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Biomarkers for Immunotherapy in Genitourinary Malignancies

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Abstract

Immunotherapy for genitourinary malignancies such as prostate, renal, and bladder cancers has experienced a resurgence since the development of three novel strategies: the autologous cellular product therapy, Sipuleucel-T for prostate cancer, the checkpoint inhibitors, anti-CTLA-4 for melanoma and anti-PD-1, and-PD-L1 for melanoma, renal cell, bladder and lung cancers, respectively. These agents have led to strikingly durable responses in several of these solid tumors but their efficacy has been inconsistent. Why all solid tumors are not equal in their response to these therapies is unclear but more importantly, demonstrating that changes in humoral or cellular responses can directly impact on the tumor's biology have not been evenly demonstrated largely as a result of differences in immune monitoring and the lack of reliable endpoints. How to design immunologic endpoints that reflect a meaningful impact on the cancer remains a challenge for clinical trial development. The issues faced by clinical investigators and the current state of immune monitoring are discussed.

Keywords

biomarkers; Sipuleucel-T; ipilimumab; nivolumab; Ki-67; MDSCs; Tregs; EliSPOT; renal cell; prostate cancer; bladder cancer; CTCs; PSA; PSMA; IL-2; immunoscore; cytokines

Introduction:

Significant enthusiasm has returned for immunotherapeutic strategies that not only target a particular aspect of the immune system but can also impact on the biology of the cancer and yield durable clinical responses. Immunotherapy is not new; preclinical studies have suggested that animals can be cured with a wide variety of approaches from conjugate and DNA vaccines, to combinatorial schemes with chemotherapy or biologic modifiers. However, stunningly successful preclinical approaches have not directly translated into success in man. To date, while multiple clinical trials have shown benefit as manifested by improvement in both survival and/or clinical response, there remains a disparity between

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immunologic monitoring parameters such as antibody titers or T cell responses, and clinical benefit. Therefore, clinical trials with immunologic endpoints that may have some relevance to the biology of the cancer are seriously lacking the necessary harmonization for the evaluation of all cancers and remains an area of debate among immunology aficionados. The concept of immune biomarkers[1] has been introduced by many with the expectation that there is an “immune signal” to indicate that the biologic or tumor target has been “hit” by the immune system and has caused a change in the biology of the cancer, ie, decrease in size of a target lesion, remission, or has correlated with some immune parameter to indicate a relevant response to the therapy. The far-reaching application of this is that a particular biomarker could aid in clinical decision making in terms of which anti-cancer therapy to use in a particular patient or can show that activation of a particular cellular population is indicative of treatment functionality and potential response [2,3], Many so-called biomarkers have included cell surface antigens that are overexpressed or biochemically altered from the normal conformation as the cancer undergoes malignant transformation. These include antigens that can be serologically measured such as Prostate Specific Antigen (PSA)[4], prostatic acid phosphatase (PAP)[2], Prostate Specific Membrane Antigen (PSMA) [5], or include those that can be evaluated by immunohistochemistry such as Six Transmembrane Epithelial Antigen of the Prostate (STEAP) [6], Prostate Stem Cell Antigen (PSCA)[7], MUC1,2[8], Globo H[8], GM2[8], EGFR[9-11] or erbB2[9-12] receptor overexpression (Table 1). While immunohistochemistry may be helpful in looking at overall expression markers on cancer cells, interrogation of the tumor milieu looking for relative increases or decreases in inflammatory or immune cell populations, or quantitation of circulating DNA [13], circulating tumor cell (CTC) numbers [14-17] continue to be evaluated in different clinical contexts as potential biomarkers of hitting the therapeutic target. One word of caution remains, the therapeutic target may be different from the immune target in question.

