Predictors of response and resistance to checkpoint inhibitors in solid tumors

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Checkpoint inhibitors (blocking antibodies to PD-1, PD-L1, *CTLA-4*) have proven effective against several tumor types. Unfortunately, only a minority of tumors within each subtype responds, and checkpoint inhibitors can cause significant toxicity. There is a flurry of activity around determining patient and tumor factors of response and resistance to this class of medication. To date, most of the data surrounds mutational burden and amount of PD-L1 staining in pre-treatment samples.

Roh *et al.* examined clinical and correlative characteristics of advanced melanoma patients as their melanoma progressed through treatments with *CTLA-4* inhibitors and PD-1 inhibitors. Of an initial cohort of 56 patients, 54 were treated with *CTLA-4* inhibitor, and 7 of them responded (1). The non-responders were then treated with the PD-1 inhibitor, and 14 (28%) responded (1). Using serial biopsies, the authors identified several tumor factors that favor response and resistance (1). Of note, their definition of response is either radiographic partial or complete remission or stable disease lasting at least 6 months (1).

The authors undertook an impressive level of investigation, building upon their previous report of deep immune profiling. In their sample, they did not find a correlation between number of mutations, tumor heterogeneity or T cell receptor clonality with response or resistance. However, they did find that a high number of copy losses was associated with lack of response to checkpoint inhibition. Specifically, they found that patients who progressed on both *CTLA-4* and PD-1 blockade were more likely to have higher numbers of copy loss than those who responded to *CTLA-4* blockade. They did not see an association between copy number loss and mutational burden. In examining the types of genes for which there were copy number alterations, they observed that those subjects who did not respond to either agent tended to have recurrent copy number loss in tumor suppressor genes on chromosomes 6q, 10q, and 11q23.3 (1).

To confirm that copy number loss is associated with lack of response to checkpoint inhibitions, they used whole exome sequencing from a separate cohort of 110 melanoma patients and RNA sequencing from data from 42 additional melanoma patients. For this analysis, copy number loss was not associated with resistance, but lower number of copy number loss was associated with benefit. While tumor suppressor genes continued to be a factor, the specific mutations were not overlapping. Using a previously described association between loss of chromosome 10 and resistance to *CTLA-4* inhibitors, the authors found that loss of copy number on chromosome 10 was also associated with lack of response to *CTLA-4*, perhaps because PTEN resides on that chromosome, and PTEN loss is a putative driver of resistance.

This analysis is an important addition to the body of knowledge surrounding response and resistance in

Table 1 Summary of clinical investigations of response/resistance to checkpoint inhibitors using human subjects

Factor (reference)	Results
Stool microbiome	Those with baseline microbiome enriched with Faecalibacterium genus and other Firmicutes had a longer progression free survival and overall survival on treatment with ipilimumab, n=26
Neutrophil to lymphocyte ratio	High neutrophil to lymphocyte ratio correlates with poor survival in ipilimumab treated metastatic melanoma patients, n=58
Neutrophil, LDH	Patients with metastatic melanoma treated with ipilimumab who have low neutrophils and low LDH tended to live longer than 24 months whereas those with high neutrophils and high LDH lived less than 24 months, n=113
Eosinophil count	Patients with melanoma treated with pembrolizumab who had a high relative eosinophil count (≥1.5%) and high relative lymphocyte count (≥17.5%) and low LDH had longer overall survival, n=616
Beta catenin expression	Patients with metastatic melanoma with high density of CD8+ T cells and beta-catenin <10% had a longer progression free survival than those without CD8+ T cell infiltration and beta-catenin ≥10%, n=64
JAK 2 and beta-2-microglobulin	In melanoma patients, JAK 2 and beta-2-microglobulin mutations were correlated with resistance to PD-1 inhibitors, n=4
TIM-3 upregulation	Upregulated TIM-3 was seen in 2 lung cancer patients treated with PD-1 inhibitor
Mutational burden	High mutational load was correlated with improved survival in melanoma for two studies, n=25 and n=38, respectively
Specific neoantigens	Specific tumor tetrapeptide sequences predicted to bind to MHC class I molecules were correlated with improved survival in all patients with melanoma treated with CTLA-4 antibodies, n=25 for discovery set, n=39 for validation set
Intratumor heterogeneity and high clonal neoantigen burden	In non-small cell lung cancer treated with PD-1 inhibition (n=34) and melanoma (n=64) treated with CTLA-4 inhibition, those with tumors exhibiting low neoantigen intratumor heterogeneity and a high clonal neoantigen burden had improved overall survival relative to those with high intratumor heterogeneity and high clonal neoantigen burden
PD-L1 expression	There were significantly greater objective response rates to PD-1/PD-L1 inhibitors in a pool analysis of more than 6,000 patients with solid tumors for those with high PD-L1 expression. 46% of patients had positive PD-L1 expression
PTEN	Melanoma patients treated with PD-1 inhibitors were more likely to respond to therapy if their tumor had at least 90% expression of PTEN, n=39
CD8+ cells	Expansion of CD8+ T cells within the tumor occurred in melanoma patients responding to PD-1 inhibitor therapy, n=46. Baseline levels of CD8, PD-1, PDL1 expressing cells at the invasive tumor margin and within the tumor, n=46 (doi: 10.1038/nature13954)
T cell receptor clonality	More restrictive T cell receptor repertoire (per beta chain) at baseline predicts for response, n=46 (ref as above)

