MINI REVIEW

Personalized Oncology Meets Immunology: The Path toward Precision Immunotherapy

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ABSTRACT

Personalized oncology aims to tailor therapy by targeting the unique genetic characteristics of a patient’s tumor, whereas cancer immunotherapy focuses on activating the patient’s immune system to control the tumor. The fusion of these ostensibly separate strategies has created a new dimension for personalized cancer immunotherapy. This entails the development of next-generation cancer vaccines that target neoantigens as well as the use of mutational signatures as predictive biomarkers for clinical response. The optimal use of immunotherapeutic agents will hinge on a robust understanding of the mutational profile of a cancer’s genome that significantly dictates antitumor immunity and immunotherapeutic response.

Significance: Cancer immunotherapy has provided substantial clinical benefit in a significant number of patients with advanced disease. However, the need for more precise immunotherapies and predictive biomarkers remains pressing. Recent progress in these areas has been promising and has created a framework for precision immune-oncology.

INTRODUCTION

The impressive successes seen with immunotherapy within the past several years have garnered increasing optimism for its use across multiple human cancers. Recent successes with melanoma, non–small cell lung cancer, and renal cell cancer have paved the way for several active trials across different cancer types (1–5). However, as the field continues to grow, the need for appropriate selection of patients for optimal therapeutic benefit is critical.

Although current clinical trials with immunotherapeutics appear promising, there are obstacles that must be addressed prior to greater expansion of this family of therapeutics in oncologic care. The current challenges that exist within immune-oncology include two particularly important issues. First, the percentage of patients who respond is low. Thus, there is a need for the development of more efficient and effective therapies to optimally activate the host immune system while limiting the toxicities of autoimmunity. The second challenge involves identifying patients who respond best to any given immunotherapy and the dependence of this response on the unique molecular profiles of their tumors. These determinants of response may be independent of classic indicators of prognosis, such as tumor grade, stage, or histology.

Understanding the precise genetic and nongenetic determinants of therapeutic response is paramount in successfully individualizing cancer care, and, as with targeted therapies, this paradigm is critical for the optimal use of immunotherapy. With the advent of next-generation sequencing, the feasibility of large-scale genomic analysis of patient tumors is becoming more attainable. As the field continues to advance, the importance of understanding cancer at the genomic/molecular levels will be critical, and genomic information will likely supplement more classic markers (such as pathologic analysis) in predicting response to immunotherapies. This review will discuss the current therapeutic strategies employed in immune-oncology as well as the development of genomic and nongenomic predictors of response to these novel therapies and their implications for individualized cancer care.

THERAPEUTIC STRATEGIES IN IMMUNE-ONCOLOGY

Immune Checkpoint Blockade

Recently, significant clinical responses have been observed in patients treated with monoclonal antibodies against immune checkpoint receptors such as cytotoxic T-lymphocyte antigen-4 (CTLA-4) and programmed cell death (PD-1). These results have garnered interest in immune checkpoint inhibition as a novel therapeutic approach applicable to multiple human cancers. The concept of immune checkpoint inhibition entails blocking the inherent immunoregulatory mechanisms that exist in the immune system, which evolved to prevent host autoimmunity...
and control excessive immune reactions against certain antigens. Immune checkpoint inhibition can thus help activate a more robust cellular-mediated immune response and facilitate tumor clearance. The CTLA-4 receptor is found on the surface of effector T cells and interacts with CD80/CD86 (B7-1 or B7-2) on antigen-presenting cells (APCs) to induce T-cell arrest (6). Similarly, the PD-1 receptor is also found on activated T cells and, when bound to its ligands PD-L1/PD-L2, induces a T-cell-inhibitory cascade (7). FDA approval in 2011 of the anti–CTLA-4 agent ipilimumab for metastatic melanoma marked the beginning of immune checkpoint inhibition as an accepted therapy for patients with advanced cancers. Since then, the anti–PD-1 agents pembrolizumab and nivolumab have been added to the armamentarium of available checkpoint inhibitors for cancer. They were approved by the FDA in 2014. Most recently, the indications for nivolumab were extended in 2015 from non-small cell lung cancer to include renal cell carcinoma. These agents have been able to produce dramatic results, including durable clinical benefit in close to one-third of patients with advanced disease in numerous recent studies (1, 8–11).

In addition to targeting the PD-1 receptor found on the T cell itself, much interest has also developed in targeting its ligands, PD-L1 and PD-L2, which can be found on tumor cells and APCs. The expression of these ligands on tumor cells or APCs contributes to the loss of function of cytotoxic T lymphocytes (CTL) and can promote T-regulatory cell function (7). Several promising trials are under way examining these agents alone or in combination with anti–PD-1 agents (3, 4, 8, 12).