Biomarkers in Cancer:

There has been a major initiative in identifying an established a platform with which to implement biomarkers into large clinical trials for validation. However, it is imperative that a consistent understanding of what is defined as a biomarker, how to use it to use and how to implement it into clinical trials be made before stating that any marker can be used to as a biomarker. The term “biomarker” has often been used liberally to indicate that some laboratory measure is indicative in the change of the cancer. In fact, a “biomarker” is a laboratory measurement that reflects the activity of a disease process[13,18-20]. This is in contradistinction to a “surrogate marker”, “a laboratory measurement used in therapeutic trials as a substitute for a clinically meaningful endpoint that is a direct measure of how a patient feels, functions, or survives and is expected to predict the effect of the therapy.” Of note, governmental agencies such as the US Food and Drug Administration (FDA) recognized that basing an approval on the effect of a drug on an “unvalidated” surrogate introduced additional uncertainty into the approval process.

Biomarker development has undergone a rapid acceleration defined by two functional categories: prognostic and predictive. A “prognostic biomarker” may be a biologic or clinical characteristic or behavior that can be measured objectively and can be correlated with an outcome for the patient. This can include patients at high risk for disease relapse

and therefore may derive benefit from earlier interventions. A “predictive biomarker” offers information that may confer a likely benefit from treatment. These benefits include tumor response or improvement in overall or disease free survival. This may be used to identify those specific patients who may derive clinical benefit from a specific treatment approach. It should be clarified that while a biomarker can be “prognostic” in predicting the probability of survival, it may also be “pharmacodynamic” to monitor treatment, may serve as a “surrogate endpoint to substitute for a clinical efficacy endpoint, and could also be “predictive” in attempting to matching a beneficial drug to the patient.

One example of the need for clear definitions of these two biomarker paths in trying correlate a potential predictive marker was exemplified by Stat5 status in breast cancer which was considered as a marker for response to estrogen therapy. Prognostic factors can define the effects of patient or tumor characteristics on patient outcome, whereas predictive factors define the effect of treatment on the tumor[18]. The rationale for pursuing the role of CTCs as biomarkers emanated from analysis of 3 retrospective randomized phase III trials in colorectal cancer[14], breast [15],]and prostate cancer [16]. CTCs can be detected in as little as 7.5 cc of peripheral blood per PAXgene™ (Qiagen, Venlo, Netherlands) tube. Patients with CTCs of five or more have been shown to have a poorer prognosis than those who have less than five[17,21]. Similarly, in prostate cancer patients, for whom the standard biomarker (PSA) may be unreliable or in discordance with the disease status, a more reliable assessment of biologic response to treatment may be gleaned via CTC measurement, ie, a patient whose post- treatment CTC count declines and reaches zero will likely derive biologic and radiographic benefit from treatment[21]. Another potentially relevant biomarker obtained from peripheral blood with relevance to prostate cancer is prostate-specific transcripts. Danila et al[22] used a validated reverse transcriptase polymerase chain reaction assay to detect prostate-specific RNA in whole blood from 97 men with castrate metastatic prostate cancer and compared it with routine CTC collection. The gene markers included KLK3, KLK2, HOXB13, GRHL2, and FOXA1, with the plan to validate these as prognostic factors for overall survival.(Danila) These genes were selected based on their overexpression in metastatic prostate cancer. A correlation was seen between detectable transcripts and CTC count. The authors concluded that the reverse transcriptase polymerase chain reaction assay was prognostic for survival. In addition, it had the discriminatory power to separate patients based on their risk phenotypes compared with standard CTC technology[22]. As in all these biomarker technologies, these observations need to be validated in larger patient cohorts and across all clinical states of the disease.

Efforts toward identifying other biomarkers have focused on associations of pre- and posttreatment levels of a known soluble antigen that served as the immunogen or the expression of which is closely associated with disease, antibody titers, immune cell populations in serum, or assessment of tumor-infiltrating lymphocytes[23,24]. Cancer is often thought of as a T-helper 2-dominant disease either with excess of interleukin (IL)-4, IL-5, IL-10, transforming growth factor-beta production with a therapeutically driven shift back towards the T-helper 1 profile. A T-cell signature that includes frequency and function of circulating T-cells via EliSpot, cytokine flow cytometry, tetramer binding can also be used[23-29]. Apoptosis of CD8+ cells, a unique B-cell signature, suppressor cells in the microenvironment, which include regulatory T-cells/myeloid-derived suppressor

cells (MDSCs) in human tumors, or observation of CD4+FOXP3+CD24^{high} regulatory T-cells (often associated with a poor prognosis) all remain important avenues for immune evaluation. Cytokine gene or protein profiling are well suited to evaluation of the tumor microenvironment. There is also the potential to capture differences in patterns of their cytokine production that may be correlated with clinical response[28-34] .

