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# Gene expression profiling to predict responsiveness to immunotherapy

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## Abstract

Recent clinical successes with immunotherapy have resulted in expanding indications for cancer therapy. To enhance anti-tumor immune responses, and to better choose specific strategies matched to patient and tumor characteristics, genomic-driven precision immunotherapy will be necessary. Herein, we explore the role that tumor gene expression profiling (GEP) and transcriptome expression may play in the prediction of an immunotherapeutic response. Genetic markers associated with response to immunotherapy are addressed as they pertain to the tumor genomic landscape, the extent of DNA damage, tumor mutational load, and tumor-specific neoantigens. Furthermore, genetic markers associated with resistance to checkpoint blockade and relapse are reviewed. Finally, the utility of GEP to identify new tumor types for immunotherapy and implications for combinatorial strategies are summarized.

## INTRODUCTION

Evidence has identified and linked loci within tumor genomes to prognostic and predictive outcomes.(1–4) While not a new concept, genomic-driven approaches have catalyzed the recent success of targeted therapeutic strategies in yielding enhanced survival benefits for patients with cancer whose tumors depend on specific growth and proliferation pathways. In parallel, immunotherapeutic pathways have been further elucidated and recent clinical successes have resulted in ever expanding indications for cancer therapy. Particularly, monoclonal antibodies against immune checkpoint molecules including programmed death receptor 1 (PD-1) and cytotoxic T-lymphocyte antigen 4 (CTLA-4) are revolutionizing



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cancer treatment for patients with advanced disease. These strategies have shown significant therapeutic impact across numerous tumor types, notably cancers traditionally considered to be non-immunogenic.(5) To enhance anti-tumor immune responses, and to better choose specific strategies matched to patient and tumor characteristics, genomic-driven precision immunotherapy will be necessary.

Significant and durable response rates have been reported with checkpoint blockade in melanoma, NSCLC, bladder cancer and Hodgkin's lymphoma.(6) However, the clinical benefit of these inhibitors has been limited to a subset of patients and only certain tumor types.(7, 8) In a pooled analysis of all patients from clinical trials employing the CTLA-4 inhibitor ipilimumab as a monotherapy, an overall 5-year survival rate approaching 20% was achieved.(9) For PD-1 blockade (nivolumab), long-term data remains immature, however, median survival appears to extend beyond 2 years with monotherapy response rates up to 43%.(10, 11) Recently, a combined ipilimumab and nivolumab regimen was utilized in advanced melanoma with a response rate close to 60% in patients receiving dual therapy, though with not insignificant autoimmune complications.(12) This emphasizes the necessity for selection stratification of those patients most sensitive to therapy, and subsequently, predictive biomarker development to identify responders from non-responders. Further benefits of a personalized approach to immunotherapy include minimization of unnecessary exposure to potentially life-threatening immune-related toxicities, particularly when regimens are combined, (9, 13) as well as reduction of the financial implications imposed on health systems by costly therapeutics.

The development of large-scale rapid throughput gene expression profiling (GEP) of tumors has enabled patient selection with targeted therapies. Subsequently, analysis of the transcriptome provides further granularity to enhance patient selection and prediction of response. In this review we explore the role that transcriptome expression and GEP may play in the prediction of an immunotherapeutic response. Genetic markers associated with response to immunotherapy will be addressed as they pertain to 1) tumor genomic landscape 2) the extent of DNA damage 3) tumor mutational load and 4) tumor-specific neoantigens. Furthermore, genetic markers associated with resistance to checkpoint blockade and relapse will be reviewed. Finally, the utility of GEP to identify new tumor types for immunotherapy and implications for combinatorial strategies will be summarized.