Additional immunoregulatory receptors are also under investigation as targets, including TIM3 (encoded by the HAVCR2 gene) and LAG3 (13, 14). TIM3 is now known to be an immune checkpoint molecule. TIM3 is found on CD4+ Th1 cells and CD8+ CTLs, and functions to limit T-cell activity primarily through decreasing T-cell cellular responses and activation of T-regulatory cell mechanisms (14, 15). TIM3 is frequently coexpressed with PD-1, and these dual-expressing cells exhibit greater defects in both cell division and cytokine production than cells that express only PD-1. The LAG3 receptor is also an immune checkpoint molecule and functions primarily to decrease T-cell activity and proliferation by binding strongly to MHC class II molecules and aiding in T-regulatory cell function (16). LAG3 has been shown to be coexpressed on T cells with PD-1 and contributes to the decreased function of CTLs on the tumor, making it an enticing candidate for combined therapy with anti–PD-1 agents (13, 17). Agents targeting TIM3 and LAG3 (among many others) are in preclinical and clinical investigation. If blockade of these molecules ultimately proves to be clinically meaningful, these agents may become part of the armamentarium of immune checkpoint adjuncts in patients whose tumors express high levels of these regulatory ligands. Figure 1 depicts examples of immunoregulatory ligands and their respective receptors.

In addition to these immune checkpoint molecules, a large number of other immunoregulatory molecules—both antagonists and agonists of host immunity—are being investigated as therapeutic targets. A definitive discussion of these targets is beyond the scope of this review. However, Table 1 lists a number of other targets, in addition to the ones discussed above, that have drawn interest from the academic and pharmaceutical communities.

**Cancer Vaccines**

Vaccines against viruses implicated in the development of human cancers, such as the human papilloma virus (HPV), can provide a preventative means against cancer development. HPV is associated with cervical and oropharyngeal head and neck squamous cell cancers, and vaccination with a recombinant HPV vaccine against specific HPV subtypes has been shown to reduce the incidence of these cancers (18–21). Similarly, vaccination with the hepatitis B vaccine can help prevent hepatitis-associated hepatocellular carcinoma (22).

Beyond preventative vaccines, therapeutic vaccines against antigens expressed by tumors provide a potentially promising strategy for immunotherapy. Vaccines have been engineered against tumor-specific and tumor-associated antigens to mount an immune response against the tumor that can persist long after administration of the vaccine secondary to the generation of immune memory cells. The first and only therapeutic cancer vaccine approved in the United States was sipuleucel-T for advanced prostate cancer. Autologous lymphocytes are extracted from the patient and exposed to prostatic acid phosphatase (PAP) in vitro with conjugated GM-CSF and then reinfused into the patient. These immune cells have mostly differentiated into dendritic cells and are able to present PAP antigen (which is found on 95% of prostate cells) to T cells in the lymph nodes to generate an immune response against the tumor (23). Sipuleucel-T was found to increase overall survival by 4 months in patients with advanced castration-resistant prostate cancer as well as provide improvements in 3-year survival rates compared with control groups (23). Vaccine trials for other cancer types are also under way, including a phase III trial for a melanoma vaccine against gp100 in combination with immunomodulators such as IL2 (24). Other agonists of innate immunity have also been shown to boost vaccine efficacy. Recently, a stimulator of interferon genes (STING) agonist vaccine has been shown to promote antitumor innate immunity and even improve response in combination with anti–PD-1 therapy (25). Another notable therapeutic vaccine is GVAX, a whole-cell cancer vaccine derived from two cancer cell lines engineered to express GM-CSF for the treatment of pancreatic cancer. Initial trials with GVAX failed to show improved overall survival, but when combined with CRS-207, a mesothelin-secreting vaccine, the resulting synergistic effect produced a modest overall improvement in patients with metastatic pancreatic cancer (26). However, the failure of many vaccine monotherapies demonstrates the tumor’s propensity for immune escape and the need for combination therapies.

Nevertheless, therapeutic vaccines have provided clinical benefit, albeit modest, to patients with advanced disease, underscoring vaccination as a key therapeutic strategy in immune-oncology for future development. Furthermore, generating personalized mRNA/DNA-based vaccines against a patient’s tumor-specific antigens affords the opportunity to increase the efficacy and specificity of these therapeutic vaccines (discussed below).

**Adoptive Cell Transfer Therapy**

Adoptive cell transfer (ACT) therapy has emerged as a promising therapeutic strategy within immune-oncology. Autologous tumor-infiltrating lymphocytes (TIL) are obtained from
Figure 1. Immunoregulatory influences within the tumor microenvironment. Stimulatory (green) and inhibitory (red) signals between T lymphocytes and antigen-presenting/tumor cells are depicted above. Drugs in clinical investigation stimulating or inhibiting these specific targets are also illustrated.

an excised tumor specimen. The TILs are then cocultured with IL2, which promotes their growth ex vivo (27). Sub-populations of the proliferating TILs are then tested against the patient's original tumor in vitro, and those cells that are found to be active against the tumor are expanded to as high as one hundred billion \((10^{11})\) cells. The expanded population of TILs (which are now primed against the patient's tumor) is then transfused back into the patient to mount an immune response in vivo against the residual disease. This approach has in fact produced significant clinical responses in patients with metastatic disease in a number of studies (28–30). The principle of ACT should be applicable across multiple cancer types; currently, however, the vast majority of studies have shown efficacy against only a few tumor types, such as melanoma. This likely relates to the immunogenicity of melanoma and the higher affinity for immune clearance of these tumors. However, much interest has developed in modifying ACT to increase its applicability across cancer types by making it more patient-specific. The ACT therapy approach itself is a highly personalized therapy, but with the increasing feasibility of genetic sequencing of each individual patient’s tumor, ACT may become even more precise, as seen with engineered T-cell receptors (TCR) and chimeric antigen receptors (CAR), which are discussed below.

