymphoma has been linked to poor outcomes—specifically, rapid progression in three patients with adult T cell leukemia-lymphoma treated with PD-1 inhibitors[95]. These clinical observations may be explained by the role of PD-1 as a tumor suppressor in T cell lymphomas and particularly by the ability of PD-1 to block oncogenic TCR signaling[96]. Overall, these findings caution against employing PD-1 blockade in tumors driven by oncogenic T cell signaling.

The frequency of *PDL1* amplification in solid tumors, in comparison, is reported to be much lower, with a prevalence of 0.7% in over 118,000 sequenced tumors, inclusive of 100 solid tumor histologic types[97]. Enrichment was seen in particular tumor types, including hepatocellular cholangiocarcinoma, head and neck squamous cell, nasopharyngeal carcinoma, undifferentiated soft-tissue sarcoma, kidney sarcomatoid, and thyroid anaplastic carcinoma, of which 12 of 13 patients with clinical annotation had co-amplification of *JAK2* (ref.[97]). PDL1-amplified tumors did not always have high PD-L1 expression as determined by immunohistochemistry, echoing an earlier report in head and neck squamous cell tumors[96]. Within a clinically annotated cohort, 9 of 13 patients with identified *PDL1* or *PDL2* amplification were treated with PD-1/PD-L1 inhibitors either alone or in combination with CTLA-4 or an investigational agent and had an objective response rate of 67% and a median progression-free survival of 15 months[97]. This small cohort illustrates the challenges of widespread applicability given the low prevalence of *PDL1* CNAs, emphasizing that this finding is preliminary

PD-L1 regulation.

Since PD-L1 associates with response to checkpoint blockade[6,7], understanding PD-L1 regulation may reveal novel molecular mechanisms that could be targeted to overcome resistance to immune checkpoint inhibitors. Recent work has demonstrated that cyclin D-

cyclin dependent kinase 4 (CDK4) and speckle-type POZ (pox virus and zinc finger) protein (SPOP) regulate PD-L1 protein abundance by promoting proteasome-mediated degradation of PD-L1 and that CDK4 and CDK6 (CDK4/6) inhibitors increased PD-L1 protein levels and enhanced response to PD-1 inhibitors in preclinical models of cancer[98]. In 97 primary human prostate cancers, 15 tumors with *SPOP* mutations had stronger PD-L1 staining and a lower number of TILs than tumors without *SPOP* mutations[98]. Additional PD-L1 regulators have been revealed by functional genomic screens in human cancer cell lines. Specifically, haploid genetic screens and CRISPR-Cas9 knockouts revealed that CMTM4 and CMTM6 (CMTM4/6), two closely related transmembrane proteins, reduced PD-L1 protein ubiquitination and enhanced T cell inhibition by PD-L1-expressing tumors[99]. Another similar CRISPR-Cas9 screen also identified CMTM6 as a regulator that prevents PD-L1 protein degradation and showed that CMTM6 depletion improved tumor-specific T cell activity in vitro and in vivo[100]. Together, these findings on PD-L1 regulators have important therapeutic implications in the enhancement of immune checkpoint blockade response. CDK4/6 inhibitors are already being investigated with PD-1/PD-L1 inhibitors in clinical trials, and CMTM4/6 first requires validation in human tumors.

DNA repair and mutational signatures

Mismatch repair deficiency and other DNA repair alterations.

Genomic analyses have also shown that mutational processes that directly alter DNA damage response and repair pathways influence response to immune checkpoint blockade[101]. Mismatch repair (MMR) deficiency has emerged as a robust indicator of response to immune checkpoint blockade[37,40]. Alterations in genes encoding MMR proteins, including *MLH1, MSH2, MSH6*, and *PMS2*, lead to uncorrected DNA replication errors and a large number of mutations, particularly small insertions and deletions in repetitive DNA sequences called microsatellites[61]. These genomic alterations often cause frameshift mutations that produce immunogenic neoantigens that are recognized and targeted by T cells[102,103]. Phase II clinical trials of single-agent PD-1 blockade in patients with MMR deficient tumors have demonstrated impressive response rates of 40–70% with durable progression-free and overall survival[37,40]. These results culminated in the first FDA approval of a cancer therapy based on a genomic biomarker regardless of tumor histology and site of origin. Notably, patients with primary resistance had similar tumor mutation burden as patients with responses, and responding patients had transient expansions of T cell clones specific for neoantigens that bound MHC proteins with > 100-fold higher affinity than wild-type peptides[37], highlighting neoantigen immunogenicity rather than mutation burden as a potential driver of response to immune checkpoint blockade.

The success of MMR deficiency as a response predictor has raised the question of whether other DNA repair alterations are similarly associated with checkpoint blockade response. Tumors with inactivating mutations in *POLE*, encoding a DNA polymerase essential to nucleotide and base-excision repair, have demonstrated impressive responses to PD-1 inhibition in multiple case studies[39,104–106]. Another study of PD-1/PD-L1 inhibitors in metastatic urothelial carcinoma showed higher response rates in tumors with DNA damage response alterations compared with those without these alterations, and the highest response

rates were in tumors with loss-of-function alterations as opposed to alterations of unknown signif-icance[107]. Importantly, statistical models that included DNA damage response alterations were superior at predicting survival compared with models accounting for mutation load[107], indicating that alterations in DNA damage response pathways may impact response to immune checkpoint inhibitors more than mutation load.

Mutational signatures.

Beyond dysfunctional DNA repair, specific mutational signatures, which are characteristic patterns of mutation types generated by different mutational processes, have been linked to checkpoint blockade response. Among patients with lung cancer treated with checkpoint inhibitors, tumors harboring mutational signatures associated with smoking had a higher response rate than those without such smoking signatures[39,53]. Another cohort of patients with lung cancer who experienced durable clinical benefit from anti-PD-1 therapy displayed an enrichment of mutational signatures associated with apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like (APOBEC), a family of RNA and DNA editing proteins capable of promoting mutagenesis in cancer[108]. Likewise, patients with bladder cancer and head and neck squamous cell carcinoma demonstrated a significant association between APOBEC-related mutational signatures and response to immune checkpoint inhibitors[53]. Finally, patients with melanoma who were treated with checkpoint blockade were more likely to experience clinical benefit if their tumors harbored mutational signatures associated with ultraviolet light exposure or prior alkylating agent treatment as opposed to other dominant mutational signatures, and stratification by mutational signature eliminated the association of mutation burden with response, suggesting that mutation burden may not be an underlying driver of response in melanoma[53]. It will be of interest to understand whether these DNA-damaging processes generate more neoantigens or act by another mechanism to improve immunotherapy response.