LDH, lactate dehydrogenase; JAK, Janus kinase; TIM-3, T-cell immunoglobulin mucin-3; PD-1, programmed death-1 receptor; PD-L1, programmed death-1 receptor ligand; CTLA-4, cytotoxic T-lymphocyte antigen 4; PTEN, phosphatase and tensin homolog.

checkpoint inhibitors. Other studies of clinical specimens have shown that there are host and tumor factors that are linked to response and resistance (*Table 1*). Some of these factors can be easily examined on routine laboratory tests, whereas others require tissue biopsy. With the exception of PD-L1 expression and microsatellite instability, both of which are most reliably gauged using a tumor biopsy, none of these is in routine clinical use.

Checkpoint inhibitors are one mechanism by which to overcome tumor-associated immune suppression. However, the upregulation of these negative regulators on T cells is not the only mechanism by which the tumor evades an active immune response. In fact, tumors utilize multiple, often redundant, "layers" of immune suppression. Blood vessels within the tumor but not adjacent normal tissue express the death mediator Fas Ligand (FasL) as a

mechanism by which to kill tumor infiltrating Fas+CD8 T cells (2). Interestingly, CD4+Foxp3+ suppressive cells express lower levels of Fas thereby protecting them from FasL mediated apoptosis (2). Tumor cells also develop mechanisms to resist immune cell killing. Some of the mechanisms described include the upregulation of IAPs (inhibitors of apoptosis) (3), increased expression of pro-survival genes such as *BCL2/BCL-XL* (3), downregulation of MHC I on the tumor cell surface (4) and upregulation of T cell inhibitory molecules such as PD-L1. In addition, because tumor cells undergo high rates of proliferation, they also have demanding metabolic needs. This rapid consumption of extracellular nutrients leads to a hostile, hypoxic, microenvironment for immune cells (5) and starves cytotoxic T cells of the necessary metabolic substrates needed for optimal effector function (6). Aside from tumor cells, the tumor microenvironment is a highly inflammatory environment rich in stromal cells and leukocytes that secrete inhibitory molecules such as TGF-β (7), IDO (8), IL-10 (9), and arginase (10). Therefore, effective immunotherapies must overcome multiple layers of immune cell suppression within the tumor microenvironment. While checkpoint blockade targets one of these layers, combination therapies that target multiple layers may find synergy in the clinic.