PERSONALIZED CANCER CARE AND PREDICTION OF IMMUNOTHERAPY RESPONSE

From the discussion above, it is clear that immunotherapy takes on many different shapes and forms—immune checkpoint blocking agents, cancer vaccines, T-cell therapy, etc. It is
<table>
<thead>
<tr>
<th>Target</th>
<th>Principal immune function</th>
<th>Source(s)</th>
<th>Principal pathway mechanism(s)</th>
<th>Select drug(s) in clinical investigation</th>
</tr>
</thead>
<tbody>
<tr>
<td>IDO1</td>
<td>Inhibitory</td>
<td>Expressed by multiple immune cell subpopulations, tumor cells, host cells</td>
<td>Involved in tryptophan metabolism causing a depletion of available tryptophan in the tumor microenvironment required by immune cells for antitumor activity; also involved in T-regulatory cell activation</td>
<td>Epacadostat (INCB024360) GDC-0919 Indoximod</td>
</tr>
<tr>
<td>B7-H3</td>
<td>Inhibitory</td>
<td>Transmembrane receptor protein found on APCs, tumor cells, host cells</td>
<td>Binds to unknown receptors on T cells causing inhibitory/stimulatory immune signals; however, primarily inhibitory</td>
<td>Enoblituzumab (MGA271)</td>
</tr>
<tr>
<td>B7-H4</td>
<td>Inhibitory</td>
<td>Transmembrane receptor protein found predominantly on APCs, tumor cells</td>
<td>Binds to unknown receptors on T cells causing an inhibitory effect</td>
<td>None</td>
</tr>
<tr>
<td>VISTA</td>
<td>Inhibitory</td>
<td>Transmembrane receptor protein found on APCs, T cells</td>
<td>Binds to unknown receptors on T cells stimulating FOXP3 expression in T cells and inhibiting T-cell proliferation/activity</td>
<td>None</td>
</tr>
<tr>
<td>CD200R</td>
<td>Inhibitory</td>
<td>Transmembrane glycoprotein found on myeloid and lymphoid cells</td>
<td>CD200-CD200R pathway signals inhibitory effects on myeloid and NK cells; also stimulates T-regulatory cell proliferation/activity; upregulates IDO activity</td>
<td>Samalizumab</td>
</tr>
<tr>
<td>ICOS</td>
<td>Inhibitory</td>
<td>Transmembrane receptor protein found on T cells (highly expressed on T-regulatory cells)</td>
<td>Exerts an immune inhibitory function by binding to ICOS-L on APCs stimulating T-regulatory cell function, thus mediating overall immune suppression</td>
<td>MEDI570</td>
</tr>
<tr>
<td>KIR</td>
<td>Inhibitory</td>
<td>Transmembrane receptor found predominantly on NK cells</td>
<td>Produces inhibitory signal upon recognition of HLA Class I inhibiting NK-cell-mediated cytotoxicity</td>
<td>Lirilumab</td>
</tr>
<tr>
<td>TIGIT</td>
<td>Inhibitory</td>
<td>Transmembrane receptor protein found predominantly on T cells and NK cells</td>
<td>Produces inhibitory signal on T cells and NK cells upon binding to its high-affinity ligand, CD155</td>
<td>None</td>
</tr>
<tr>
<td>OX40</td>
<td>Stimulatory</td>
<td>Transmembrane receptor protein found on T cells</td>
<td>Binds to OX40-L on APCs to stimulate T-cell proliferation and activity</td>
<td>MEDI6469 MOXR0916</td>
</tr>
<tr>
<td>4-1BB</td>
<td>Stimulatory</td>
<td>Transmembrane receptor found on activated T cells</td>
<td>Produces stimulatory signal upon binding to 4-1BBL on APCs stimulating T-cell proliferation and activity</td>
<td>Urelumab (BMS-663513) PF-05082566</td>
</tr>
<tr>
<td>GITR</td>
<td>Stimulatory</td>
<td>Transmembrane receptor found on T cells</td>
<td>Produces stimulatory signal upon binding to GRITL on APCs stimulating T-cell proliferation and activity; also involved in T-regulatory cell function</td>
<td>TRX518</td>
</tr>
</tbody>
</table>

Abbreviations: IDO, indoleamine 2,3-dioxygenase; NK, natural killer.
not surprising, then, that personalization of these treatment strategies will involve evaluation of the different determinants of response.

