Review Article

Personalized Cancer Immunotherapy: Today's Challenge and Tomorrow's Promise

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Abstract

Precision medicine continues to be the benchmark toward which we strive in cancer research. Conventionally, it is the term applied to the use of genomic information to guide molecularly targeted therapy. However, the advent of clinically effective cancer immunotherapies has posed a challenge for this concept of precision medicine, as robust biomarkers that can differentiate responders from nonresponders have not been described. Here, we review the current scientific efforts using novel technologies to develop biomarkers for immunotherapeutics, to ultimately achieve "personalized immunotherapy." We first examine the role of programmed death ligand 1 expression and tumor mutational burden, the two most-studied tumoral response biomarkers; and subsequently discuss innovative candidate biomarkers including integrated "omics" approaches utilizing serial tumor, blood, and microbiome sampling. We also detail the challenges in unifying these approaches into a patient-focused immunogram to truly personalize immunotherapy.

Keywords: *Biomarkers, CTLA-4, immunotherapy, PD-1, PD-L1, recision medicine*

Introduction

The recognition of specific molecular aberrations that conferred sensitivity of cancer cells to targeted therapies led to the concept of "personalized medicine." This concept of a tailored approach to patient treatment based on the molecular analysis of tumor genes and proteins has been validated with highly efficacious kinase inhibitors that target specific subgroups of patients.[1-3] The advent of cancer immunotherapy has created new challenges to this approach. Numerous immunotherapeutics have shown efficacy across tumor types recently,[4-8] with durable long-lasting responses mainly seen in subgroups of patients. This clinically apparent asymmetry to the benefits of immunotherapy combined with the high cost of these agents has fostered a search for response biomarkers. Certainly, to date, currently available predictive biomarkers for response to immunotherapy have lacked precision. In this review article, we summarize currently used cancer immunotherapy biomarkers and focus on how recent developments in "omics" technologies can be leveraged to better clarify the biological and clinical nuances of immunotherapy responses.

Background

Although the immune system's importance in cancer control has been long established,[9] the paucity of nontoxic immunotherapeutics had previously limited their role in the oncological armamentarium. This has changed since the discovery of the importance of regulatory immune checkpoints and the recognition of their potential for therapeutic manipulation.[10] The two best-described checkpoints are the CTLA4-B7 checkpoint, for which ipilimumab is a licensed antagonist and the programmed cell death 1 (PD-1) – programmed death ligand 1 (PD-L1) checkpoint. The landmark study demonstrating the efficacy of ipilimumab in metastatic melanoma first validated immune checkpoint inhibition as a viable therapeutic strategy.[8] Therapeutic manipulation of the PD-1/PD-L1 checkpoint has been even more successful, and there are now multiple Food and Drug Administration (FDA) licensed therapeutics targeting both receptor and ligand. Although immune checkpoint inhibitors are often grouped together, the differences in their mechanisms of action

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result in meaningful differences in activity profile, toxicity, and synergy in combinations. CTLA4-B7 inhibitors target priming and antigen-presentation aspects of the cytotoxic T-cell response, whereas PD-1/PD-L1 inhibitors target the effector mechanism.

Given the enormous pipeline of immunotherapy drugs undergoing clinical testing currently, clinicians are faced with many challenges regarding their optimal use. Clinical challenges include deciding when to cease treatment given an observed lack of correlation between progression-free survival and overall survival.[11] Furthermore, pseudoprogression, or appearances suggestive of radiological progression due to immune infiltration, further complicates clinical decision-making.[12] There is also confusion regarding the optimal duration of treatment. Much of the initial excitement of immunotherapy was in the apparent durability of responses, especially seen with ipilimumab in melanoma.[13] However, recent evidence has suggested that ongoing treatment is necessary for disease control, arguing against the durability of immune responses created by PD-1 inhibition.[14] These commonly encountered clinical difficulties are accompanied by concerns regarding the sustainability of funding these therapies. As the indications for the use of immunotherapeutics continue to expand, there is a resultant spiraling, unsustainable cost to health-care systems globally.[15]

The combination of the clinical challenges of assessing immune response and the emphasis on cost containment systemically contextualizes the importance of developing robust response biomarkers. Biomarker development is complex, as their importance and utility are context-dependent. There are two general types of predictive biomarkers. The first group of biomarkers is characterized by binary outcomes (present/absent) and is highly predictive of response to therapy. Examples of such biomarkers include oncogenic driver mutations for which highly effective targeted kinase inhibitor therapy is available (e.g., gefitinib for epidermal growth factor receptor's-mutant nonsmall cell lung cancer [NSCLC][1]). The second group of biomarkers is more nuanced in their application and is not binary in nature. Most currently available biomarkers for immune checkpoint therapy fall into the latter category.