Oncogenic signaling pathways

Certain cancer signaling pathways may influence response to immune checkpoint blockade by promoting immune evasion. Increasing evidence points to intratumoral immune exclusion as a dominant strategy employed by oncogenic pathways to limit response to immune checkpoint inhibitors[109]. Tumor immune microenvironments containing cytotoxic lymphocytes surrounding but not infiltrating the tumor core are termed immune-excluded or cold tumors, while those microenvironments with activated cytotoxic lymphocytes infiltrating the tumor core are referred to as immune-infiltrated or hot tumors[110,111]. In patients with metastatic melanoma, immune-excluded tumors demonstrated poorer responses to both CTLA-4 and PD-1 inhibitors than immune-infiltrated tumors[14,112]. Here, we discuss alterations in oncogenes and tumor-suppressor genes that contribute to intratumoral immune exclusion and checkpoint blockade resistance.

Tumor suppressors and oncogenes.

Lung adenocarcinomas with inactivation of the tumor suppressor gene serine/threonine-protein kinase *(STK11*, also known as *LKB1)* have been shown to have a reduced number of TILs and worse responses to immune checkpoint inhibitors in multiple studies[45,113–115]. An

emerging mechanism by which *LKB1* alterations facilitate immune exclusion is methylation-induced suppression of stimulator of interferon genes (STING) expression that blocks cytoplasmic double-stranded DNA sensing, which otherwise induces type 1 interferons and T cell recruit-ment[116]. Furthermore, inactivation of *LKB1* in a KRAS-driven genetically engineered mouse model of non-small-cell lung cancer was associated with increased interleukin-6 expression, enhanced recruitment of neutrophils, and decreased T cell infiltrate, phenomena that could be reversed with interleukin-6 blockade or neutrophil depletion[113].

Loss of *PTEN*, another tumor suppressor, was similarly associated with increased immunosuppressive cytokines, decreased T cell tumor infiltration, and inferior responses to PD-1 inhibitors in patients with melanoma and xenograft models of melano-mas[117], as well as in a patient with uterine leiomyosarcoma[118]. In addition, *PTEN*-deficient melanomas displayed reduced autophagy-related transcript and protein levels that led to resistance to T cell killing in vitro[117]. Importantly, autophagy has been reported to enhance tumor immunogenicity by recruiting dendritic cells, increasing cross-presentation and priming T cells[119,120], an essential step in anti-tumor immunity in which dendritic cells introduce T cells to neoantigens and trigger cytotoxic differentiation[121]. Whether defective T cell priming contributes to immune exclusion and PD-1 resistance in *PTEN*-deficient tumors remains to be studied[109].

Activating oncogenic alterations associated with intratumoral immune exclusion and checkpoint blockade resistance include WNT/β-catenin and transforming growth factor-β (TGF-β)[122,123]. β-catenin signaling has been shown to prevent T cell tumor infiltration by decreasing dendritic cell recruitment and T cell priming in autochthonous mouse melanoma models[124,125]. Conversely, multiple lines of evidence suggest that TGF-β signaling in fibroblasts creates a stromal barrier to physically exclude T cells from the tumor core in patients who did not respond to immune checkpoint inhibitors[123,126]. Hence, strategies to enhance checkpoint blockade response need to be tailored to the specific immune exclusion mechanisms employed by each oncogenic pathway.

Clonal driver alterations.

Certain clonal driver alterations also correlate with immune exclusion and checkpoint blockade response. Murine models of *EGFR*-mutated lung tumors showed higher levels of immunosuppressive growth factors and a lower ratio of cytotoxic ($CD8^+$) to regulatory ($Foxp3^+$) tumor-infiltrating T cells compared to normal lung[127], and both human and murine *EGFR*-mutated lung tumors displayed lower numbers of infiltrating $CD8^+$ T cells compared with KRAS-mutated lung tumors[128]. Notably, patients with *EGFR*-mutated non-small-cell lung cancer have demonstrated a low response rate to anti-PD-1/PD-L1 therapy[45,129]. Moreover, a large meta-analysis of patients with lung cancer who were randomized to anti-PD-1/PD-L1 therapy or chemotherapy found no overall survival benefit of checkpoint blockade in 271 patients with *EGFR*-mutation-positive lung cancers and a statistically significant treatment-*EGFR* mutation interaction in the overall cohort of more than 2,000 patients with known *EGFR* status[130], suggesting that *EGFR* mutations are indeed a robust predictor of PD-1 blockade resistance.

Conversely, the relationship of *KRAS* with checkpoint blockade response is more variable. In the same meta-analysis, patients with *KRAS*-mutation-positive tumors had improved overall survival with anti-PD-1/PD-L1 therapy as compared to chemotherapy, but the interaction between mutation status and treatment effect was not significant for *KRAS* mutations[130]. Likewise, another smaller study found no enrichment of *KRAS* in lung cancers responding to PD-1/PD-L1 inhibitors[45], and many studies have implicated *KRAS* in chemokine upregulation and immunosuppressive cell recruitment[131,132]. Therefore, the association of *KRAS* mutations with checkpoint inhibitor response likely depends on additional factors, such as tissue context and coexisting mutations. Specifically, *KRAS*-mutated pancreatic tumors have fewer infiltrating T cells than KRAS-mutated lung tumors[132], and KRAS-mutated lung tumors with *TP53* mutations have better responses to PD-1 inhibitors[133], particularly when compared with *KRAS*-mutated lung tumors with *LKB1* mutations[114]. Ultimately, the impact of tissue stroma and concurrent mutations on the immunologic effects of clonal driver mutations requires further clarification and must be considered in future biomarker-development efforts.

Transcriptional signatures and the tumor microenvironment

Transcriptional signatures linked to immune checkpoint inhibitor response encompass the broader tumor immune microenvironment and expose underlying pathways of response and resistance. These signatures have been derived from bulk RNA and thus represent an amalgamation of tumor cells, immune cells, and stromal cells. Gene expression analyses of clinical tumor samples have found that checkpoint blockade response correlates with T cell infiltration, cytotoxic function, antigen processing, checkpoint molecules, and decreased suppressor cells[85,134–137], including immune checkpoint relationships derived from spontaneously regressing neuroblastoma tumors[138]. One important caveat is that all of the signatures presented here require confirmation by independent groups and prospective clinical validation.

Immune infiltration and stromal signaling.

From a functional standpoint, higher T effector and IFN-γ response gene expression, including of *CD8A, EOMES, GZMA* and *GZMB*, and *IFNG*, was strongly associated, in randomized phase II studies, with improved overall survival in patients with non-small-cell lung cancer treated with a PD-L1 inhibitor[134] and improved progression-free survival in patients with metastatic renal cell carcinoma treated with a PD-L1 inhibitor with or without anti-vascular endothelial growth factor (VEGF) therapy[52]. More than likely, this gene signature points to the existing presence of an effector T cell response, poised to respond to anti-PD-1 therapy. Another immune infiltration transcriptional signature is a T-cell-inflamed gene expression profile that comprises 18 genes involved in antigen presentation, chemokine production, cytolytic activity, and T cell exhaustion[85,139]. This signature has been shown to predict clinical response to anti-PD-1 inhibition in over 300 patients across 22 tumor types[115].