Despite its successes, the efficacy of immune checkpoint blockade is widely variable across individual patients and tumor types. Early examination of tumor tissue from patients treated with anti–PD-1 antibody suggested that PD-L1 levels (as assessed by immunohistochemistry) on tumor cells correlated with positive clinical response to treatment. This was demonstrated by Topalian and colleagues, who observed a 36% objective response rate in patients with PD-L1-positive expression of their tumors as compared with no responses from PD-L1-negative patients, albeit seen only in this specific cohort (10). Similar associations between response and PD-L1 expression have been corroborated in a number of subsequent studies across tumor types since then, and PD-L1 expression remains the strongest currently accepted biomarker for response to anti–PD-1 therapy (3). As a result, a number of academic and pharmaceutical companies have developed a variety of immunohistochemical assays for evaluating pretreatment expression of PD-L1 in solid tumors. However, it should be noted that the majority of patients who are PD-L1 positive will not demonstrate objective responses to anti–PD-1 therapy; furthermore, approximately 15% of patients who are PD-L1 negative have demonstrated objective responses, which is in contrast to the lack of response seen in PD-L1–negative patients in Topalian’s original study. Moreover, PD-L1 levels are inducible and have region-specific intensities that can be difficult to measure.

Recent efforts have focused on protein expression profiles that could potentially be used to predict response to immunotherapy using DNA barcoding–nanostring technology. This technology entails the use of antibodies linked to DNA “barcodes” which have photocleavable linkers (31). The unique DNA barcodes can then be measured and allow for the quantification of the protein of interest. A major advantage of this approach is that it permits quantification of even small amounts of proteins from a small number of cells, such as a fine-needle aspirate sample, making its use highly feasible in the clinical setting. Furthermore, this approach may permit sampling of multiple sites of a tumor in order to overcome issues with tumor heterogeneity. Large-volume biopsy specimens from a single region of a tumor may not accurately depict the characteristics of the tumor as a whole if significant heterogeneity is present. Ultimately, this technology could potentially provide valuable gene expression information, such as expression of checkpoint molecules, immune cytokines (i.e., IFNγ), cancer testis antigens, etc., in order to help guide immunotherapy treatments and predict response. Currently, however, the expression of checkpoint ligands alone or immune-related proteins has not materialized as an accurate predictor of response to immunotherapies, which underscores the need for more precise genomic and nongenomic markers of response to more effectively personalize immunotherapeutic cancer care.

**Mutation Burden, Neoantigens, and Immunotherapeutic Response**

Recently, it has become clear that the genetic landscape of tumors has an important role in dictating tumor immunity and response to immunotherapy (32). Nonsynonymous mutations in cancer cells have the potential to generate novel antigens that are presented to the immune system. These neoantigens can be detected as non-self-epitopes, triggering the immune system for removal (33). The exact role of tumor neoantigens in immune-mediated clearance is an area of intense investigation, particularly in regard to their role in the response to immunotherapy. Higher overall mutation burden is correlated with increased immune CD8+ T-cell cytolytic activity in human cancers (34, 35). Importantly, recent studies have also shown that a higher mutational burden is accompanied with increased durable clinical benefit (DCB) and progression-free survival (PFS) in patients treated with immune checkpoint inhibitors (32, 36, 37). Rizvi and Chan demonstrated that higher nonsynonymous mutational load was associated with DCB in patients with non–small cell lung cancer treated with the anti–PD-1 agent pembrolizumab (32). Snyder and colleagues demonstrated the same relationship between high mutation load and clinical benefit to anti–CTLA-4 therapy in patients with melanoma (36). However, important exceptions were noted, including a few patients with high levels of nonsynonymous mutational burden who did not respond to checkpoint blockade and some patients with a very low nonsynonymous mutational burden who did respond to the therapy.

How can this be reconciled with our current understanding of mutational burden and the likelihood of response? We hypothesize that the absolute number of nonsynonymous mutations trends with but may not be the root factor driving improved immunogenic response in patients treated with checkpoint blockade. Rather, it is likely the creation of specific immunogenic neoantigen peptides that drives tumor clearance and subsequent improved survival. Rizvi and colleagues demonstrated that neoantigen-specific T-cell reactivity increased in concert with clinical response in a patient who responded well to treatment with pembrolizumab, giving further credence to the theory that specific neoantigens are the principal drivers of clinical response to immunotherapy (32). However, the probability of the creation of these specific immunogenic neoantigen peptides by the tumor increases with a higher total mutational burden. As such, tumors with increased neoantigen burden are more likely to respond to checkpoint blockade, as evidenced by the aforementioned recent studies (32, 36, 37).