The Present

Programmed death ligand 1 immunohistochemistry

The rapid emergence of multiple therapies targeting the PD-1/PD-L1 axis achieving FDA approval across tumor types[4,5,7] has been accompanied by a focus on developing biomarkers to enrich patients for response. Tumoral PD-L1 expression by immunohistochemistry was first identified in the initial Phase I study of nivolumab as a candidate biomarker and is the best-studied biomarker to date.[16] Table 1 notes reported results of various PD-L1 assays across tumor types. Taken together, data from these trials suggest that PD-L1 positive tumors, particularly NSCLC and melanoma, are enriched for response to PD-1/PD-L1 inhibitors compared with PD-L1 negative tumors. Theoretical advantages of PD-L1 immunohistochemistry as a biomarker include its mechanistic validity, the widespread availability of immunohistochemistry in clinical laboratories and its relatively low cost.

Out of all tumor types, clinical trials in NSCLC have demonstrated the best and most consistent correlation between the degree of PDL1 expression and magnitude of benefit to immune checkpoint therapy.[11,25,26] The recent KEYNOTE 042 presented as ASCO 2018 is consistent with this theme, showing the superiority of pembrolizumab over chemotherapy in all patients with PDL1 expression of >1%, although higher PDL expression clearly equated to greater degree of benefit in terms of progression-free survival (PFS) and response rate.[30]

However, there are several limitations with PD-L1 immunohistochemistry as a biomarker when used in isolation. Most significantly, the absence of PD-L1 expression does not necessitate lack to benefit to immune checkpoint inhibition. Indeed, there is a growing body of literature demonstrating robust responses in patients who are PD-L1 negative in various tumor types.[21,23] Even in NSCLC, the lack of PDL 1 expression can no longer be used as a "Go versus no Go" decision-making tool for choosing immunotherapy in the first line. A number of recent Phase III trials presented at the ASCO and AACR in 2018, including KEYNOTE 189,[31] KEYNOTE 407,[32] CHECKMATE 227,[33] and ImPOWER150[34] have all shown that even in the low and absent PDL1 expressing NSCLC population, the addition of an immune checkpoint inhibitor to cytotoxic chemotherapy shows a significant survival advantage compared to chemotherapy alone. These studies also give impetus to the strategy that the addition of chemotherapy to immune checkpoint inhibitors may make a PDL1 negative, otherwise checkpoint inhibitor monotherapy unresponsive patient actually respond. Although to truly verify this hypothesis, Phase III studies of immunotherapy plus chemotherapy verses immunotherapy alone in the PDL1 negative or low expression NSCLC population need to be performed.

Denying patients potentially highly efficacious therapy without absolute evidence of lack of benefit is a consequent clinical dilemma. This fundamental inadequacy is compounded by several interrelated issues.

First, there is contention in the literature as to whether tumoral PD-L1 expression is more important than its expression in the microenvironment or in immune cells. While the initial trials suggested positive PD-L1 tumor expression was associated with higher response rates, it now appears that the expression of PD-L1 in the tumor microenvironment also appears to be crucial for therapeutic

Table 1: Selected studies of programmed death ligand 1 immunohistochemistry

Tumor type	Study	Drug	Antibody	Cutoff	PD-L1+ rate	Response rate PD-L1+
Melanoma	Grosso et al.[17]	Nivolumab	28-8	5%	NR	44%
	Kefford et al.[18]	Pembrolizumab	NR	1%	NR	51%
	Weber et al.[19]	Nivolumab	28-8	5%	46%	44%
	Taube et al.[20]	Nivolumab	5H1	5%	53%	39%
	Robert et al.[21]	Nivolumab	28-8	5%	35%	53%
	Weber et al.[22]	Nivolumab	28-8	1%	52%	39%
NSCLC-Squamous	Brahmer et al.[23]	Nivolumab	28-8	1%	83%	18%
				5%	NR	21%
				10%	NR	19%
NSCLC-Non-squamous	Borghaei et al.[24]	Nivolumab	28-8	1%	78%	31%
				5%	NR	36%
				10%	NR	37%
NSCLC	Garon et al.[5]	Pembrolizumab	22C3	50%	23%	37%
	Reck et al.[25]	Pembrolizumab	22C3	50%	30%	45%
	Herbst et al.[11]	Pembrolizumab	22C3	50%	28%	30%
	Fehrenbacher et al.[26]	Atezolizumab	Ventana SP142	50% (tumor) OR 10% (immune cell)	16%	38%
	Rittmeyer et al.[27]	Atezolizumab	Ventana SP142	50% (tumor) OR 10% (immune cell)	16%	31%
RCC	Motzer et al.[28]	Nivolumab	28-8	1%	24%	NR
				5%	11%	NR
	Motzer et al.[29]	Nivolumab	28-8	5%	27%	31%