Transcriptional signatures associated with resistance to checkpoint immunotherapy have focused on stromal and vascular biology. The innate anti-PD-1 resistance signature (IPRES),

derived from bulk RNA sequencing of tumor biopsies, was associated with primary resistance to PD-1 blockade in patients with melanoma and included enhanced expression of genes involved in stromal and vascular pathways, such as mesenchymal transition, cell adhesion, extracellular matrix remodeling, angiogenesis, and wound healing[140]. Similarly, resistance to anti-PD-1 inhibition in over 300 patients across 22 tumor types correlated with enriched expression of genes related to vascular endothelium, myeloid infiltrates, and stromal Wnt signaling[115]. These studies highlight the contribution of stromal and endothelial components of the tumor microenvironment to checkpoint blockade resistance, which requires further clarification in future work.

T cell dysfunction and exclusion.

Another transcriptional signature predictive of checkpoint blockade response integrated both T cell exclusion in cold tumors and T cell dysfunction in hot tumors in a computational framework termed tumor immune dysfunction and exclusion (TIDE) using gene expression and survival data from over 33,000 human tumor samples[141]. Whereas the TIDE T cell exclusion signature was derived from expression profiles of immunosuppressive cells, including cancer-associated fibroblasts, myeloid-derived suppressor cells, and tumor-associated macrophages, the TIDE T cell dysfunction signature employed Cox proportional hazards models to identify genes that interact with cytotoxic T lymphocyte function to influence survival, producing a signature enriched for immune inflammatory pathways and depleted of T cell activation pathways[141]. The combined overall TIDE signature predicted checkpoint immunotherapy response better than tumor mutation load and uncovered *SERPINB9*, a gene encoding a protein inactivator of granzyme B and normally expressed in immune cells and immune-privileged sites, as a novel mechanism underlying immune evasion in cancer cells[141]. Identifying additional mechanisms of resistance in immune-infiltrated tumors that have high levels of IFN-γ will facilitate preclinical investigation of combination agents aimed at overcoming acquired resistance to checkpoint blockade.

Unlike bulk RNA sequencing, single-cell RNA sequencing allows the detection of resistance mechanisms specific to tumor subpopulations, such as cancer cells, immune cells, and fibroblasts. One such single-cell RNA sequencing study in melanoma revealed a transcriptional signature in malignant cells associated with T cell exclusion and checkpoint immunotherapy resistance that consisted of reduced antigen presentation, IFN-γ signaling, complement response, and immune modulation pathways, as well as enriched expression of chromatin regulators, transcription factors, and cyclin-dependent kinases or targets, particularly CDK4 and CDK7 (ref.[142]). Interestingly, this malignant cell transcriptional signature, which outperformed other signatures in predicting response to PD-1 inhibitors, was more pronounced in cycling cells and repressed by CDK4/6 inhibitors[142].

T cell functionality and heterogeneity

T cell clonality.

Efforts to define a functional T cell response to checkpoint blockade using genomic approaches initially focused on the clonality of the T cell response using T cell population RNA sequencing approaches. Assessments of T cell clonality traditionally relied on

sequencing the β chain of the hypervariable complementarity-determining region 3 (CDR3) of the TCR to identify a unique T cell clone because the extreme polymorphism of this region is thought to be the primary determinant of antigen-recognition specificity[143]. Greater T cell clonality, ranging from 0 to 1, with 1 indicating a monoclonal population, suggests a more restricted T cell response to a small number of antigens, such as those within the tumor microenvironment. Increased T cell clonality has been correlated with improved response to PD-1 blockade in melanoma, with a higher abundance of pre-treatment clones identified after PD-1 blockade[14,144]. Although there was no difference in clonality among responders and non-responders to anti-CTLA-4 therapy, those patients who had received anti-CTLA-4 blockade before PD-1 therapy showed an increase in T cell clonality[144].

In comparison, patients with non-small-cell lung cancer with acquired resistance to PD-1 blockade with or without CTLA-4 inhibition demonstrated diminished TCR clonality[41]. This decrease in clonal TCR frequency contrastsd with the clonal TCR expansion seen during initial response[41]. While this work highlights the overall diversity of the T cell response as a potential critical factor for checkpoint inhibitor response, this approach cannot match the TCR to its cognate neo-epitope. Instead, advances in single-cell RNA sequencing that identify the full-length TCR-α and TCR-β chains[145], when combined with improved algorithms for prediction of neoantigens, may provide more definitive evidence about the relationship between T cell clonality and corresponding neoantigens in response to checkpoint blockade.

T cell exhaustion and stemness.

Based on work in mouse models of chronic viral infections, a nuanced view of T cell functionality has emerged with the recognition that the ‘exhausted’ $CD8^+$ $PD\text{-}1^+$ T cell population itself is heterogenous, and a subset of the T cells respond to PD-1 blockade[146,147]. These populations may be more broadly broken into two classes: a subset that is considered to be ‘progenitors’ or ‘stem-cell-like’, which expresses the chemokine receptor $CXCR5^+$ and the transcription factor TCF7 (also known as TCF1), lacks the co-inhibitory receptor TIM-3, and is capable of responding to checkpoint blockade and giving rise to effector T cell progeny; and a ‘terminally exhausted’ subset that lacks CXCR5 or TCF7 but expresses TIM-3 and does not proliferate in response to PD-1/PD-L1 blockade[147,148]. The stem-like population of $Cd8^+$ $PD\text{-}1^+$ $CXCR5^+$ $TIM\text{-}3^-$ T cells has been identified in lung cancer tumors via multiparameter flow cytometry and is similar to a subset of $CD8^+$ T cells in melanoma identified by single-cell RNA sequencing approaches[149]. More recently, this $CD8^+$ $TCF7^+$T cell population, also expressing the IL-7 receptor, has been found to be enriched in melanoma tumors that respond to checkpoint blockade, which was confirmed in an independent cohort[150]. A $CD8^+$ T cell population expressing TIM-3 and CD39, an ectonucleotidase involved in adenosine signaling, was enriched in non-responders[150]. While this work focused on the $CD8^+$ T cell compartment, additional analyses of single-cell data will likely further illuminate the role that heterogeneity plays in other T cell subsets, including regulatory T cells, $CD4^+$helper and effector cells, and tissue-resident T cells, as well as the myeloid cell compartment, and the contributions of these immune cell subtypes to responsiveness to immunotherapy.