In addition, it was observed that a validated molecular smoking signature strongly correlated with response to pembrolizumab (32, 38). Patients with high levels of transversions (smoking signature high) were significantly more likely to respond to anti–PD-1 therapy as compared with patients with low levels of transversions. Interestingly, patients’ self-reported smoking history did not correlate as well with either DCB or PFS. Molecular footprints of tobacco-induced carcinogenesis may be a better marker here compared with clinical smoking history, for the following reasons: (i) patient smoking history may not be reliable, or (ii) there are patient-to-patient variations in how much DNA damage any given amount of tobacco can induce. Immune checkpoint inhibitors have demonstrated efficacy in other human malignancies that are linked to smoking, such as esophageal cancer, head and neck squamous cell cancer, and bladder cancer. It will be very interesting to determine whether a similar smoking signature can help identify immunotherapy responders in these tumor types as well. These findings can potentially alter the management of patients in the clinical setting.
setting. By combining whole-exome sequencing and predictive neoantigen computational algorithms, clinicians will be able to better tailor therapy to an individual’s specific tumor (Fig. 2).

It is likely that not merely the quantity, but rather the quality, of neoantigens is more important for initiation of anti-tumor immunity after treatment with immune checkpoint inhibitors. Several studies have now shown that functionally active antigens or neoantigens have homology to antigenic peptides from microbes or viruses (36, 39). Birnbaum and colleagues used degenerate libraries and showed that single TCRs that each recognized single MHC class II–presented peptide antigens could cross-react with hundreds of related peptides. They observed that core sequences of 4- to 5-mers dictated T-cell cross-reactivity (39). Interestingly, many of these cross-reacting peptides shared extensive similarities to environmental microbes. The authors synthesized these environmental antigens and showed that the TCRs did indeed cross-react with these peptides and induced proliferation (39). Similarly, Snyder and colleagues identified shared candidate neoantigen features present only in responders to anti–CTLA-4 treatment and noted the presence of shared stretches of 4-mers in these peptides (36). Perhaps not coincidentally, these features were also homologous to validated antigens from microbes. Given this homology, it is conceivable that these common tetrapeptide sequences exist in patients who respond to anti–CTLA-4 therapy because they are essentially “primed” against these microbial-resembling antigens and can thus more effectively clear the tumor as compared with patients without these common antigens. It should be noted, however, that in a study by Van Allen and colleagues, these recurrent tetrapeptide sequences were not enriched in their cohort (40). The reasons for this discrepancy have yet to be elucidated but are being examined. Possible explanations may involve potential broad differences in the tumor genetic profiles or perhaps even in the TCR clonal repertoires between these distinct patient cohorts. It could also be that the microbiomes of the German patients differ from those of the American cohorts. If JAX and Taconic mice have differing responses to immune checkpoint blockade, it is not outside of reason that humans from different continents undergo a similar phenomenon. Lastly, the Van Allen study used different methodologies including NetMHCpan for MHC binding prediction, which is much more speculative and
inaccurate for less-represented alleles. This may have introduced a significant amount of noise to their dataset, which is supported by the fact that they report more neoantigens than mutations in their tumors, which is highly unlikely. Lastly, they reported their own recurrent peptides that they claim to be specific for responders; however, we found these peptides in our nonresponders. Clearly, these discrepancies underscore the need for validation in larger patient cohorts before recurrent peptides can be made broadly applicable as predictive biomarkers in the clinical setting.

Another study identified a large population of memory T cells with affinity for Melan-A peptide-MHC (pMHC) tetramers in an individual with no history of melanoma. It was noted that this specific T-cell clone was strongly reactive to a Mycobacterium-derived peptide (41). Interestingly, a number of public, shared TCRs with defined antigen specificity are TILs and present in the tumor microenvironment (42). The CDR3β sequences in tumors contained numerous matches to known TCRs with viral specificity. These included TCRs specific for Epstein–Barr virus and Influenza A. Moreover, specific bacteria are needed for the anti–CTLA-4 and anti–PD-1 treatments to exert their antitumor effects (43, 44), and the former could be transferred by adoptive transfer of microbe-specific T cells. Together, these data suggest that immunologic memory and how this memory is shaped by environmental exposures may need to be taken into account in order to personalize immunotherapy. More critically, it points to the idea that there may be some shared, public features in the neoantigen landscape.

Genomic Stability and Response to Immunotherapy

Mutations in cancer cells that affect genes involved in DNA replication error correction are of particular interest in cancer immunotherapy. These genetic alterations have the ability to decrease the cell’s DNA replicative fidelity and thus can confer a “mutator phenotype” to the cell. This genomic instability can induce malignant transformation in human cells (45, 46). Faithful DNA replication is principally executed by the accuracy of the nuclear DNA polymerases (Polymerase δ and Polymerase ε) and their respective exonuclease proofreading domains (47). These exonuclease domains have the ability to excise misplaced DNA nucleotides and allow the placement of the correct nucleotide in accordance with the template DNA strand (48). Murine models with inactivating mutations of the genes encoding the proofreading domains of DNA polymerases (POLE and POLD, respectively) have been shown to develop spontaneous tumors (47, 49, 50). Inactivating mutations of the exonuclease domains of these polymerases are found in human cancers. A number of colorectal and ovarian cancers, for example, have been shown to harbor inactivating mutations of the POLE and POLD genes (51–53). Mutations in the POLE and POLD genes were also seen in patients with non–small cell lung cancer (32).