Host factors of response and resistance

Studies of host factors have identified composition of the gut microbiome through characterization of stool specimens at baseline is associated with response. In a study of 26 patients receiving ipilimumab for metastatic melanoma, fecal microbiota was assessed using 16S ribosomal RNA sequencing before and after ipilimumab infusion. Researchers found that patients with progression free survival of more than 6 months had a significant presence of Faecalibacterium genus and other Firmicutes bacteria in their microbiota compared to non-responders in whom Bacteroides was predominant. Autoimmune colitis was found more in patients with a high proportion of Firmicutes in their microbiota, while no colitis occurred in Bacteroides predominant microbiota patients. It is intriguing that the gut microbiota could have a significant effect on checkpoint inhibitor therapeutic efficacy and adverse effects. Some have proposed manipulation of gut microbiota prior to treatment administration to maximize therapeutic efficacy and minimize adverse effects. Fecal transplant is a recognized treatment for antibiotic refractory Clostridium difficile infections and may be utilized for patients undergoing checkpoint inhibitor therapy (11).

Aging results in thymic involution suggesting that aging results in a systemic reduction in number of T-cells and worse antitumor response. One study testing the effect on age on ipilimumab efficacy found that age did not significantly affect response to ipilimumab. The study was conducted on 188 patients over the age of 70 with unresectable advanced (stage III, stage IV) melanoma and found an immune related disease control rate (immune related complete response/partial response/stable disease) of over 3 months in 38% of elderly patients compared to an immune related disease control of 33% in 645 patients less than 70. The differences between age groups in median progression free survival and overall survival were not statistically significant (over 70, n=193; less than 70, n=662). The results of this study suggest that age is inconsequential factor on checkpoint inhibitor therapeutic efficacy (12).

Certain laboratory biomarkers can be predictive of increased response to checkpoint inhibitor therapy. One study conducted on 58 melanoma patients treated with ipilimumab found that a neutrophil to lymphocyte ratio greater than or equal to four before treatment was correlated with worse overall survival. Another study also examining biomarkers for survival for 113 metastatic melanoma patients treated with ipilimumab found that low serum LDH and neutrophils were significantly correlated with patients who lived more than 2 years while high serum LDH and neutrophils were significantly correlated with patients who lived less than 2 years (13). Another study looked for biomarkers associated with overall survival in 616 melanoma patients treated with pembrolizumab and found that a high relative eosinophil count (≥1.5%), high relative lymphocyte count (≥17.5%), and low LDH were significantly correlated with better overall survival (14).

Another study tested the effect of ipilimumab on median overall survival in 187 HLA-A*0201-positive patients and 266 HLA-A*0201-negative patients (15). They found that median overall survival was similar in both patients, suggesting that the mechanism of ipilimumab is not dependent on HLA haplotype.

Tumor factors of response and resistance

Activation of the WNT pathway may be predictive of resistance to checkpoint inhibitor therapy due to downregulation of CD8+ T-cell infiltrate in the tumor microenvironment. One study testing the effect of baseline beta-catenin expression and prognosis of 64 metastatic

melanoma patients found that greater beta-catenin expression correlated with decreased CD8+ T-cell infiltrate in the tumor microenvironment and vice versa. The results of this study suggest that activation of the Wnt pathway could be assessed before checkpoint inhibitor administration because adequate CD8+ T-cell presence is necessary in the tumor microenvironment for therapeutic efficacy (16).

JAK-2 and beta microglobulin mutations may be predictive of resistance to pembrolizumab, while mismatch repair deficiency mutations can be predictive of response to pembrolizumab. However, HLA status does not affect response to ipilimumab. Biopsy samples were analyzed from four relapsing patients with metastatic melanoma who progressed on pembrolizumab. Two of four patients had mutations in the JAK kinase while a third patient had a mutation in the beta-2-microglobulin antigen presenting protein. A cell line from a patient with a JAK2 mutation found lack of *PD-L1* upregulation in response to stimulation with interferon gamma. Beta-2-microglobulin is part of the MHC I complex and mutations in beta-2-microglobulin resulted in loss of outer membrane localization of the MHC I complex which would result in subsequent immune evasion (17).

Another study compared the response to pembrolizumab in 32 patients with progressive metastatic colon cancer with or without mismatch repair deficiency (mismatch repair deficient, n=11; mismatch repair proficient, n=21) and 9 patients with mismatch repair tumors that were not colorectal. Patient with mismatch repair defects in their tumors had a significantly greater immune related objective response rate and progression free survival rate on pembrolizumab than patients without mismatch repair defects (18). The results suggest that mismatch repair defects are predictive of therapeutic efficacy in response to pembrolizumab.