Chromatin modifier genes

SWI-SNF chromatin remodeling complexes regulate genomic architecture[151] and impact response to checkpoint inhibitors. Inactivating mutations in the *PBRM1* gene, which encodes a subunit of the PBAF SWI-SNF chromatin remodeling complex, conferred susceptibility to dual PD-1 and CTLA-4 inhibition in mouse melanoma tumors otherwise resistant to checkpoint blockade, and *PBRM1*-deficient cells showed enhanced chromatin accessibility and significantly enriched expression of IFN-γ response genes, including genes encoding chemokines that recruit effector T cells, resulting in increased intratumoral cytotoxic $CD8^+$ T cells[152]. Moreover, *PBRM1*-deficient tumors in the Cancer Genome Atlas co-associated with expression of genes related to stimulated 3' antisense retroviral coding sequences (SPARCS), a subclass of endogenous retroviruses that triggers innate immunity by generating double-stranded RNA[153]. *PBRM1* loss additionally correlated with checkpoint inhibitor response in patients with metastatic renal cell carcinoma[154]. Notably, the benefit conferred by *PBRM1* loss was predominantly observed in patients who had been previously treated, the majority of whom received VEGF inhibitors, and not in patients receiving anti-PD-1/PD-L1 as first-line treatment[154]. Prior analyses of patients with metastatic renal cell carcinoma demonstrated that *PBRM1* mutations correlated with improved response to first-line VEGF inhibitors[155,156]. A subsequent randomized phase II study of patients with treatment-naive metastatic renal cell carcinoma also found that *PBRM1* mutations were associated with improved responses in patients for whom first-line treatment was a VEGF inhibitor, but not in patients for whom it was a PD-L1 inhibitor with or without a monoclonal anti-VEGF antibody[52]. Altogether, these works indicate that prior anti-angiogenic therapy influences the association between *PBRM1* status and response to anti-PD-1/PD-L1 therapy, underscoring the need for further investigation of this pathway and the conclusion that *PBRM1* mutations should not presently be advanced as a clinical biomarker.

AT-rich interactive domain-containing protein 1A *(ARID1A)* and SWI-SNF related, matrix associated, actin dependent regulator of chromatin, subfamily A, member 4 *(SMARCA4)*, two other genes encoding SWI-SNF chromatin remodeling complex subunits, have similarly been linked to response to checkpoint inhibitors. In mice with *ARID1A*-deficient ovarian tumors, PD-L1 inhibition led to reduced tumor burden and prolonged survival compared with controls[157]. This study found that *ARID1A*loss and impaired ARID1A binding to the MMR protein MSH2 similarly reduced MMR and increased mutation frequency, leading the authors to hypothesize that *ARID1A* loss may increase response to checkpoint blockade through MMR deficiency[157]. Another study reported that four patients with ovarian small-cell carcinoma, a low-mutation cancer driven by loss-of-function *SMARCA4* mutations, had impressive responses to anti-PD-1 therapy[158]. Taken together, these studies demonstrate a role for chromatin-modifier genes in response to checkpoint inhibitors and highlight the need to confirm these findings and investigate the relationship between other epigenetic changes and checkpoint blockade response.

Additional recurrent genetic alterations

Other recurrent genetic alterations have been linked to response to immune checkpoint blockade, including *SERPINB3* and *SERPINB4 (SERPINB3/4)*, genes encoding serine protease inhibitors involved in apoptosis and autoimmunity[159], and CNAs. In two independent melanoma cohorts, it was found that *SERPINB3/4* mutations were associated with clinical benefit and longer survival after anti-CTLA-4 therapy[159]. Inactivating mutations in *SERPINB3/4* cause proteins to misfold and form inflammatory aggregates capable of triggering autoimmune disease, leading to the hypothesis that *SERPINB3/4* mutations initiate a broad tumor immune response reactivated by checkpoint inhibitors[159].

In contrast, CNAs have been connected to resistance to checkpoint blockade. A high number of somatic CNAs in patients with melanoma treated with anti-CTLA-4 therapy correlated with worse survival, and over 5,000 tumors of 12 cancer types with high levels of whole-chromosome and chromosome-arm CNAs exhibited reduced expression of cytolytic immune cell genes, specifically *CD247, CD2, CD3E, GZMH, NKG7, PRF1*, and *GZMK*[160]. Similarly, an analysis of patients with melanoma treated with checkpoint inhibitors found that non-responding tumors had a higher burden of copy-number loss and reduced expression of immune-related genes, including cytolytic markers, HLA molecules, IFN-γ pathway genes, chemokines, and adhesion molecules[144]. In sum, these studies indicate that tumor aneuploidy may be associated with decreased tumor immune infiltrates and resistance to checkpoint blockade, suggesting that CNAs may represent a survival strategy for tumors, allowing them to escape strong anti-tumor immune responses. The mechanism underlying this association may be related to lost immunogenic neoantigens enhancing immune evasion or another pathway that has yet to be discovered.

Toward clinical translation

The aforementioned genomic correlates of immune checkpoint response may serve as a blueprint around which to develop targeted therapies to overcome immune checkpoint inhibitor resistance. This section presents examples of potential therapeutic applications sparked by these genomic observations (Table 2). Numerous combination regimens with immune checkpoint blockade exist beyond those related to genomic alterations and are reviewed comprehensively elsewhere[23,161,162].

Neoantigen vaccines.

One of the most advanced areas in immunogenomics therapeutic development is personalized cancer vaccines. These efforts utilize next-generation-sequencing-based analyses to identify and target predicted neoantigens with patient-specific vaccines that stimulate an immune response tailored to each individual's tumor mutational profile[163]. Two recent studies have demonstrated that personalized neoantigen vaccines based on individual tumor whole-exome and transcriptome sequencing augmented existing T cell responses and activated new T cell responses in patients with advanced or newly metastatic melanoma[164,165]. In addition, three patients with radiologically detected metastatic lesions at the start of neoantigen vaccination, who subsequently progressed, experienced complete responses with the addition of anti-PD-1 therapy[164,165]. These neoantigen vaccines are

currently being examined in multiple clinical trials in other cancers with lower neoantigen burden, including renal cell carcinoma (NCT02950766), glioblastoma (NCT03422094), follicular lymphoma (NCT03361852), and chronic lymphocytic leukemia (NCT03219450) with and without immune checkpoint inhibitors. Whether the enhanced T cell priming and activation effects of neoantigen vaccines can overcome checkpoint blockade resistance has yet to be established.

Interferon therapies.

Another approach to addressing checkpoint blockade resistance focuses on therapies that boost anti-tumor immunity through IFN activation. IFN-γ-responsive genes involved in antigen presentation, cytokine signaling, cytotoxic activity, and adaptive immunity have been shown to correlate with response to checkpoint inhibitors[85,115]. Phase I/II trials investigating combinations of IFN-γ and checkpoint inhibitors are underway in advanced solid tumors and refractory lymphomas (NCT02614456, NCT03063632). The IFN-γ receptor depends on the JAK-STAT signal transduction pathway to regulate transcription of IFN-γ-inducible genes[166], so JAK inhibitors may curtail the efficacy of checkpoint inhibitors. In support of this concept, a phase I clinical trial in advanced solid tumors showed that the JAK1 inhibitor itacitinib combined with the PD-1 inhibitor pembrolizumab reduced peripheral T cell activation and did not affect intratumoral $CD8^+$ T cell or T regulatory cell levels, leading to lower-than-expected response rates[167]. Alternatively, T cell priming may depend on IFN-β activation by the STING protein complex acting as an endoplasmic reticulum receptor that propagates cytosolic double-stranded DNA sensing to trigger an anti-tumor immune response[113,168]. Although many STING agonists are under clinical development in combination with checkpoint inhibitors (NCT02675439, NCT03172936, NCT03010176), the single-agent activity of these therapies has been limited[169,170]. Compelling evidence indicates that STING expression is repressed by hyper-methylation in LKB1-deficient lung cancers, perhaps explaining the limited efficacy of current STING agonists and raising the question of whether combinations of hypomethylating agents and antibody-STING agonist conjugates would enhance responses to checkpoint inhibitors[116].