In addition to the DNA polymerases, mismatch repair (MMR) proteins play a critical role in maintaining the cell’s DNA replicative integrity. Nonmutated DNA polymerases will generate replicative errors at a rate of 1/10^10 to 1/10^14 nucleotides (47, 54). This intrinsic error rate is almost entirely corrected by the cell’s MMR machinery, decreasing the error rate to only 1/10^10 nucleotides (47, 55). Mutations in MMR genes can generate a higher mutational load in the cell by disabling repair of base pair mismatches. This loss of fidelity can be observed by the alteration of DNA microsatellites that are normally corrected by the MMR machinery. Microsatellite instability (MSI) therefore serves as a surrogate for MMR dysfunction in the cell (56).

Hereditary nonpolyposis colorectal cancer, also known as Lynch syndrome, is due to an inherited defect in MMR genes (57). These patients have approximately an 80% lifetime risk of developing colorectal cancer (58). Defects in the MMR machinery are also seen in patients with other cancer types as well (36, 59–61). Similar to mutations in the POLE/POLD genes, mutations in the MMR genes will allow for the generation of oncogenes (or loss of tumor-suppressor genes) and thereby increase the likelihood of malignant transformation in the cell.

Recent work has demonstrated a strong association between the presence of MSI and clinical benefit from treatment with an immune checkpoint inhibitor. What is the connection between mutator phenotypes and response to immunotherapy? The answer may in part relate to the concept of immunogenic neoantigens discussed previously. Increased nonsynonymous mutations generate more neoantigens, some of which may be immunogenic and can elicit the host immune response (alone or in combination with immune checkpoint blockade) to clear the tumor. Therefore, tumors with very high mutational burdens (such as those conferred by mutations of the polymerases and the MMR machinery) may be more susceptible to immunotherapy, as explained by the neoantigen theory of immunotherapeutic response. Le and colleagues demonstrated that patients with colorectal cancer whose tumors were MMR-deficient had significantly better clinical responses to treatment with pembrolizumab as compared with patients who were MMR-proficient (37). Furthermore, they noted that MMR-deficient patients of other noncolorectal cancer types (endometrial, etc.) also displayed clinically significant responses to treatment with pembrolizumab (37). These results validate the concept that high mutation count could be used as a predictor of immunotherapeutic response across multiple cancer types. In addition, mutations in the POLE and POLD genes in non–small cell lung cancer were noted to have higher mutational burdens and better objective clinical responses after treatment with pembrolizumab (32). Therefore, it may be possible for clinicians to tailor therapy based on the status of a patient’s DNA error corrective ability (or its surrogate markers, i.e., MSI) to determine the best course of treatment with immunotherapies. Further investigation is needed in order to refine the use of these tumor characteristics as predictors of immunotherapeutic response across human cancers.

Tumor Antigens, Vaccines, and Adoptive Cell Therapy

The history of cancer vaccine development spans several decades, with most attempts unfortunately resulting in failure. These earlier attempts likely failed because the choice of vaccine target was inadequate or immune checkpoints were still intact and prevented the activation of antitumor immunity. In the age of personalized oncology, the search for more effective vaccine targets against tumor-specific antigens provides an enticing strategy. These targets may be specific to an
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individual patient’s tumor or seen in a subset of patients with a particular tumor type. A particularly notable example is a set of tumor-specific antigens known as cancer testis antigens (CTA). These antigens are found in germ line cells and, specifically, on many cancer cells (62). CTAs are thought to contribute to the oncogetic and metastatic potential of tumor cells across many cancer cell types (63–65). The first CTAs identified were the MAGE family of antigens in 1991 (66). Since then, many more tumor-specific CTAs have been identified. As these antigens are predominantly expressed on tumor cells, they provide a site-specific target for immunotherapy. Although promising, results from preliminary studies using vaccines against CTAs have produced variable and underwhelming clinical results. A trial targeting the cancer testis antigen NY-ESO-1 in patients with melanoma showed some moderate clinical responses (67). However, two large phase III randomized studies using the cancer testis antigen MAG-MAGE-A3 in patients with non–small cell lung cancer and melanoma failed to show improvement in disease-free survival as compared with placebo (68). The reasons for the lack of clinical response are multifold and likely involve T-cell exhaustion, clonal editing, and other mechanisms of immune escape by the tumor.

In addition to CTAs, tumor self-antigens provide attractive targets for vaccine development as well. These include nonmutated antigens which are overexpressed in tumor cells as compared with normal cells. Some examples of differentiation tumor self-antigens in melanoma include gp100, Melan-A/Mart-1, and tyrosinase. In addition, many more overexpressed tumor self-antigens have been identified as well, including notable examples in breast cancer such as HER2/neu and MUC1. Together with CTAs, these tumor self-antigens offer an appealing opportunity for targeted immunotherapy.