NR: Not reported, PD-L1: Programmed death ligand 1, NSCLC: Nonsmall cell lung cancer, RCC: Renal cell carcinoma

activity. PD-L1 is upregulated by activated effector T-cells but is also very highly expressed by tolerant and exhausted T-cells.[35] In addition, there is heterogeneity within the exhausted T-cell populations with differing potential for reinvigoration by PD-1 pathway blockade.[36]

This confusion over the primacy of tumoral versus immune PD-L1 expression has been further corroborated in clinical trials. Clinical trials of atezolizumab have been paired with companion diagnostics evaluating PD-L1 expression in immune cells rather than tumor alone,[7,37] with some suggestion that PD-L1 expression in immune cells may better correlate with the clinical outcomes. In contrast, the POPLAR study suggests that PD-L1 expression on tumor cells and immune cells may have nonredundant roles in the regulation of antitumor immunity and predicting response to therapy. However, it must be noted that PD-L1 expression on immune cells in this study correlated with a previously validated interferon-gamma-associated gene signature which correlated with survival in the patients treated with atezolizumab. Although many studies have primarily reported tumoral PD-L1 expression [Table 1], the debate over the role of immune PD-L1 expression casts some doubt on this as a biomarker.

In addition, the second major issue with PD-L1 expression as a biomarker is the dynamic nature of the tumor/tumor microenvironment interaction. This spatial and temporal heterogeneity argues against the validity of static immunohistochemistry performed on mostly archival tumor biopsies. Unsurprisingly, significantly discordant results (>20%) have been noted between matched nodal and primary specimens of tumors, for example, in renal cell carcinoma, raising concerns that this approach may result in clinically significant false-negative results.[38] Furthermore, the majority of clinical trials have utilized archival noncontemporaneous tissue samples that may not reflect the tumor/microenvironment at the time of immunotherapy commencement.[39] Exploratory efforts to overcome this issue of heterogeneity are ongoing, such as the use of liquid biopsies to obtain circulating tumor cells in real time and the refinement of assays to detect PD-L1 on circulating tumor cells.[40]

The final issue limiting the widespread applicability of PD-L1 immunohistochemistry is the technical limitations of the assay. To date, studies have used different antibodies with significant interantibody variability, membranous versus cytoplasmic staining patterns, and varying cutoff points for identifying positive from negative samples.[41,42] Efforts are, however, underway to harmonize assessment of PD-L1 status, including standardized pathological training to assess PD-L1 status.[43] The blueprint industrial-academic collaborative partnership in NSCLC has already published Phase I results looking at four different PD-L1 assays (22C3, 28–8, SP142, and SP263), demonstrating reproducible assay performance bar the SP142 assay, which exhibited lower staining overall.[44] Significantly, there was more interantibody variability with staining of immune

cells compared to tumor cells, which has been observed previously.[42] Thus, for individual patients, despite broadly similar results between assays, interchanging assays may lead to different results with therapeutic implications. This Gordian knot has driven the immense efforts to develop more precise biomarkers for immunotherapy as will be discussed in the following sections.