CDK4/6 inhibitors.

Another promising therapeutic strategy to overcome checkpoint inhibitor resistance is boosting T cell infiltration of tumors by blocking immune exclusion pathways. Targeting *CDK4*, a defining component of the malignant cell transcriptional resistance signature discovered in single-cell sequencing analyses of melanoma[142], embodies an emerging tactic to transform immune-excluded tumors into immune-infiltrated tumors. In mouse models of cancer, human breast cancer cell lines, and clinical breast cancer biopsies, CDK4/6 inhibitors have been shown to promote anti-tumor immunity by reducing regulatory T cell proliferation, enhancing effector T cell activation, and improving antigen presentation through decreased methylation of endogenous retro-viruses[171–173]. Moreover, mouse models of colon, lung, and breast cancer have shown that CDK4/6 inhibitors augment responses to PD-1 blockade[171–173], and preliminary clinical data suggest that CDK4/6 inhibition improves responses to PD-1 blockade in hormone-receptor-positive metastatic breast cancer as compared with historical response rates for either as a monotherapy[174]. Numerous phase I/II trials combining CDK4/6 inhibitors with checkpoint immunotherapy are ongoing across

tumor types, including breast, ovarian, lung, and head and neck cancers (NCT03147287, NCT03294694, NCT02779751, NCT03498378).

Anti-TGF-β therapies.

Finally, TGF-β signaling in fibroblasts constitutes another critical mechanism of immune exclusion and resistance to checkpoint inhibitors[126], as demonstrated by the finding that combined blockade of TGF-β and PD-L1 reduced stromal TGF-β signaling and augmented T cell tumor infiltration and tumor regression in a mouse model of immune-excluded mammary carcinoma[123]. Likewise, in mouse models of colorectal adenocarcinomas, TGF-β inhibition conferred susceptibility to anti-PD-L1 therapy with augmented infiltrating lymphocytes and type 1 T helper cell activation that led to metastasis eradication[175]. On the basis of this evidence, combined TGF-β and PD-1/PD-L1 inhibition is under evaluation in phase I/II trials in advanced solid tumors (NCT02947165, NCT02734160, NCT02423343). Moreover, the bifunctional fusion protein M7824, consisting of a PD-L1 monoclonal antibody linked to TGF-β receptor II, which traps all three TGF-β isoforms, displayed more effective tumor suppression than single-agent therapy in syngeneic mouse models, including long-term survival and anti-tumor immunity[176]. The dose-escalation component of a phase I study (NCT02517398) investigating this agent demonstrated a manageable safety profile and some durable responses[177], although whether this agent is more effective than PD-L1 inhibition alone has yet to be established. Table 2 displays ongoing clinical trials of therapeutic mechanisms targeting these genomic correlates of response to improve efficacy of immune checkpoint inhibitors.

Clinical trial innovation.

Innovation in trial design, patient recruitment, and clinical endpoints will help determine the best therapies informed by genomic data to combine with checkpoint blockade to overcome resistance. Basket trials organized around a specific molecular alteration constitute an important strategy to test immunotherapy combinations across tumor types in biomarker-selected patients with the highest probability of benefit (Fig. 3a)[178]. Adaptive trials may similarly augment enrollment into subgroups with promising response rates by using interim analyses to make sample size modifications that ensure statistical power in the subgroup of interest (Fig. 3b)[179]. Novel trial designs for combination dose-escalation studies include run-in periods that allow sequential combination after monotherapy within the same patient, zig-zag schedules that alternate agents for dose increases, and bifurcated arms with monotherapy escalation and combination escalation at a lower dose[180]. Beyond trial designs, patient recruitment can be improved with automated tools that prompt clinicians to consider patient-specific trials matched to actionable genomic alterations at key clinical and research time points, including when scans or labs indicate progressive disease, when tumor genetic testing is completed, and when new trials open[178]. To address the nonlinear dose-response and dose-toxicity kinetics of checkpoint inhibitors, early-phase trials should explore novel biomarker-driven response criteria and surrogate endpoints, such as circulating tumor DNA and target receptor occupancy, while avoiding additive dosing without efficacy benefits[181,182]. Likewise, new endpoints for later-stage studies, such as immune-related response criteria and durable response rate, require continued investigation[180]. Most importantly, in light of the rapidly ballooning quantity of immuno-oncology trials, academic

institutions and pharmaceutical companies need to coordinate trial efforts and sharing of correlative science data to prevent redundancy and prioritize the most promising checkpoint inhibitor combinations[161].

Future directions

Much work remains to develop these correlates of checkpoint blockade response into reliable biomarkers that can guide treatment decisions and therapeutic development. First and foremost, many of these concepts need to be validated in functional preclinical models and prospective clinical cohorts. Moreover, the search for additional genomic correlates requires clinically annotated patient cohorts with sample sizes into the hundreds and even thousands to detect rare response-associated variants[53]. This effort necessitates populations with greater diversity of race, ethnicity, age, and tumor histology[183]. Future discovery efforts should focus on detailed genomic and clinical characterization coupled with thorough investigation of less explored areas, including epigenomics, proteomics, and metabolomics, to fully characterize the relationship of host immunity, tumor biology, and the microbiome to checkpoint inhibitor response. Comprehensive clinical databases of patients treated with checkpoint inhibitors must be effectively integrated with preclinical models and screens. Ultimately, systems biology and bioinformatic approaches are needed to coordinate this vast array of information, to assess the relative importance of each component, and to develop risk scores to capture the complexity of multiple driving alterations influencing response. Altogether, these efforts to contex-tualize genomic correlates into the larger tumor-immune biology will build a deeper quantitative and conceptual understanding of response and resistance to checkpoint blockade to better promote the development of rational biomarkers and therapies.