## GENETIC MARKERS ASSOCIATED WITH RESPONSE TO IMMUNOTHERAPY

Gene expression profiling of many tumors, including lymphoma, NSCLC, and breast cancer, has been a powerful technique to identify prognostic gene expression signatures.(14–17) More recently, gene expression signatures predictive of response to chemotherapy have been described, including for cetuximab in colorectal cancer(18) and for cisplatin and fluorouracil in gastric adenocarcinoma.(19) Gene expression profiling has subsequently been successfully applied in the detection of the global tumor transcriptome to gain mechanistic insights into the activity and toxicity of immunotherapy agents.(20) An immune responsiveness phenotype in cancer that can be further enhanced by immune stimulation has previously been defined in various tumors, notably in colon adenocarcinomas resected from patients who are likely to experience prolonged relapse-free survival.(21) Thus, gene

signatures that predict how patients will respond to immunotherapy are of particular interest. Recently, genome-wide analysis of serial melanoma biopsies from patients treated with recombinant IL-2 revealed a signature predictive of clinical response from pretreatment biopsies.(22) Other examples include a 12-chemokine gene expression signature, also in melanoma, that was associated with improved survival. This gene signature is under validation in other histologies, including lung cancer.(23) In advanced melanoma, complex immune signatures have also been explored. For example, Ribas and colleagues described an interferon-inflammatory immune gene expression signature associated with both enhanced overall response rates (ORR) and progression free survival (PFS) in patients treated with pembrolizumab,(24) which is subsequently being investigated in other malignancies.

More recently, an eight-gene signature reflecting preexisting immunity, the T-effector/IFNg signature, was explored in a phase II trial of previously treated non-small-cell lung cancer (NSCLC) (POPLAR trial). High gene expression signature levels appeared to be prognostic of prolonged overall survival, though not PFS or ORR, in patients treated with atezolizumab. (25) A similar signature was determined for patients receiving MAGE-A3 immunotherapy. (26) This 84-gene expression signature that associated with clinical response was identified in metastatic melanoma and confirmed in resected NSCLC patients. The genes were mainly immune-related involving interferon gamma pathways and specific chemokines, supporting data that the pretreatment tumor immune microenvironment was associated with clinical response to this immunotherapy. However, in the recently reported NSCLC MAGRIT trial (MAGE-A3 as Adjuvant Non–Small-Cell Lung Cancer Immunotherapy trial) (27), adjuvant MAGE-A3 did not increase disease-free survival compared with placebo in patients with MAGE-A3-positive tumors, and a gene signature predictive of clinical response could not be determined. The genes selected for the panel comprised of immune-related Th1/interferon-γ genes previously discovered in the phase 2 melanoma study(26) with additional genes related to biological pathways of the previously described predictive signature. No difference in outcome was evident despite application of the gene signature as a potential predictive biomarker. These results support that immunological responses to vaccines may not translate into clinical responses(28–30), which highlights the difficulty and complexity in creation of predictive gene signatures using GEP in these trials and amongst different tumor types. Thus, data on the creation of predictive gene signatures will be reviewed in the specific context of genomic landscape, DNA damage, mutational load, and neoantigens.

## Response to immunotherapy in the context of the intratumoral genomic landscape

The immunologic and biological mechanisms underlying response or resistance to immune-checkpoint blockade remain poorly understood. Despite observations of clinical benefit from blockade in many cancer types, pretreatment correlates of response are incompletely characterized. Immune checkpoint blockade is hypothesized to function by attenuating immunosuppressive signals, thus releasing the brakes and activating antigen-specific immune-mediated destruction of tumor cells.(31, 32) This pro-inflammatory tumor microenvironment can be characterized through genomic and transcriptomic analyses and correlated with response to therapy.

Common characteristics of inflamed tumors responsive to anti-PD1 therapy include dense CD8+ T-cell infiltrates, a broad chemokine profile, PD-L1 expression on immune cells, a type 1 interferon (IFN) signature, and elevated expression of IFN-gamma induced genes.(33, 34) High cytolytic activity is also correlated with expression of IFN-stimulated chemokines (CXCL9, CLCL10 and CSCL11) capable of attracting T cells to the tumor site.(35) For anti-CTLA-4 therapy, early on-treatment proliferation of ICOS+ CD4+ T-cells can be used as a pharmacodynamic biomarker for monitoring of treatment efficacy.(36) In melanoma several cytokine and chemokine signaling genes, as well as the cytotoxic factors granzyme A and perforin 1, are differently expressed prior to ipilimumab therapy between responders and non-responders.(37, 38)