Mismatch repair testing

Pivotal work by Alexandrov et al.[45] demonstrated a strong correlation between immunotherapy responsive tumor types and typical somatic TMB. This tied in neatly with work from other laboratories showing increased numbers of effector CD8+ tumor-infiltrating lymphocytes, expressing high levels of PD-1, in tumors with a high mutational load.[46] Deficiencies in mismatch repair (MMR) result in microsatellite instability (MSI). Tumors with deficient MMR may give rise to substantial nonsynonymous single-nucleotide variants, resulting in more neoepitopes and a theoretical enhanced responsiveness to PD-1 inhibition. This hypothesis was tested in a proof-of-principle early phase trial by Le et al. with striking findings.[47] First, the correlation between MMR status and total nonsynonymous TMB was observed. More significantly, the study demonstrated marked clinical divergence in outcomes: Patients with MMR proficient tumors did not respond to PD-1 inhibition, in stark contrast to patients with MMR deficient (MMRd) tumors where a high response rate (40%) was observed.[47] This finding has been corroborated in other settings. Two siblings with recurrent, highly mutated constitutional MMRd glioblastoma multiforme treated with nivolumab had profound and durable responses, compared to poor survival outcomes normally expected in this clinical scenario.[48]

Pooled data from 5 uncontrolled, multicenter, single-arm clinical trials involving 149 patients with microsatellite instability or MMRd tumors led to the accelerated approval of pembrolizumab for the treatment of adult and pediatric patients with metastatic MMRd tumors.[49-52] Most patients on these studies had advanced metastatic disease and taken together, the pooled patients on the study had an overall response rate of 39.6% with pembrolizumab, including 11 (7.4%) complete responses and 48 (32.2%) partial responses. For those who responded, 78% had responses that lasted for at least 6 months. Given the FDA approval, rapid and accurate identification of patients with MMRd tumors who could benefit from immunotherapy is of paramount importance.

Currently, two types of MMR testing are in clinical use: immunohistochemical staining, which assesses the expression levels of MMR proteins (MLH1, MSH2, MSH6, and PMS2) within the tumor and polymerase chain reaction (PCR), which detects mutations in microsatellite regions. For PCR tests, revised Bethesda criteria recommend using a "pentaplex" of five mononucleotide microsatellites (BAT25, BAT26, NR21, NR24, and NR27).[53] Tumors with three or more unstable markers are called MSI-high and tumors with one unstable marker are called MSI-low. Although PCR and immunohistochemical techniques are sensitive, specific, and have high concordance (>95%),[54,55] these studies have largely been carried out in colorectal cancer and their utility across cancer types is unknown. Moving forward, concerted efforts to catalog large series of microsatellite loci that are frequently altered in numerous cancers[56] would serve to further refine the identification of patients who are likely to benefit from immunotherapy. In addition, non-MSI DNA repair defects resulting in hypermutation, such as those in polymerase ε (POLE),[57] which may confer susceptibility to checkpoint inhibition[58] will not be routinely identified with current testing protocols.

The Future: Key Technologies in Precision Medicine for Cancer Immunotherapy

Rapid advances in technology with decreasing costs and improved throughput are now enabling the collection of large amounts of information on different cancer "omic" landscapes. The criteria for the use of omics-based predictors in clinical trials have recently been published by the US National Cancer Institute[59] and will be key in ushering in the era of personalized immunotherapy. Table 2 lists some of the key technologies that will be leveraged in an era of personalized immunotherapy.

Next-generation sequencing of DNA

Numerous clinical studies have utilized next-generation sequencing technology to build on the work of Alexandrov et al.,[48] showing that total nonsynonymous TMB, which correlates with neoantigen load, is predictive of response to immunotherapy.[60,61] The has led to the addition of TMB – a quantitative measure of the total number of nonsynonymous mutations per coding area of a tumor genome to commonly performed commercially available next-generation sequencing assays, including both Foundation Medicine©[61] and Caris©.[62]

While some overlap exists between high PDL1 IHC expression and high TMB, clearly both also function independently to predict efficacy of immune checkpoint inhibitors. Using the Foundation Medicine CDx assay, with a cutoff in TMB of 10 mutations per megabase, the recent Phase III CHECKMATE 227 trial clearly demonstrated superiority of combination ipilimumab and nivolumab over chemotherapy as the first-line therapy in patients with high TMB, in terms of response rate (45.3% vs. 26.9%) and PFS (1 year PFS 42.6% vs. 13.2%), independent of PDL1 expression.[63]

While tumors harboring more mutations are more likely to respond to immunotherapy; there remains significant variability between panels and bioinformatics algorithms