Concerns arise as to whether or not “immune responses” to cancer are really potential biomarkers of prognosis. Whiteside[35] examined the numerous challenges trying to associate correlative immunologic parameters with clinical endpoints. She notes that expression of IgG kappa chain on tumor-infiltrating lymphocytes as well as B-cell and T-cell signatures have been validated as immunologic biomarkers. She postulated that based on preclinical data, tumor- induced immune suppression exists and promoted tumor escape. This assertion could be further extrapolated in the pre- and posttreatment milieu by measuring changes in immune suppression using a variety of parameters, many of which under validation. Another issue that often arises is whether a panel of immune assays can yield a signature that is applicable for every immune therapy or possibly for every disease as specific biomarkers may be needed for different diseases and different drugs.

Exploring potential biomarkers within the tissue milieu:

The neoadjuvant setting can provide direct interrogation of the tumor and its associated normal tissue microenvironment (Tables 2A,B). The benefit of testing an immune therapy either by directly introducing the immune agent into the tumor or tumor-bearing organ or by systemic delivery may provide immediate if not measureable effects. This includes ascertaining whether or not the drug has caused regression of the cancer, but also may provide an indication of a direct immune effect by assessment of the cellular population infiltrating the tumor or stromal elements. The potential success of the neoadjuvant approach is predicated on the fact that pre and post treatment biopsies can be easily and safely executed and that the tissue is appropriately processed and stored such that subtle changes in the milieu can be easily detected. Neoadjuvant administration (Table 3) of immune therapy provides a window to directly assess for possible on-target *in situ* effects yet it remains uncertain whether effects on the local tumor environment, including cancer control can lend itself to systemic control of micrometastatic disease. Krejci et al,[36] set precedence for this in a small clinical trial of patients with bladder cancer[35] who underwent neoadjuvant treatment with ipilimumab followed by cystectomy. They showed that an increased frequency of CD4+ICOShi T-cells sustained over 12 weeks of therapy correlated with an increased likelihood of clinical benefit consisting of overall survival. The identification of ICOS as an immunologic marker in both tumor tissues and in the peripheral blood of patients treated with anti-CTLA-4 may be of some potential benefit as a marker of immune monitoring upon which other neoadjuvant trials may be built. Using a similar approach, a Phase II trial of the impact of neoadjuvant sipuleucel-T is ongoing in patients destined to undergo prostatectomy.

Immune drugs for probing biomarker discovery:

Significant information regarding the nature of immunologic correlates as well as a recent publication by the Society for Immunotherapy of Cancer[39] which established new guidelines regarding evaluation of response parameters has been highlighted in melanoma. This has been based on the successes in this disease of two novel checkpoint inhibitors, ipilimumab[37] and nivolumab[26,27,38]. Despite the effectiveness in generating durable responses in melanoma, renal cell, bladder and lung cancers, the corresponding immune monitoring components have been variable despite recommendations that include standardization and harmonization of immune assays. One caveat remains, that is, the same criteria that subserve the response analysis for one particular solid tumor may not hold true for another cancer. There may still be discordance between radiographic responses as determined by RECIST and those by immune monitoring. Why one solid tumor such as melanoma had dramatic and durable responses to ipilimumab while those with the same drug in prostate cancer[40-42] were largely negative except for a rare patient also remains an unresolved challenge. Other questions that may be important include whether more bone trophic cancers such as prostate may not be ideal targets for immune therapies compared with those that metastasize largely in viscera or skin such as lung, bladder, renal and melanoma. As such, circumspection is advised as to whether independent criteria that are specific for a particular cancer and/or a specific immune drug are important. To this end, recent work by a number of authors suggests that several parameters may potentially be considered to assess immune response to a particular drug. These include B-lymphocyte and T-lymphocyte profiling, assessment of absolute lymphocyte count (ALC), absolute and relative eosinophil counts pre and post treatment, expression of antigen and antigen ligand, and myeloid-derived suppressor cells (MDSCs).