IHC-based studies support the notion that the density and location of CD8+, CD4+, PD-1+, and PD-L1+ in tumor cells and/or tumor-infiltrating immune cells in pretreatment biopsies can predict therapeutic response to immune checkpoint inhibitors.(39, 40) While pre-treatment PD-L1 expression in tumor biopsies does appear to predict response to anti-PD-1 therapies,(10, 34, 41) many tumors scored as PD-L1 positive do not respond, while some responses occur in PD-L1-negative tumors.(12, 42) Such conflicting results likely reflect the limitations related to IHC staining and scoring techniques, as well as to the complexity related to PD-L1 expression in a single biopsy across cells present in the tumor microenvironment, including infiltrating myeloid cells; therefore, better biomarkers are clearly needed to optimize therapeutic decisions. Expression of PD-L1/PD-L2 and CTLA-4 represent mechanisms of immune regulation and opportunities for gene expression-based targeting with immune checkpoint blockade.(34, 38) High pretreatment levels of CTLA-4 mRNA are predictive of response to both anti-CTLA-4 and anti-PD-L1 therapy. Further, amplification of the PD-L1/PD-L2 regions has been demonstrated to be associated with high cytolytic activity in cervical, gastric and colorectal cancer.(35) In Hodgkin’s lymphoma, amplification of PD-L1/PD-L2 is associated with an approximately 90% response rate to nivolumab.(43)

A gene expression profiling mechanism to characterize the tumor microenvironment has been created using a recently developed computational method to characterize tumor-infiltrating immune cell subsets (CIBERSORT) using RNA-sequencing (RNA-seq). This technique addresses many limitations of IHC, has enabled detailed transcript analysis of the tumor leukocytic infiltrate, and is capable of predicting prognosis from pretreatment biopsies.(44) Further, useful insight can be gained into the relationship between regulatory cell types (T-regulatory cells and myeloid derived suppressor cells) and checkpoint inhibition responses.(45)

Another significant benefit of transcriptomic analysis beyond quantitative IHC assessment is the extent of supplementary information assessable regarding inflammation associated gene signatures, oncogenic drivers, checkpoint protein expression and cell subtypes. Yet this approach lacks the spatial and structural fidelity (invading edge, density) afforded by detailed characterization of immune infiltrates which has established prognostic utility(46), therefore a complementary approach may be optimal.

Defects in antigen processing and presentations machinery (APM) are also frequently observed in the tumor microenvironment as an immune evasion mechanism.(35) However, in the report linking microsatellite instability with clinical outcomes following anti-PD1 therapy in colorectal cancer patients, despite 60% of mismatch-repair deficient tumors lacking MHC class I expression, 40% responded to anti-PD1 blockade.(47) Furthermore, MHC class II expression on tumor cells was also associated with response to anti-PD1 in two independent cohorts of patients with melanoma.(48) This suggests that transcriptomic and genomic factors may contribute to the response patterns of PD-1 therapeutics. Mutation of coding regions, promoter regions, epigenetic modification and alterations in IFN-gamma-signaling can all result in APM inactivation,(49) therefore both genomic and transcriptomic analysis are necessary to establish the integrity of the antigen presentation mechanism.

## Response to immunotherapy in the context of DNA damage

The accumulation of large-scale cohort-based cancer genome sequence data in multiple cancer types has provided the opportunity to describe and classify mutational signatures, and to infer underlying mechanisms. Rather than being a random assortment of base changes spread across the genome, somatic tumor mutations reflect the sum of mutagenic exposures and mutational processes active during cancer evolution and progression. Following the pioneering work that defined five common mutational signatures active in a breast cancer cohort(50), a large Sanger Institute led collaborative analysed 4,942,984 mutations across 7042 tumors that incorporated 30 cancer types and identified greater than 20 distinct mutation signatures.(50–52) Many signatures were associated with the age of the patient at diagnosis, specific mutagenic exposures, or DNA damage response (DDR) defects, and approximately half were entirely novel signatures.