Table 2: Key technologies and their applications for personalized immunotherapy

Technology	Methods	Advantages	Limitations	Possible applications
Massively paralleled DNA sequencing (next-generation sequencing)	Targeted sequencing panels	Low cost High sensitivity High coverage of genes of interest	Limited by panel size	Tumor mutational burden
	Whole-genome sequencing	Breadth and depth of coverage Ability to identify copy number changes and rearrangements Identification of pathological variants in noncoding regions	Large data output High cost	Tumor mutational burden Identification of novel pathogenic variants and mechanisms of resistance Combination with transcriptomics/proteomics approaches to identify markers of primary and secondary resistance
RNA-based sequencing technologies	Transcriptome assessment	Differential expression of genes implicated in tumorigenesis and resistance	Large data output High cost	Development of gene expression signatures for response/resistance Identification of pathways implicated in primary/secondary resistance
	T-cell repertoire sequencing	Allows tracking and evaluating of distinct T cell clones	Single-cell approaches are required to identify pairs of TCR receptors High cost	T cell repertoire sequencing
Protein-based technologies	Single protein detection (immunohistochemistry, ELISA)	Low cost Readily available Ease of implementation	Limited by immunohistochemical expression and assay reproducibility	Ready to use clinical biomarkers such as PD-L1 and MMR protein expression
	Multiple protein detection (mass spectrometry-based assays), SERPA	High sensitivity	High cost	Identification of immune cell landscapes that predict sensitivity/resistance to immunotherapy
Cell-based assays	Flow cytometry	Relatively simple Relatively low cost	Limited number of immune markers Limited to analysis of peripheral immune cell subsets (not within the tumor microenvironment)	Immune cell profiling

SERPA: Serologic proteome analysis, ELISA: Enzyme-linked immunosorbent assay, PD-L1: Programmed death ligand 1, TCR: T cell receptor

used, combined with lack of validated cutoffs which limits clinical use currently. For instance, a study by Qiu et al. has demonstrated that although there is high concordance in the raw data achieved from whole-exome sequencing, the final outputs can demonstrate substantial variability.[64] There are, however, some advantages in next-generation sequencing approaches to TMB. Novel bioinformatic algorithms such as mSINGS[65] and MANTIS[66,67] have been used to manipulate sequencing data to derive MSI status. Moreover, TMB may also enable identification of non-MSI hypermutated tumors which respond to immunotherapy such as POLE mutations and be utilized in tumor types that currently do not undergo routine MMR testing.

Of greater importance, are the emerging studies suggesting that T-cell reactivity may rely on a small proportion of neoantigens that are particularly immunogenic, rather than an overall quantitative neoantigen load.[68,69] This is corroborated by other studies showing that only a fraction of predicted neoepitope peptides are actually expressed on major histocompatibility complex I molecules on tumors,[70] indicating that there are many other factors contributing to the antigen presentation apparatus that are yet to be fully delineated. One such example is renal cell carcinoma which is typically immunogenic but does not have high TMB as measured by nonsynonymous single-nucleotide variant counts; recent work has identified novel immunogenic mutational classes such as insertions and deletions which create novel open reading frames and a large quantity of mutagenic peptides highly distinct from self that contribute to the immunogenic phenotype.[71]

Increasing evidence suggests that the immune system interacts actively with tumor antigens in a process now termed "cancer immunoediting."[72] Neoantigen evolution occurs with ongoing DNA alterations that accumulate with tumor growth over time and immune silencing of these neoantigens occurs through T-cell modulation.[73] This leads to intratumor neoantigen landscape heterogeneity. An integrated analysis of intratumoral heterogeneity and neoantigen burden on a series of lung adenocarcinomas showed that decreased neoantigen intratumoral heterogeneity was associated with improved clinical benefit.[46] Immunoediting, therefore, poses challenges with interpretation of neoantigen data as a biomarker. Accurate capture of neoantigens that are responsible for tumor immunogenicity going forward may require serial monitoring of tumors to accurately reflect neoantigen heterogeneity, as well as developing algorithms to filter data obtained from whole exome sequencing.

The widespread use of next-generation sequencing technology has also generated a wealth of insights into key genes/pathways involved in mediating immune resistance. This is likely to increase in importance with the broader use of immune checkpoint therapies in multiple indications. As the denominator of treated patients increases, acquired resistance will become an increasing problem. For example, loss of PTEN, which enhances PI3K signaling and is a common genomic defect across multiple cancers, was found to be associated with resistance to immune checkpoint therapy.[74] Loss of PTEN in tumor cells in preclinical models of melanoma inhibits T-cell-mediated tumor killing and decreases T-cell trafficking into tumors. PTEN loss in tumor cells was associated with increased expression of immunosuppressive cytokines, which results in decreased autophagy and reduced T-cell-mediated tumor apoptosis. In melanoma patients, PTEN loss correlated with decreased T-cell infiltration at tumor sites, reduced likelihood of successful T-cell expansion from resected tumors, and inferior outcomes with PD-1 inhibitor therapy.[57]