Alternative immune checkpoint inhibitors in tumors can be predictive of resistance to checkpoint inhibitor therapy. One study assessed two lung patients who had progressed on pembrolizumab and compared their tumor antigens to five lung patients who had not been treated with immunotherapy. The two lung cancer patients treated with pembrolizumab had significant TIM-3 upregulation on the T-cells compared to patients who had not been treated with pembrolizumab (19). Perhaps pembrolizumab should be combined with an antibody to TIM-3 to maximize patients' overall survival.

Higher mutational load in a tumor improves overall survival and could be predictive of response to checkpoint inhibitor therapy. Number of intratumoral mutations can influence overall survival. A study performed on 25 patients treated with ipilimumab aimed to elucidate whether the certain aspects of the tumor genome resulted in improved response to anti-*CTLA-4* therapy. Whole exome sequencing compared DNA from tumors to normal blood from the same patient. Increased number of mutations was significantly correlated with improved survival and improved response to anti-*CTLA-4* therapy; however, there were tumors with a large number of mutations that did not respond to therapy (20). Perhaps treatments prior to checkpoint inhibitor therapy should attempt to increase the number of mutations in tumors since a higher mutational load improves overall survival. In 2017, the Food and Drug Administration approved the use of pembrolizumab in any cancer that exhibits microsatellite instability (21).

The tumor landscape can be predictive of response to checkpoint inhibitor therapy. A study of 25 patients treated with ipilimumab also identified a highly significant association with peptide signatures of tumors and benefit from *CTLA-4* blockade. The researchers searched for conserved stretches of amino acids predicted to bind to MHC class I molecules amongst multiple tumors. They identified many tetrapeptide sequences that were highly correlated with strong response to *CTLA-4* blockade and increased overall survival. High mutational load likely increased the probability of formation of the tetrapeptide sequences (20).

Another study investigating the relationship between tumor landscape and response to checkpoint inhibitor therapy was conducted on 34 patients with non-small cell lung cancer treated with pembrolizumab. Researchers found that tumors that responded to pembrolizumab had significantly lower neoantigen intratumor heterogeneity than tumors with no clinical benefit. Overall, tumors with low neoantigen intratumor heterogeneity were associated with better overall survival (22).

A systematic review investigating the utility of using PD-L1 expression in solid tumors as a predictive biomarker of response to PD-1/PD-L1 inhibitors, compiled data from 41 clinical trials with 6,664 patients with solid tumors with a focus on objective response rates. The odds of patients responding to PD-1/PD-L1 inhibitor therapy with PD-L1 positive tumors were significantly higher than patients whose tumors were PD-L1 negative (23).

One study showed that PTEN loss correlated with poor response to PD-1 inhibitor treatment in 39 melanoma patients. Tumors with less than 10% of cells expressing

PTEN via immunohistochemistry were classified as PTEN absent. Patients with PTEN present in their tumors had significantly greater tumor reduction than patients with PTEN absent (24).

Another study trying to elucidate patterns predictive for PD-1 inhibitor response in 46 patients with metastatic melanoma, found that tumors that responded to therapy had significantly more CD8 T-cells and PD-1 and PD-L1 expressing cells within it and at the invasive tumor margin as well as a more restrictive T cell receptor beta chain repertoire (25).

These studies suggest that the tumor landscape of the patient should be analyzed before administration of checkpoint inhibitor therapy for predictive peptide signatures, ligands, and low neoantigen intratumor heterogeneity.

We need reliable biomarkers to identify patients who are most likely (or even least likely) to respond to checkpoint inhibitors. Preferably these markers would not require biopsy, as some tumors cannot safely be biopsied. It is very likely that a single marker is insufficient and that an algorithm, or molecular signature, is needed. To definitively answer this question, we need more collaboration to increase the power of our analyses. Roh and colleagues are getting us one step closer.

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Footnote

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