Both *BRCA1* and *BRCA2* proteins are integral to cross-linking DNA repair via homologous recombination,(53) therefore deficient cells accumulate DNA double-strand breaks and generate genomic instability with subsequent malignant transformation. Furthermore, they interact with Fanconi Anaemia pathways: *FANCC, FANCG* and *PALB2*.(54) Undoubtedly, mutational mapping of cancers, such as in this example, will be correlated with response to immunotherapies. The best example of this concept is in MSI instable colon cancer, where specific DNA mismatch repair machinery mutations have correlated with response to PD-1 inhibition.(47) In addition, the presence of extensive DNA damage is likely to increase the probability of neoantigen formation such that the most mutationally unstable tumors are likely to be the most strongly immunogenic.(52)

## Response to immunotherapy in the context of mutational load

Through the development of next generation sequencing (NGS) technologies including whole genome sequencing (WGS) and RNAseq, a high mutational burden of greater than 100 non-synonymous single-nucleotide variants (nsSNV) per exome could be defined and found to be associated with improved clinical outcome in melanoma patients receiving anti-CTLA-4 treatment.(38, 55) This observation was subsequently validated, with high mutational load associated with prolonged survival in independent cohorts of NSCLC patients treated with Pembrolizumab.(56) Those responders demonstrating the highest mutational burden harbored multiple specific DDR gene mutations (*BRCA2, MSH2,*

*POLD1, POLE, RAD51c*, and *RAD17*) that likely intensified tumor immunogenicity through somatic mutation generation. Higher response rates to PD-1 blockade in MMR-deficient tumors (47, 57) as well as in *BRCA2*-mutated melanoma support this concept.(58)

In the *BRCA2*-mutated melanomas, pretreatment samples revealed that although the overall somatic mutational loads of anti-PD-1-responsive melanoma tumors were not greater than those of non-responsive tumors, higher mutational loads were significantly associated with prolonged survival after immune checkpoint blockade. These data remain consistent with the concept that neoepitopes derived from somatic non-synonymous mutations may be critical for determining clinical benefits from anti-PD-1 therapy.

Case studies of exceptional responders to immune checkpoint inhibitors provide opportunities to identify potentially useful patient and tumor-specific predictors. In the study of an exceptional responder to anti-PD1 therapy in metastatic bladder cancer, somatic and germline JAK3 mutations were identified in the same allele.(59) Transfection of this mutant allele into cell lines increased PD-L1 expression, which may explain this patient's response to anti-PD-L1 therapy. In support of this finding, NSCLC tumors with the same mutant allele also exhibited higher rates of PD-L1 positivity than tumors with other JAK3 genotypes. This example highlights the increased information that can be added to our current understanding by integrating gene expression germ-line mutation analysis in further studies.

## Response to immunotherapy in the context of tumor-specific neoantigens

Recent technological innovations have made it possible to dissect the immune response to patient-specific neoantigens that arise as a consequence of tumor-specific somatic mutations, and emerging data suggest that recognition of such neoantigens is a major factor in the activity of clinical immunotherapies. These tumor antigens are recognized by T-cells as foreign based on the changes in the peptide sequence compared to the wild-type peptide. While it is likely that highly mutated tumors form neoantigens more readily, the stochastic nature of neoantigen generation calls for a functional validation as not all formed neoantigens will be immunologically relevant. Bioinformatics approaches have been successful in correlating mutational load with predicted neoantigen load.