Constitutive activation of the beta-catenin/WNT signaling pathway has also been shown to induce resistance to T-cell checkpoint blockade.[75] In a comparison of the genomic profiles of T-cell inflamed versus noninflamed melanoma tumor samples, Spranger et al. found that 94% of tumors lacking infiltration of T-cells exhibited high levels of beta-catenin signaling, while only 4% of T-cell infiltrated tumors expressed high beta-catenin activation.[75] Tumors with elevated B-catenin expression lacked a subset of dendritic cells (DCs) known as CD103+ DCs, due to decreased expression of the chemokine ligands 4. Murine tumors lacking B-catenin responded effectively to immune checkpoint therapy, whereas b-catenin-positive tumors did not due to defective recruitment of CD103+ DCs and impaired priming and infiltration of T-cells into the tumor microenvironment.[58]

Another mechanism by which cancer cells could escape cytotoxic T-cell killing is by downregulating or mutating molecules involved in the interferon-gamma signaling pathway, which goes through the interferon-gamma receptor chains janus kinase 1(JAK1) and/or JAK2 and the signal transducer and activators of transcription (STATs). Zaretsky et al. demonstrated copy-number-neutral loss-of-function mutations in JAK1 or JAK2, concurrent with loss of heterozygosity due to deletion of the wild-type allele, in two out of four cases of late acquired resistance to pembrolizumab, which were absent in the baseline biopsies.[76] More recently, the relevance of the JAK-STAT pathway has been corroborated by Shin et al. in preclinical models of tumors with high TMB which would theoretically predispose to innate immunogenicity.[77] Functional abrogation of the JAK-STAT pathway due to inactivating mutations resulted in impairment of PD-L1 expression upon interferon-gamma exposure, thereby rendering PD-1 inhibitors ineffective.

Acquired resistance can also occur through loss of the shared component of human leukocyte antigen (HLA) Class I molecules, beta 2 microglobulin (B2M), which leads to the absence of surface expression of HLA Class I.[60] B2M is crucial to the antigen presenting machinery, playing an essential role in HLA Class I folding and transport to the cell surface. Mutations in B2M thus can disrupt major histocompatibility complex-restricted antigen presentation, which is crucial to generating a cytotoxic T-cell response to cancer.

Gene expression profiling

Nongenomic signatures are also being studied as predictive biomarkers for treatment response. Gene expression profiling using transcriptomics in a series of patients with melanoma treated with immune checkpoint inhibitors led to the identification of a unique innate PD-1 resistance signature featuring a distinct set of genes, particularly associated with the epithelial–mesenchymal transition, which typically conferred resistance to PD-1 inhibition.[78] Importantly, genes contributing to this signature such as *AXL* have also been identified as contributing to T-cell exclusion in preclinical models of immune resistance.[79] Other small studies have suggested immune activation (T-cell/interferon-gamma) signatures may be able to predict response and benefit from therapy.[26,80,81]

A complementary approach using targeted gene expression profiling with NanoString panels composed of immune-related genes identified a distinct adaptive immune signature of differentially expressed genes between responders and nonresponders.[82] This study in patients with melanoma treated with immune checkpoint inhibitors showed a profound and highly statistically significant difference in the expression of markers for T-cell subsets and immunomodulatory molecules in responders versus

nonresponders to therapy in early on-treatment tumor samples. Intriguingly, the investigators suggest that early on-treatment biopsies may better predict response compared to pretreatment biopsies (although rather than be a bona fide predictive biomarker, this signature may be a result of the treatment itself).

Immune cell profiling

Flow cytometry-based techniques have been used to examine immune cell populations in peripheral blood. Huang et al. used immune profiling of peripheral blood from patients with advanced melanoma before and after treatment with the PD-1-targeting antibody pembrolizumab and identified pharmacodynamic changes in circulating exhausted-phenotype CD8 T-cells (T_{ex} cells).[83] He demonstrated that response to PD-1 inhibition was functionally correlated to reinvigoration of exhausted T-cells, rather than expansion of the effector T-cell pool. Other groups including Ghoneim et al. used a similar approach to analyze T-cell subsets to evaluate mechanisms of resistance to immunotherapeutics.[84] By obtaining serial samples of peripheral blood in a preclinical model, they demonstrate progressive T-cell exhaustion and show that de novo DNA methylation occurring during and after the peak effector, T-cell response is critical for establishing exhaustion. These approaches have been hypothesis generating and may be exploited clinically in the future.