The checkpoint inhibitors; Ipilimumab and nivolumab as paradigms for developing immunologic response criteria:

Multiple clinical trials in melanoma[37,43-48] have highlighted the clinical value of ipilimumab and, for the first time, demonstrated immunologic impact through a variety of different parameters. These parameters have shown some level of surrogacy as biomarkers in that they are associated with a biological change in the disease as well as survival. The anti-PD-1 monoclonal antibody nivolumab also had unexpected efficacy[26,27]. Programmed cell death protein 1 (PD-1) is a protein that in humans is encoded by the *PDCD1* gene which encodes a cell surface membrane protein of the immunoglobulin superfamily. Its two ligands, PD-L1 and PD-L2, members of the B7 family[49,50], with PD-L1 protein being upregulated on macrophages and dendritic cells in response to treatment with lipopolysaccharide and granulocyte-macrophage colony-stimulating factor. It has similar effects on T-cells and B-cells upon T-cell receptor and B-cell receptor signaling. PD-1 is expressed on the surface of activated T-cells, B-cells, and macrophages in resting mice, suggesting that, compared with CTLA-4, it can more broadly and negatively regulate immune responses. This has been further confirmed in PD-1 knockout mice that develop lupus-like glomerulonephritis and cardiomyopathy on C57BL/6 and BALB/c backgrounds, respectively[50,51]. T-cells stimulated *in vitro* with anti-CD3 and treated subsequently with

PD-1 ligand resulted in reduced T-cell proliferation and interferon-γ production[30]. The broader applicability and efficacy of anti-PD-1 was affirmed by Topalian et al[26] and Brahmer et al[27]. Unexpectedly, it was observed that overexpression of the PD-1 ligand could correlate with treatment response. In addition, this was the first time that concurrent clinical testing of antibodies blocking an immune regulatory receptor and one of its cognate ligands has been reported. This has led to more recent work by Snyder, et al [52] suggesting that patient who can respond to checkpoint inhibitors can be identified via array, thereby enriching the patient population who earlier on can be identified has been responsive to this therapy.

The availability of clinical material from patients enrolled in several clinical trials using ipilimumab alone and in combination with chemotherapy and biologics in melanoma has enriched efforts toward immunologic biomarker discovery and profiling compared with other solid tumors. Recent data reported by Schindler et al[43] suggest that pretreatment levels of absolute and relative eosinophil counts are associated with improved overall survival in patients with metastatic melanoma treated with ipilimumab. A retrospective multicenter analysis was performed in 123 patients with stage III or IV melanoma who had received treatment with ipilimumab in the first-line and second-line setting at the approved dose of 3 mg/kg every 4 weeks. A baseline absolute eosinophil count of ≥ 0.1 (10^9/L) was associated with improved overall survival (P=0.002), with significantly improved survival rates of 79%, 60%, and 48% at 6, 12, and 18 months compared with rates of 48%, 37%, and 19%, respectively, for patients with a baseline eosinophil count below 1. Although retrospective, relative eosinophil counts also seemed to have similar benefits, suggesting the potential of these values as an immune-mediated response biomarker.

An early report by Ku et al, [44] detailed a single-institution experience with ipilimumab used as a compassionate drug for patients with advanced melanoma. Patients with an ALC >1,000/μL after two ipilimumab infusions (week 7) had a significantly improved clinical benefit rate (P=0.01) and a median overall survival of 11.9 versus 1.4 months for patients with an ALC <1,000/μL. These observations served as the foundation for further studies by Postow et al[45] who evaluated data from six studies of ipilimumab with or without dacarbazine, a standard chemotherapy used in melanoma. ALC was measured at baseline, prior to each dose during induction at the established times of weeks one, 4, 7, and 10, and at the end of induction at week 13. In all studies, the ALC increased significantly over time in patients who received ipilimumab irrespective of whether they were being treated with or without chemotherapy. A positive association between rate of ALC increase and overall survival was seen after two treatment doses. However, the increase in ALC was not specifically predictive of an overall survival benefit from ipilimumab based on analysis of the original ipilimumab trial with treatment arms using single-agent ipilimumab versus combination with the glycoprotein 100 vaccine. An overall survival benefit was observed for ipilimumab relative to the vaccine arm irrespective of the rate of change in ALC (P=0.14). Therefore, these data suggest that ALC cannot be regarded as a reliable biomarker in disease management at this time.