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Box 1 |

Tumor immunogenicity

The foundation of anti-tumor immunity rests on the generation or reactivation of cytotoxic T cell responses. T cell activation is a highly coordinated and regulated activity, requiring initial stimulation through both the T cell receptor (TCR) and co-stimulatory molecules, such as CD28, a protein expressed on T cells that interacts with the ligands B7–1 (CD80) and B7–2 (CD86) on antigen-presenting cells. CTLA-4 competitively binds with high affinity to these ligands to limit initial co-stimulatory signals in lymph nodes[184]. Although murine models initially suggested that anti-CTLA-4 therapy also depleted regulatory T cells[185–187], which constitutively express CTLA-4, recent human studies have shown conflicting results[188,189]. PD-1, in comparison, is induced following initial T cell activation to regulate T cells[190]. PD-1 binds the ligands PD-L1 and PD-L2 to attenuate TCR signaling[191], thereby leading to decreased T cell proliferation, cytotoxicity, and cytokine production[192].

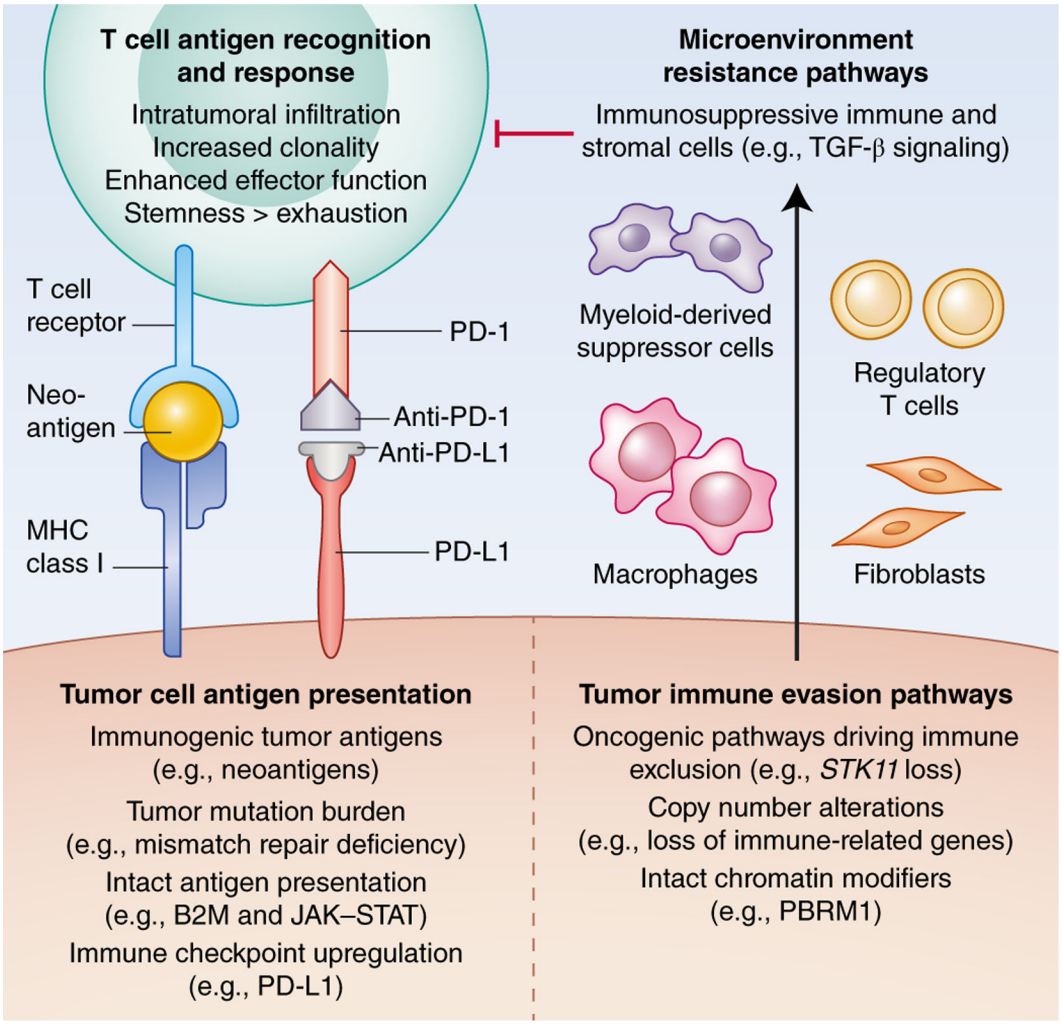

Fig. 1 |. Framework for genomic correlates of response to immune checkpoint blockade within the tumor immune microenvironment.

The left side outlines correlates of response, focusing on antigen presentation and recognition; the right side delineates resistance pathways that promote tumor immune evasion and induce immunosuppressive cells, which in turn inhibit the T cell-mediated anti-tumor response. Credit: Debbie Maizels/Springer Nature