How can precision oncology approaches increase the efficacy of vaccine strategies against tumor-specific and tumor-associated antigens? One possibility entails the use of tumor-specific vaccines targeted to an individual patient’s tumor. Next-generation sequencing allows for efficient and timely sequencing of individual patient tumors to determine the expression level of a specific cancer testis antigen (or other immunogenic epitope). This could allow for the identification of which tumor-specific neoantigens are expressed and in which patient—two pieces of data that are critical for improving vaccine efficacy. Subsequently, vaccines against these specific neoantigens are engineered and delivered to the patient to facilitate immune clearance of the tumor. However, not all mutant peptides are capable of eliciting an immune response. The successful recognition of a given antigen by adaptive immunity requires that key criteria are first met. The peptide must be successfully degraded by intracellular proteasomes and shuttled to the endoplasmic reticulum for further antigen processing. Antigen presentation by MHC class II molecules occurs via a separate pathway. Unlike antigen presentation by MHC class I molecules (which occurs primarily via the ubiquitin-proteasome pathway), antigen presentation by MHC class II molecules occurs via the lysosomal degradation pathway. Mutations in tumors affecting the cell’s antigen-presenting machinery (APM) may result in an inability for a predicted antigenic peptide to be presented. A variety of computational programs exist for the in silico prediction of possible neoantigens. These algorithms incorpo-
rate the likelihood that a given peptide sequence will undergo appropriate cleavage by the APM and bind to the MHC. MHC class I molecules generally present intracellular antigens that are 8 to 11 amino acids in length, whereas MHC class II molecules can present extracellular antigens of larger sizes generally ranging from 11 to 20 amino acids. Computational algorithms exist for prediction of antigen binding to MHC class I/II molecules as well as for binding within the MHC-TCR complex. Whole-exome sequencing of patient tumors allows for the identification of tumor-specific mutations in the clinical setting. These gene mutations can be translated in silico, and resultant peptide sequences are tested against the described neoantigen prediction algorithms. Thus, these neoantigen prediction tools can help direct the selection of candidate neoantigens for vaccine development by predicting which mutations are most likely to create potentially presented neoantigens.

The principle of tumor neoantigen-based vaccination was demonstrated in a murine model by Castle and colleagues, who utilized whole-exome data from B16-F10 mouse melanoma tumors (69). The authors identified 563 expressed, non-synonymous gene mutations, of which 50 were selected using neoantigen prediction algorithms for in vivo validation. Mutation-specific peptide vaccines were then engineered and administered to syngeneic hosts. One third of these vaccines elicited a host immune response, and two were found to have significant antitumor effects. This study demonstrated the concept that vaccines engineered against private tumor-specific mutations could be employed as a personalized vaccine strategy.

In a recent study by Carreno and colleagues, tumor-specific neoantigens were identified from resected specimens from patients with cutaneous melanoma (70). Selected neoantigen peptides were incorporated into dendritic cell–based vaccines that were administered to the patients from whom they were derived. The authors noted robust vaccine-induced neoantigen-specific T-cell responses (70). However, rigorous clinical outcomes from these treatments were not reported, and it will be critical moving forward to openly and accurately report these important data.

Typically, CD8+ T cells are implicated in neoantigen-based vaccination efficacy, but recent work in murine models has also validated that tumor-associated neoantigens are specifically recognized by CD4+ T cells as well (71). After exome sequencing and appropriate vaccination with CD4+ T-cell reactive epitopes, activated CD4+ T cells are able to reshape the tumor immune microenvironment and facilitate tumor clearance. A growing body of evidence has demonstrated that these cells play a critical role in promoting antitumor immunity. In an additional study specifically evaluating CD4+ T cells, Linnemann and colleagues demonstrated the presence of intratumoral neoantigen-specific CD4+ T cells in melanoma, which promoted antitumor immunity (72). The authors noted that these CD4+ T cells were reactive against private mutations specific to the original tumor. These studies demonstrate the feasibility of potentiating personalized T-cell immunity against patient-derived neoantigen peptides.

A second strategy involves removing the operative immune checkpoints that hinder the vaccine’s antitumor capability. As previously discussed, CTAs and lineage-derived self-antigens are wild-type proteins and may be subject to central tolerance. Here, transcriptome analysis can reveal which immune
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checkpoints are active. Treatment with the correct inhibitors to target the operative immune checkpoints can facilitate improved vaccine efficacy.

A similar strategy can be employed with the use of adoptive cell therapy. Traditionally, TILs are isolated from the patient and expanded in vitro, often with stimulation from cytokines such as IL2 (27). These TILs are reinfused into the patient and induce cellular-mediated immune clearance of the tumor (28). This approach can potentially be improved by sequencing the individual patient’s tumor exome and identifying specific neoantigens. Subsequently, the patient’s circulating autologous T cells can be extracted and engineered to express a CAR or an engineered TCR against the neoantigen(s) identified by the sequencing of the tumor. This approach produces a highly specific T-cell population that can be reinfused into the patient to clear the tumor. The Schumacher group demonstrated the ability to target neoantigens by a technique known as “TCR gene capture” (73). Using this approach, they were able to demonstrate how sequencing TCR genes from a set of tumor-infiltrating CD8+ T cells can provide information on the TCR repertoire active against tumor antigens. Subsequently, with this knowledge, T cells with reactive TCRs could then be engineered and delivered to the patient to mediate clearance without necessary prior knowledge of antigen specificity.