Wolchok, et al[46] demonstrated improved efficacy of nivolumab (anti-PD-1) in combination with ipilimumab compared with using either drug alone with a manageable

safety profile.. An analysis of single versus combined use of these agents was done by Callahan et al,[47] who investigated the question of whether previously identified putative biomarkers for ipilimumab or nivolumab monotherapy are relevant in the combination setting. Interestingly, objective responses were seen in patients who were negative and positive for PD-L1 by immunohis- tochemistry. Evaluation of this two-drug combination did not document a rise in ALC relative to baseline, but there were phenotypic changes in peripheral blood T-cell subsets, including increased percentages of CD4 and CD8 expressing HLA-DR, ICOS, and/or Ki67. Low ALC (<1.0 at week 6–7) did not preclude overall response, with three of 12 patients with low ALC seen to be responders. This is in contradistinction with prior reports of achievement of ALC >1.0 after 6 weeks of treatment with ipilimumab being associated with favorable clinical outcomes. Of note, another potential biomarker, myeloid-derived suppressor cells (MDSC) levels, which were considered to be at a lower frequency before treatment, were associated with an improved overall response when compared with patients showing high MDSC levels[47]. The results of this analysis suggest that the overall response was independent of PD-L1 status or ALC compared with either drug alone in this small subset of patients.

Potential biomarkers in other genitourinary malignancies:

Mahoney and Atkins[28] in a recent review, have suggested that IL-2 responses has been associated with normal serum lactate dehydrogenase levels or low plasma (Vascular Endothelial Growth Factor (VEGF) and fibronectin levels [30], as well as tumors containing mutations in the BRAF or NRAS family. However, these remain to be validated prospectively. Other biomarkers associations include responses to IL2 as predicated on histology [31, 32] or carbonic anhydrase (CA9) expression [28,31,32].

A recent report in bladder cancer [53] demonstrated that patients whose tumors expressed PD-L1-positive TIL cells had higher than expected response rate to the PDL-1 inhibitor MPDL3280a. This inhibitor is a high-affinity engineered human IgG_1 anti-PD-L1 antibody that inhibits the interaction of PD-L1 with PD-(PDCD1) and B7.1(CD80). However, more recent observations[54] have indicated that an antitumor response can be sustained irrespective of PD-L1 expression. This was thought to be due PD-L1's possible waxing and waning expression. A larger study of the MPDL3280A in several solid tumors[49], showed that the drug was associated with T-helper type 1 (TH_1) gene expression, CTLA4 expression and the absence of fractalkine (CX3CL1) in pretreatment tumor specimens. It was suggested that MPDL3280A was more effective in patients in which pre-existing immunity was suppressed by expression of PD-L1, and was re-stimulated upon introduction of antibody treatment. In an attempt to further identify immune biomarkers in bladder cancer, Se-Feng, et al [55] utilized a microarray approach to interrogate the milieu of non-muscle-invasive and muscle-invasive bladder(MIBC) cancers. Of the specimens evaluated, the results of the functional classification analysis allowed them to explore immunological or inflammatory functions. The gene expression patterns of representative immune or inflammation-associated genes, were analyzed comparing MIBC and control samples with ten of these genes (IL-5, IL-26, IL-22RA1, IL-1RAPL1, IL-1F5, IL-17RB, IL-17RE, IL-20, IL-28A, and TRAF2) showed increased expression; 26 genes were down-regulated in MIBC samples. The functionality of these genes was also studied. Overall, the gene

expression profiling yielded three cytokines, IL-5, IL-20, IL-28A that were involved in the migration, invasion and matrix metalloproteinase (MMP) expression without effecting cell proliferation [55]. The implication of these results is that these cytokines may be involved in the progression of the disease, whereas other cytokines, IL-5, IL-8, IL-17 may promote tumor growth. These cytokines and their role in bladder carcinogenesis may ultimately be translated into biomarkers for early diagnosis and treatment. To date, there is still no one particular biomarker of disease as it transitions through its clinical states.