Although the complete response rate to anti-CTLA-4 therapy is low for patients with metastatic melanoma, durable clinical benefit is consistently observed in approximately 20% of patients undergoing therapy.(60–62) Clinical studies of exceptional responders and small cohorts of melanoma patients attribute this response to NRAS mutation status,(63) total neoantigen load, and a neoantigen-derived consensus tetrapeptide signature thought to be necessary for activation of an antitumor T-cell response.(55) The recent whole exome analysis confirmed that neoantigen loads were significantly correlated with clinical benefit in melanoma patients treated with ipilimumab(38), though the tetrapeptide signature was not confirmed in additional analyses. (38, 58)

Subsequently, it has been suggested that tumors with high mutational frequency may produce sufficient neoantigens to generate anti-tumor immunogenicity, while tumors with low frequency may not.(64) Similar observations have been made in DDR deficient tumors,

notably high MSI tumors,(57) BRCA-mutant ovarian tumors(65) and melanoma.(58) While favoring immunogenic neopeptide generation, the duality of genomic instability and mutation accumulation also supports tumor heterogeneity and emergence of less immunogenic novel clones cable of escaping immune surveillance.

A high degree of intratumoral heterogeneity is associated with poor outcome, while sensitivity to immune checkpoint blockade is associated with low heterogeneity and high numbers of clonal neoantigens.(66) Therefore an incongruous role exists for DDR defects in regulating immune checkpoint blockade response. Although DDR deficient tumors demonstrate profoundly unstable genomes and a high mutational burden, these tumors are the most likely to exhibit high intratumoral heterogeneity as a result random mutation induction.(67)

A significant challenge is the determination of those epitopes that will prime a T-cell response. In melanoma following ipilimumab treatment, only two of 448 immunologically relevant epitopes triggered a patient-specific antitumor T-cell response.(68) In NSCLC patients, response to PD-1 blockade was associated with a T-cell response against only a single neoantigen resulting from a single nucleotide variant in *HERC1.* (56)

Mutational burden and predicted neoantigen load also mold the landscape and functional capacity of the antitumor immune microenvironment. RNA-seq data has correlated tumor-infiltrating cytotoxic T-lymphocytes with a higher immunogenic mutation rate.(69) WGS studies of RNA expression in the tumor microenvironment also revealed that the predicted neoantigen load correlates with the cytolytic activities of intratumoral cytotoxic T-lymphocytes and natural killer cells.

These data are supportive of high mutational burden correlating with increased neoantigen formation and tumor immunogenicity. However, the high attrition rate from a high mutational burden to the small number of neoepitopes that can generate an anti-tumor immune response illustrates the complexity of neoantigen prediction using genomic data in isolation.

## GENETIC MARKERS ASSOCIATED WITH RESISTANCE TO IMMUNOTHERAPY

Resistance to checkpoint blockade is increasingly observed and only recently being dissected. Multiple mechanisms underlying resistance are being described, including increased expression of T-cell immunoglobulin mucin 3 (TIM-3) and other checkpoint inhibitors,(70) as well as the presence of somatic differences in tumor cells. These include the loss of PTEN, for example, which mediates reduced T-cell infiltration into tumors and decreased T-cell-mediated tumor killing, suggesting that resistance to anti-PD-1 or anti-CTLA-4 agents in these tumors could be predicted, and perhaps overcome, if used in combination with selective PI3Kb inhibitors.(71)

Recently Hugo and colleagues utilized WGS technology to uncover a translational signature that correlated with resistance to anti-PD-1 therapy.(58) This ‘innate anti-PD-1 resistance’

(IPRES) describes a transcriptomic subset across distinctive histological subtypes of advanced cancer. In patients failing to respond to PD-1 inhibition the IPRES signature encodes up-regulation of genes involved in epithelial mesenchymal transition, monocyte/macrophage chemotaxis, cellular adhesion, extracellular matrix remodeling, wound healing, and angiogenesis. These data suggest that attenuating the mechanics underlying the IPRES signature may have the potential to enhance anti-PD-1 responses in melanoma and other cancers. Interestingly, the IPRES signature was not predictive of resistance to anti-CTLA-4 therapy.

In this study, expression of CD8, PD-L1, PD-L2, CTLA-4, and cytolytic molecules did not differ between patients according to treatment response. However, the density of CD8 T-cell infiltration and PD-L1 expression on immunohistochemistry were associated with response to PD-1 blockade in melanoma(40), NSCLC, and genitourinary cancer.(72) This highlights one of the potential limitations of a gene expression profile analysis of either resistance, as in this case, or response, in that the spatial localization of immunocytes and expression of markers on their surface are perhaps more critical in their architectural arrangement within the tumor microenvironment than their absolute levels.