Interrogating the complex host of factors in the tumor microenvironment, which collectively influence the immune response to cancer has been more challenging. The density and distribution of CD8+ lymphocytic infiltration within the tumor microenvironment has been shown to be associated with improved patient outcomes in other tumor types, including melanoma, lung, and bladder cancers.[42,85-88] Efforts are underway to further refine the mapping of immune cell infiltrates in tumor, for example, using multicolor immunohistochemistry,[82] multiplex immune fluorescence,[89] or mass cytometry (CyTOF) technology.[90]

A combination of these techniques, termed "imaging CyTOF" offers an unparalleled method for evaluating the immune microenvironment.[91] First, antibody labeling using traditional immunohistochemical methods occurs. CyTOF using probes that are labeled with heavy metal ions through covalently coupled chelation polymers, rather than fluorescent probes is then applied. The sample is then positioned in a high-resolution laser ablation system before the subsequent readout by the mass cytometer. This allows simultaneous detection of many more unique probes (32 compared to traditional flow cytometry techniques which are limited by spectral overlap), with little or no spillover between detector channels. Together with advancing bioinformatics software, this enables the automated generation of maps of the tumor microenvironment. With spatial resolution of protein expression in samples at the single-cell level, this approach promises the study of immune cell subpopulations in the tissue microenvironment allowing delineation of cell subpopulations, cell–cell interactions, as well as highlighting tumor heterogeneity.[91,92] The adoption of this technology may help further elucidate the conceptual framework through which the tumoral immune microenvironment is viewed. Currently, the immune microenvironment can be segregated into three separate phenotypes: "inflamed," "noninflamed," or immune-excluded phenotype and an "immune desert."[93] Accurately delineating the lymphocyte subsets correlating with these phenotypes could guide novel clinical trial designs. For instance, this could enable the rational design of combinatorial strategies to overcome the hurdles of immune-excluded versus immune desert phenotypes, which are resistant to currently available immunotherapies.

Proteomics

Measuring cytokine production is an integral part of measuring immune response during immunotherapy and has been explored as potential blood-based biomarker. Sanmamed et al. longitudinally monitored serum interleukin (IL)-8 levels using a sandwich ELISA and found that in responding patients, serum IL-8 levels significantly decreased between baseline and best response and subsequently significantly increased upon progression.[94] In nonresponders, IL-8 levels significantly increased between baseline and progression suggesting that peripheral changes in serum IL-8 levels could be used to monitor and predict clinical benefit from immune checkpoint blockade.

Several other immunoproteomics approaches, such as *Ser*ologic *P*roteome *A*nalysis (SERPA) which screens an antibody reactivity profile in sera from patients are currently being explored as surrogate marker for measuring the adaptive immune response to cancer.[95]

Commensal microbiota

Perhaps, the most interesting research on personalized medicine in the immunotherapeutic era has been conducted regarding the human microbiome, which consists of over 100 trillion microbes, the majority of which resides in the gastrointestinal tract. The modification of commensal microbiota in the gut has been shown to correlate with immune checkpoint inhibitor efficacy in multiple murine models.[96,97] Three separate studies recently simultaneously published in *Science* have suggested the importance of the commensal gut microbiota in predicting response to immunotherapy.[98-100] In a cohort of 112 patients with melanoma, Gopalakrishnan et al.[99] assessed the oral and gut microbiome by 16S RNA sequencing and found that PD-1 responsive patients had a significantly higher degree of biodiversity of gut microbiome compared with nonresponders. Specifically, responders had an increased abundance of Clostridiales bacteria, while

nonresponders had higher proportions of Bacteroidales bacteria. Importantly, no correlations were observed in the oral cavity, suggesting the primacy of gut commensal microbiota for any potential immune synergy between microbiota and therapy. The groups of Matson et al.,[100] as well as Routy et al.[98] made similar observations, finding relative abundance of some species in responders compared to nonresponders. Taken together, the authors hypothesized that microbial diversity increased responsiveness to PD-1 inhibition. Antibiotic therapy taken during immunotherapy negatively correlated with response while a role for fecal microbiota transplantation was hinted at by the accompanying preclinical work. This arena remains an active area of study, and how this translates clinically will remain to be seen.