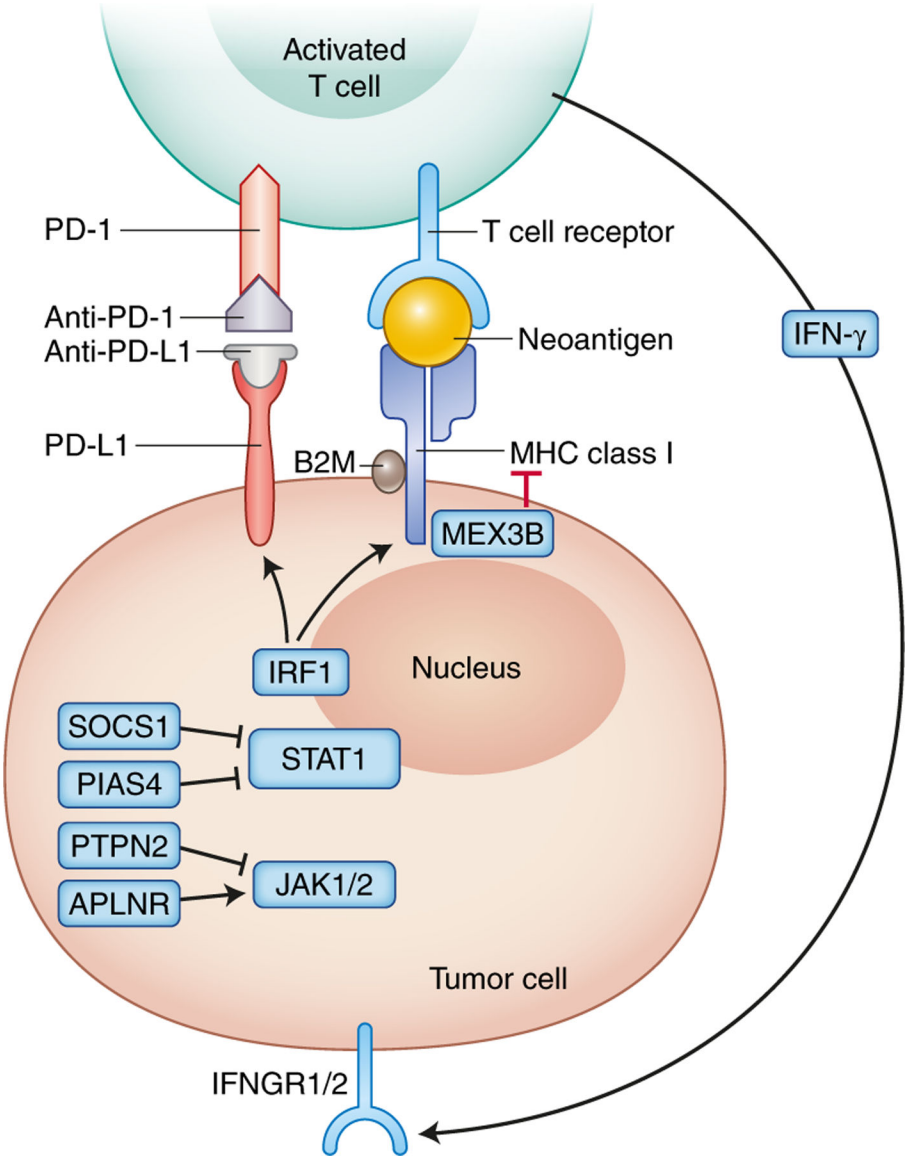

Fig. 2 |. Antigen presentation and genomic correlates of response to immune checkpoint blockade.

HLA genes *HLA-A, HLA-B*, and *HLA-C* encode the MHC class I proteins that present intracellular peptides on the cell surface to T cell receptors (TCRs) and require B2M for stabilization on the cell surface. IFN-γ, which can be released by activated T cells, binds IFNGR1/2 on tumors, which triggers JAK-STAT signaling that activates IFN response genes, including IRF1, which induces the transcription of other genes to increase the surface expression of PD-L1 and MHC molecules. Genomic mechanisms of checkpoint blockade response include upregulation of IFN-γ response gene expression[85,86], loss of protein tyrosine phosphatase non-receptor type 2 (PTPN2)[74], and germline HLA heterozygosity[82]. Conversely, genomic pathways of resistance include *B2M* loss[37,71–73], somatic HLA loss of heterozygosity[82], reduced HLA gene expression[84], increased expression of *MEX3B*[83], *IFNGR1/2* or *IRF1*copy-number loss[87], *JAK1/2* inactivation[72,88,89], *APLNR* (apelin receptor) loss[76], and amplification of negative regulators of the IFN-γ pathway, including

SOCS1 (suppressor of cytokine signaling 1) and *PIAS4* (protein inhibitor of activated STAT4)[87]. Credit: Debbie Maizels/Springer Nature

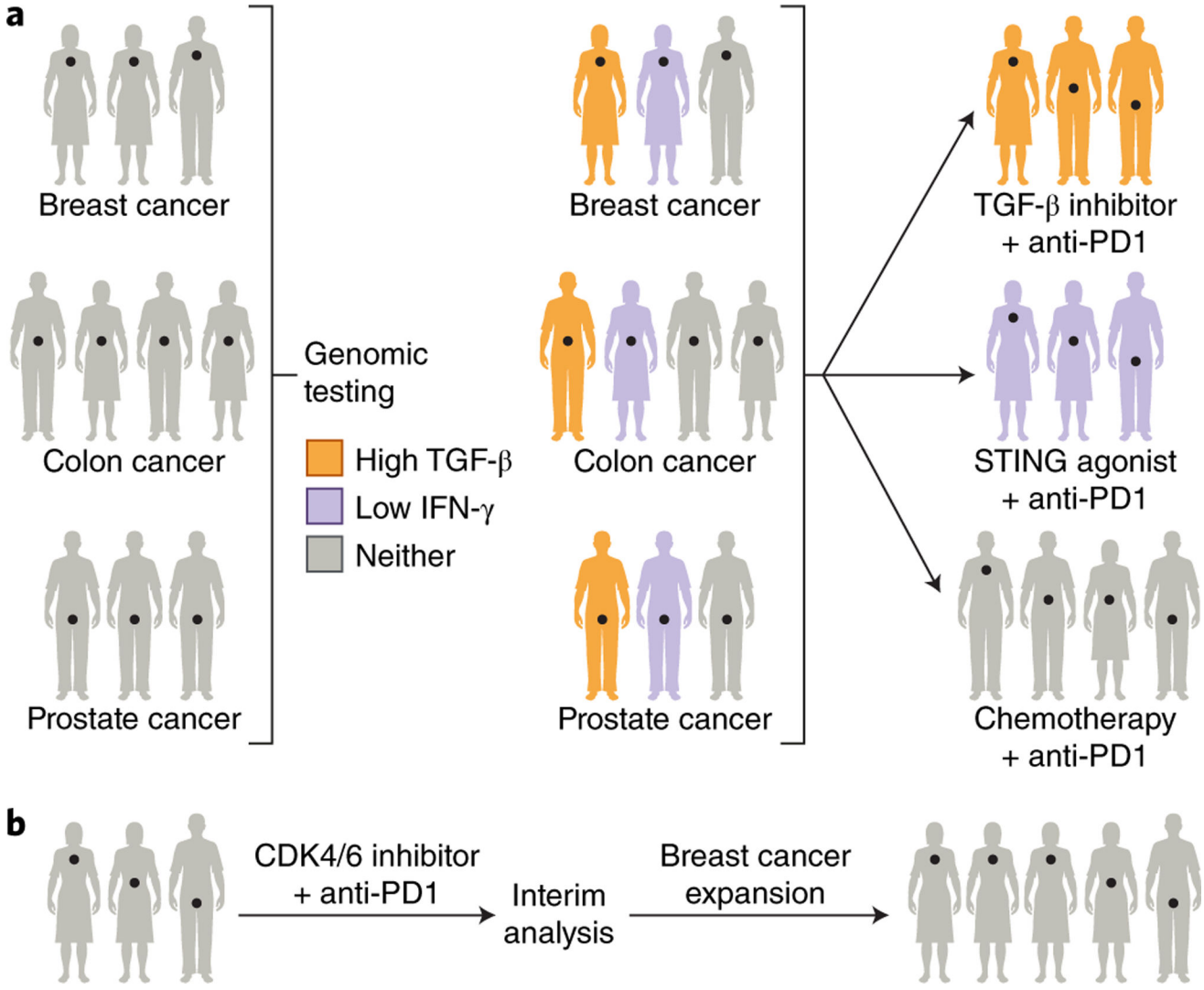

Fig. 3 |. Trial designs to overcome checkpoint blockade resistance.

a, A basket trial matches therapeutic agents based on genomic alterations irrespective of tumor site of origin. In the illustrated example, breast, colorectal, and prostate tumors are tested for TGF-β and IFN-γ transcriptomic signatures. Those patients whose tumors possess high TGF-β or low IFN-γ signatures are treated with TGF-β inhibitors or STING agonists, respectively, in addition to anti-PD-1 therapy, while tumors without genomic targets are treated with chemotherapy and anti-PD-1 therapy. **b**, An adaptive trial design employs interim analyses to make modifications, such as expansion of patient cohorts with more promising responses, illustrated in the figure as an expansion of the breast cancer cohort. Credit: Debbie Maizels/Springer Nature.