Additionally, the finding of IPRES in MAPK inhibitor (MAPKi) treated tumors indicated that cross-resistance to immunotherapy in tumors resistant to MAPKi may be a common characteristic.(73) Furthermore, the IPRES signature was enriched amongst melanoma metastases (32%) when compared to primary lesions (9%). Notably this signature was present across a number of tumor histologies suggesting that it is representative of a general mechanism of disease progression and treatment resistance.

## GENETIC MARKERS ASSOCIATED WITH RELAPSE AFTER IMMUNOTHERAPY

With the increasing use of checkpoint inhibitors for the treatment of patients with advanced cancers, it is anticipated that cases of late relapse after initial response will undoubtedly increase with time. In an effort to comprehend the biological mechanisms of acquired resistance, Zaretesky and colleagues compared biopsy samples from paired baseline and relapsing lesions using WGS technology.(74) In patients treated with anti–PD-1 that acquired treatment-resistance, identified escape mechanisms included inactivation of *JAK1* or *JAK2*; mutations that were not present prior to therapy commencement. This finding suggested that resistance to *JAK* ligands, including interferon gamma signaling, likely contribute to immune resistance and relapse. Another acquired mutation was found in melanoma patients that were initially responsive to immunotherapy, but were found to acquire immunoresistance secondary to the loss of *B2M*.(75) This protein is an integral subunit of MHC I without which tumor specific CD8 T-cells cannot recognize tumor antigens. Identification of these specific acquired gene defects while on treatment may enable options for the realistic design of salvage strategies. For example, activation of cytosolic double-stranded DNA can be employed to activate the stimulator of interferon genes (STING) (76) in a JAK-independent fashion. Furthermore, knowledge of resistance to

interferon gamma may guide mechanistic biomarker studies for the selection of patients prior to treatment commencement who have a low probability of response.

Another potential marker that may be associated with relapse is increased expression of indoleamine 2.3-dioxygenase 1 (IDO-1). IDO-1 induction and ensuing tryptophan conversion induces effector T-cell dysfunction and T-regulatory cell induction that supports a tolerogenic environment.(77) Increased expression has been observed in hypermutated colorectal cancer suggesting that it may negate an immune response directed against neoepitopes in these tumors.(78)

## EVOLVING GENETIC MARKERS ASSOCIATED WITH RESPONSE TO IMMUNOTHERAPY IN SINGLE TUMOR TYPES

### Pancreatic Adenocarcinoma

The role of immunotherapy in many “immunogenic” histologies is well established, however, clinical trials testing immune checkpoint inhibitors in pancreatic ductal adenocarcinoma (PDAC) remain to demonstrate clinically significant responses. Yet, as with other targeted therapeutic approaches in this disease, it is becoming apparent that immune therapies will likely require a predictive biomarker based approach. Using a RNAseq based expression analysis of 100 tumors; four classes of PDAC were identified, including a “pancreatic progenitor subtype”.(79) This subtype contained subclasses with active transcriptional networks characteristic of late stages of pancreas development termed Abnormally Differentiated Endocrine and eXocrine (ADEX). One of these subtypes was an “immunogenic subtype” characterized by elevated expression of multiple transcripts derived from immune cells and immune modulatory cytokines. It is suspected that this cognate molecular signature may possess an inherent vulnerability to targeted immunotherapy. Further, gene expression analysis of the other subtype (squamous) identified an immunosuppressive signaling pathway. This pathway included the expression of PD-LI, PD-L2 and IDO-1 within tumor cells. Therefore, GEP of specific tumor subtypes in this disease may allow patient specific stratification for immunotherapies.