Challenges for the future

We have discussed the challenges of each individual high-throughput assays above, but it will be the combination of these technologies that will truly personalize immunotherapy. The concept of a personalized immunogram as a framework for describing the different interactions between cancer and the immune system for individual patients has been suggested, reflecting relative ascendancy of various domains of the cancer immunity cycle.[101,102] Karasaki et al.[101] used this approach integrating genomic, transcriptomic, and immunohistochemical data to categorize the immunogenicity of NSCLC. The authors recognized eight domains which created axes for the immunogram for the cancer immunity cycle. These included T-cell immunity, tumor antigenicity, priming and activation, trafficking and infiltration, recognition of tumor cells, absence of inhibitory cells, absence of checkpoint expression, and absence of inhibitory molecules. Utilizing gene set enrichment analyses of RNA sequencing data and immunohistochemical evaluation, scores were added from each domain to derive an output category of either T-cell rich, T-cell intermediate, or T-cell poor tumors. Interestingly, some of the findings were broadly counterintuitive, such as overrepresentation of neoantigens in T-cell poor tumors and increased inhibitory molecules and checkpoints seen in T-cell rich tumors. This highlights the dynamic nature of this concept. Although the immunogram is still experimental, its logical categorization enables simpler conceptualization of the underlying interactions between the immune microenvironment and tumors and provides a springboard from which personalized immunotherapy can become a reality.

In the future, patients with advanced cancer could conceivably have their cancer immunogram interrogated utilizing multiple validated biomarker assays and technologies in "real-time," and have this reassessed longitudinally by the evaluation of fresh serial human specimens (tumor, blood, serum, and microbiome) during treatment (at baseline pretreatment, early-on-treatment, and progression time points). This could allow deep analysis to unveil potential mechanisms of therapeutic resistance [Figure 1]. Furthermore, novel biological metrics may be developed with the potential to monitor immune-related adverse events on treatment to minimize the risk to patients.

To make personalization of cancer immunotherapies a reality, a continuous effort is required translate the insights from translational clinical studies to validated, high-throughput immune assessments. Collaborative efforts such as the Immune Biomarkers Task Force convened by the Society for Immunotherapy of Cancer and further nimble academic-industry partnerships will be required to fully harness the prodigious potential of personalized immune oncology.

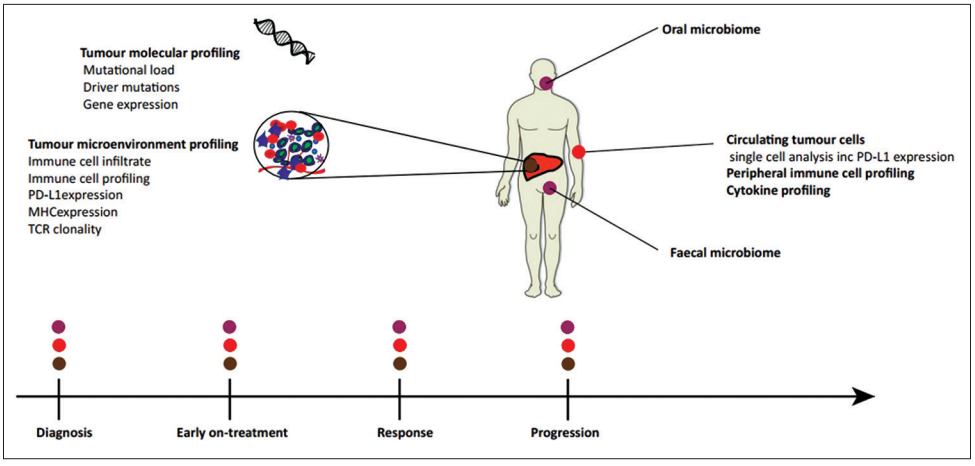

Figure 1: Pathway for personalized immunotherapy. Baseline assessment of a cancer patient includes profiling of the tumor for driver mutations, mutational load, and gene expression profiles; the tumor microenvironment including programmed death ligand 1 expression and analysis of infiltrating immune populations; and host immune responses and the microbiome. Longitudinal evaluation of fresh serial samples – tumor (brown), blood (red), and microbiome (purple) during treatment (pretreatment, early-on-treatment, response, and progression time points) may unveil potential predictive response biomarkers, resistance mechanisms, and further therapeutic strategies.

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