In an interesting recent study by Tran and colleagues, the investigators identified a CD8+ TIL targeting the KRASG12D driver mutation in a patient with metastatic gastrointestinal cancer (74). The authors suggested that engineering TILs targeting specific neoantigen mutations (such as KRASG12D) found after sequencing the patient’s individual tumor provides a highly effective targeted, personalized T-cell therapy (74). And indeed, adoptive transfer of TILs targeting a neoantigen in a patient with metastatic cholangiocarcinoma yielded impressive tumor control as well (75). Interestingly, tumor regression in this study was found to be mediated by neoantigen-specific CD4+ T cells.

However, even with these individualized approaches, the current generation of vaccines and T-cell therapy is unable to provide durable clinical responses in all patients. T-cell exhaustion and anergy are often seen as mechanisms of immune escape secondary to immune counter-regulatory mechanisms (76–78). This provides a rationale for combination therapy with the use of immune checkpoint inhibitors to prevent immune escape by the tumor. In addition, deficient numbers of reactive T cells contribute to the decreased efficacy of tumor clearance. This can in part be combated with the addition of T-cell–activating cytokines, including IL2 (79). Indeed, several trials combining these adjuvant therapies (cytokines, immune checkpoint inhibitors) with therapeutic vaccines or adoptive cell therapy have been performed or are under way (80, 81).

Challenges Facing Personalized Immunotherapy

As with all individualized therapies, challenges with personalized immunotherapy include demonstrating large-scale efficacy as well as cost-effective implementation of such strategies in the clinical setting. In the past, the enormous costs of exome sequencing made personalized genomics-based immunotherapies cost-prohibitive on a large scale. It may be possible to develop smaller gene panels (independent of cancer-driver genes) which could perhaps serve as cheaper surrogate measures for tumor mutational burden. However, as the cost of high-throughput sequencing continues to decline, the feasibility of sequencing individual patient tumors on a large scale is becoming more attainable.

Another issue that needs to be overcome is the lack of uniform efficacy which is difficult to achieve even when these strategies are personalized to individual patients. Even if similar antigens are identified in patients, the antitumor responses from identical vaccines in separate patients may be highly variable given potential differences in functional immune status and patient-specific TCR repertoires. Thus, host immunity itself is a variable that complicates the demonstration of broad efficacy with these approaches. Immune agonists, such as IL2 or TLR agonists, have been used to boost host immunity with therapeutic cancer vaccines, and this may help overcome differences in functional immune status between patients.

The importance of individual patient-specific differences in the microbiota has been demonstrated in recent work highlighting the microbiota’s ability to shape antitumor immunity. Sivan and colleagues demonstrated in mice that introduction of commensal bacteria (Bifidobacterium) to the host could confer spontaneous antitumor immunity, as well as improve response to checkpoint blockade (44). The authors suggest the mechanism of this enhanced antitumor immunity may be occurring in an antigen-independent fashion given the alterations in innate immune observed, but nonetheless demonstrates the variable response to immunotherapy that is host microbiome–specific. We believe, in fact, that this phenomenon is antigen dependent, as (i) adoptive T-cell transfer of bacteria-specific T cells transfers the ability to reap benefit from checkpoint blockade, and (ii) only specific species of microbes can elicit the effect, ruling out a nonspecific inflammatory response. Indeed, in another recent study, Vétizou and colleagues demonstrated the ability to induce responsiveness in mice to anti–CTLA-4 therapy by transfer of B. fragilis to germ-free mice as well as bacteria-specific T cells (43). These studies demonstrate that intersubject variations in microbiota could play a role in determining response to immunotherapies. Perhaps future adjuncts may serve to enhance the microbiota prior to treatment to make immunotherapeutic responses more uniform among patients. Nevertheless, the large-scale efficacy and mass distribution of personalized therapies will become more feasible as these challenges are inevitably overcome in the future.

CONCLUSION

The advancements in immunotherapy in recent years have led to some of the largest strides in cancer care in decades. Durable clinical responses have been seen in a significant number of patients treated with immunotherapies. However, the fact remains that only a minority of patients benefit from immunotherapy, and immunotherapy is not without its toxicities. Complications using immune checkpoint blockade agents have produced significant grade 3/4 toxicities (37), with death resulting in severe cases. Thus, it is imperative for investigators in the field to appropriately select patients who have the highest likelihood of durable response. We believe that the process of improving immunotherapies
should incorporate the genetic and nongenetic predictors of response. Establishment of such a paradigm will reveal individual immunotherapeutic vulnerabilities and help tailor immunotherapies to specific genetic profiles.

Disclosure of Potential Conflicts of Interest

T.A. Chan is a co-founder of Gritstone Oncology and has ownership interest (including patents) in the same. No potential conflicts of interest were disclosed by the other author.

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