### Urothelial Carcinoma

In the multi-center, single-arm, phase II trial of PD-L1 blockade with atezolizumab in patients with locally advanced or metastatic urothelial carcinoma, patient response correlated with genetic tumor subtyping.(80) The Cancer Genome Atlas (TCGA) was utilized on paraffin-embedded tumor samples finding higher response rates (34%, p=0·0017) in the luminal cluster II subtype. These tumors were characterized by transcriptional signatures associated with T-effector cell activation. By contrast, luminal cluster I tumors were associated with low CD8+ effector genes expression, lower PD-L1 immune cell or tumor cell expression, and lower responses to atezolizumab. Moreover, the median mutation load was significantly higher in responders than in non-responders assessed through focussed genomic profiling of a 315-gene panel (12·4 vs. 6·4 per megabase, p<0·0001).

Although PD-L1 immune cell status is clearly associated with atezolizumab response, incorporation of TCGA gene expression subtype, mutation load, or both of these novel

biomarkers into a model based on PD-L1 immune cell staining significantly improved the association with response. Thus, disease subtype and mutation load do not simply recapitulate the information provided by PD-L1 expression in immune cells, but rather, they provide independent and complementary information.

## EVOLVING GENETIC MARKERS ASSOCIATED WITH RESPONSE TO IMMUNOTHERAPY WITH COMBINATORIAL STRATEGIES

An important gap in our understanding is the lack of information regarding the immunologic effects of "non-immunotherapies" that may become promising combination partners with established immunotherapy agents. A combination of immunotherapy strategies and molecular targeting to alter aspects of the immune milieu may be necessary in tumor subgroups refractory to treatment.

In preclinical PDAC models, KPC mice are traditionally resistant to anti-PD1 therapy, however, combining checkpoint inhibition with CXCL12/CXCR4 axis interference has enhanced efficacy of PD-1 inhibition.(81) More recently it was demonstrated that loss or inhibition of CXCR2 could profoundly suppress metastasis. When mice with late-stage tumors were treated with the combination of a CXCR2 small molecule inhibitor and anti-PD1 therapy, their survival dramatically increased.(82) When the microenvironment of these tumors was examined, a profound increase in infiltration of CD3+ T cells was observed.

In addition, a recent report has identified generation of damage associated molecular patterns indicative of immunogenic cell death (ICD) in colon cancer treated with cetuximab. (83) In this report, ICD depended on the mutational status of the EGFR signaling pathway and on the inhibition of the splicing of X-box binding protein 1; genetic targets that can be evaluated *a priori*. Specific immunotherapeutic targets have been identified in advanced colon cancer through GEP; therefore, mechanisms to increase the immunogenicity of these tumors may play a critical role in clinically efficacious treatment strategies.(3, 4) Moreover, newer agents previously thought to be solely cytotoxic to colon cancer are now being identified as potent inducers of ICD and necrosis.(84) Treatment with these ICD-inducing agents may release tumor-specific antigens and promote a pro-inflammatory state where immunotherapies, including checkpoint blockade, can be utilized based on pre-treatment GEP.

## CONCLUSIONS

The advent of NGS technologies and a greater understanding of genomic datasets have characterized tumor-specific responses to various therapies. Undoubtedly, this approach will have a profound transformation on the management of cancer through stratification and personalization of therapy. It is certain that a combination of GEP strategies to characterize individual tumors based on the tumor genomic landscape, extent of DNA damage, mutational load, and neoantigen presentation will be employed to determine those patients who will derive optimal benefit from immunotherapy. Identification of genetic markers associated with immunotherapy resistance and relapse will continue to fine-tune patient selection, and further investigation into the cross-talk between cellular and molecular

pathways and immunogenicity will further shape the landscape to likely utilize combination molecular and chemotherapeutics with immunotherapies. The rapidly falling costs of NGS as well as advancements in bioinformatics pipelines are enabling broader integration of these technologies within clinical management.

Moving forward, it will be necessary to accumulate larger datasets, set minimal tissue purity thresholds, standardize tissue processing, and establish sequencing depth such that mutational load can be easily calculated in a robust fashion. Certainly, it has been established that mapping the immune contexture of specific tumors using predictive gene signatures can help predict prognosis. Translating this effort to target specific immunotherapies to individual patients is the ultimate goal.

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