Table 1 |

Genomic correlates of response and resistance organized by primary location

Primary location	Response category	Defining characteristics or examples
T cell	Intratumoral infiltration[85,115,135–137,139]	Transcriptional signatures of cytotoxic lymphocytes infiltrating the tumor core
	Enhanced effector function[52,134,141]	Increased expression of *PRF1*, *GZMA/B*, *CD8A*, and *IFNG*
	Increased clonality[14,144,41]	Ranging from 0 to 1, with 1 indicating a monoclonal population
	Greater sternness[147,150]	Express chemokine receptor CXCR5 and transcription factor TCF7; lack TIM-3/CD39
	Reduced exhaustion[147,150]	Express co-inhibitory receptor TIM-3 and ectonucleotidase CD39; lack CXCR5/TCF7
Tumor cell (response mechanisms)	Tumor antigens[31,32,34–40,54,57,65,67]	Neoantigens, viral antigens
	Increased tumor mutation burden[9,37,48]	Mismatch repair deficiency
	Immunogenic alterations[159]	Inactivating mutations in *SERPINB3* and *SERPINB4*
	Mutational signatures[39,53,108]	Smoking, ultraviolet light, alkylating agent therapy, APOBEC
	Genomic upregulation of PD-L1 (refs.[50,92–94,97–100])	*PDL1* amplification and loss of *CDK4*, *SPOP*, and *CMTM4* and *CMTM6*
	Chromatin modifier loss[152,154,157,158]	Inactivating mutations in *PBRM1*, *ARID1A*, and *SMARCA4*
Tumor cell (resistance mechanisms)	Tumor antigens[68]	Cancer/testis antigens similar to self and less immunogenic
	Deficient antigen presentation[37,53]	Inactivating mutations in *B2M*, *HLA*, JAK/STAT, and IFN-γ response genes
	Oncogenicpathways[45,113–115,117,118,124,125,129,130,133]	Inactivating *STK11* and *PTEN* mutations, WNT/β-catenin, *EGFR* and *KRAS* mutations
	Immune evasion alterations[141]	Increased expression of *SERPINB9*
	CNAs[144,160]	High levels of copy-number loss, chromosome arm and whole-chromosome CNAs
Microenvironment	Immunosuppressive stromal cells[115,123,126,140]	Transcriptional signatures of fibroblasts, endothelial cells, and TGF-β signaling
	Immunosuppressive immune cells[136,141]	Transcriptional signatures of myeloid-derived suppressor cells and regulatory T cells

Table 2 |

Trials targeting genomic correlates of response to immune checkpoint blockade

Intervention	Trial number	Patient population	Phase	Setting	Status
Neoantigen vaccines					
NeoVax + anti-CTLA-4 ipilimumab	NCT02950766	Stage III or low-volume stage IV RCC	I	Adjuvant/1st line	Not yet recruiting
NeoVax + anti-PD-1 nivolumab ± anti-CTLA-4 ipilimumab	NCT03422094	Newly diagnosed, unmethylated glioblastoma	I	1st line	Recruiting
NeoVax + ant-CD20 rituxumab	NCT03361852	Follicular lymphoma	I	1st line	Not yet recruiting
NeoVax + alkylating agent cyclophosphamide	NCT03219450	Chronic lymphocytic leukemia	I	1st line	Not yet recruiting
Interferon therapies					
Interferon-γ-1β + anti-PD-1 pembrolizumab	NCT03063632	Mycosis fungoides and Sézary syndrome	II	Refractory	Recruiting
Interferon-γ + anti-PD-1 nivolumab	NCT02614456	Advanced solid tumors	I	2nd line	Recruiting
JAK1 inhibitor itacitinib or PI3Kδ inhibitor + anti-PD-1 pembrolizumab	NCT02646748	Advanced solid tumors	I/II	Refractory	Recruiting
JAK2 inhibitor ruxolitinib + anti-PD-1 pembrolizumab	NCT03012230	Metastatic triple-negative breast cancer	I	2nd line	Recruiting
JAK1 inhibitor itacitinib + anti-PD-1 pembrolizumab	NCT03425006	Metastatic PD-L1$^+$ NSCLC	II	1st line	Recruiting
STING agonist MIW815 + anti-PD-1 spartalizumab	NCT03172936	Advanced solid tumors or lymphomas	I	Any line	Recruiting
CDK4/6 inhibitors					
Fulvestrant ± CDK4/6 inhibitor palbociclib ± anti-PD-L1 avelumab	NCT03147287	Metastatic HR$^+$HER2$^-$ breast cancer	II	2nd-3rd line	Recruiting
Tamoxifen ± CDK4/6 inhibitor palbociclib followed by anti-PD-LI avelumab	NCT03573648	Stage II or III HR$^+$ breast cancer	II	Neoadjuvant	Recruiting
CDK4/6 inhibitor ribociclib + anti-PD-1 spartalizumab (+ fulvestrant if breast)	NCT03294694	Metastatic ovarian cancer or HR$^+$HER2$^-$ breast cancer	I	Any line	Recruiting
CDK4/6 inhibitor abemaciclib + anti-PD-1 pembrolizumab	NCT02779751	Metastatic NSCLC or HR$^+$HER2$^-$ breast cancer	I	1st-3rd line	Recruiting
Anti-PD-L1 avelumab, cetuximab, + CDK4/6 inhibitor palbociclib	NCT03498378	Recurrent/metastatic headand neck squamous cell carcinoma	I	Any line	Recruiting
Anti-TGF-β therapies					
Anti-TGF-β NIS793 + anti-PD-1 spartalizumab	NCT02947165	Advanced solid tumors	I	Refractory	Recruiting
TGF-β receptor 1 inhibitor galunisertib + anti-PD-LI durvalumab	NCT02734160	Metastatic pancreatic cancer	I	3rd line	Recruiting
TGF-β receptor 1 inhibitor galunisertib + ant-PD-1 nivolumab	NCT02423343	HCC or recurrent NSCLC	I/II	2nd line	Recruiting
Anti-PD-L1/TGF-β trap M7824	NCT02517398	Metastatic or locally advanced solid tumors	I	Refractory	Recruiting
Anti-PD-LI/TGF-β trap M7824 + treatment of physician's choice	NCT03620201	Stage II-III HER2$^+$ breast cancer	I	Neoadjuvant	Recruiting
Anti-PD-LI/TGF-β trap M7824 versus anti-PD-1 pembrolizumab	NCT03631706	PD-L1$^+$ NSCLC	II	1st line	Recruiting

HCC, hepatocellular carcinoma; HER2, human epidermal growth factor receptor 2; HR$^+$, hormone receptor positive; NSCLC, non-small-cell lung cancer; Refractory, failed prior standard therapy (including subject refusal or intolerance); RCC, renal cell carcinoma