Autoimmune responses that have also served as biomarkers to therapy included, vitiligo, colitis, thyroiditis, transaminitis, hypophysitis as seen with ipilimumab or interferon [33,34,37,41].

The evaluation of MDSCs was performed in a recent analysis of trials in melanoma. These cells can increase in both tumor tissue and blood in cancer patients, and that they correlate with a poor clinical outcome. But pitfalls to using these cells exist, including their phenotypic and functional heterogeneity within the myeloid compartment. The fact that there are different subsets, leads to difficulty when comparing results, ie, subset of $HLA\text{-}DR^{-}Lin^{-}$ MDSC in peripheral blood has cells with monocytic and granulocytic features for which each can be divided: CD33+, CD11b+, CD15+, and CD14+. Each was different with respect to its mechanism of suppression.

Kitano et al[50] evaluated MDSCs in peripheral blood as a biomarker of clinical outcome in a pilot correlative study of patients with stage IV melanoma treated with ipilimumab 10 mg/kg. A lower MDSC quantity before treatment predicted improved overall survival (P=0.002), with a trend towards a clinical benefit at week 24 imaging. This effect appeared to be independent of pretreatment or week 7 ALC. A general trend of increasing MDSC numbers by week 24 compared with pretreatment baseline seemed to be associated with patients who did not appear to achieve clinical benefit. The authors concluded that there may be some predictive benefit of MDSCs as a biomarker, but this issue will need further retrospective and prospective validation.

Reichenbach and Finn[56] offered the premise that better understanding of immune responsiveness to therapy involves instances of crosstalk, whereby context and cell type in signaling pathways can be activated in an attempt to predict later effects on the immune response. They point out that signal transducers may have various upstream activators, ie, the IL-6 receptor, IL-21 receptor, and CD40 L, all signaling through STAT3. The role of STAT3 signaling in the differentiation of T-helper subsets has been well defined, confirming that signaling profiles can be generated to demonstrate the response to a vaccine by virtue of a CD4+ T-cell activation “fingerprint” in vivo.

Broadening Biomarker Applicability:

While immune biomarker studies in melanoma have set precedence for their potential extrapolation into other malignancies, there have been attempts to introduce an “immunoscore” that could be used in real time in routine clinical settings. The markers for incorporation into an immunoscore have been variable and not well-established. In

fact, it appears that an immunoscore may be different for each cancer being interrogated. A study Chhieng, et al [57] examined the extent of intratumoral heterogeneity as a potential biomarker in breast cancer. The markers included HER-2neu ($p185^{erbB-2}$), epidermal growth factor receptor (EGFR), Bcl-2, p53, and proliferating cell nuclear antigen (PCNA) in 30 breast carcinomas using archived, paraffin embedded tissue. Each tumor analysis incorporated the entire lesion and four regions for immunohistochemical analysis for expression of these markers. Scores of both membrane and cytoplasmic staining of HER-2neu and EGFR, scores of cytoplasmic staining of Bcl-2, and scores of nuclear staining of both p53 and PCNA were recorded and the intensity of staining and the proportion of stained cells were evaluated. The authors then developed a semiquantitative “immunoscore” that was calculated by determining the sum of the products of the intensity and corresponding proportion of stained tumor cells. Interestingly, there were no intratumoral biomarker expression differences between the samples that had intraductal (IDC) or ductal carcinoma *in situ*. However, there was a positive correlation of immunoscores was observed between the entire tumor and each region as well as across all four regions for IDC.

In addition to attempts made at classifying immunotherapies [58], a recent paper by Galon, et al [59,60] has attempted to define a system that validates how the presence of immune cells in a tumor can influence prognosis. This was based on the type, the density and the location of immune cells within the tumor and was thought to be independent of the TMN stage of the tumor. The approach was based on a methodology termed "immunoscore" that would allow in real time practice to assess quantitatively the immune infiltrate. This is currently under validation. It has been observed that the number, type and location of tumor immune infiltrates in primary tumors, are prognostic for DFS and OS. This has been validated in two large studies (with sample sizes n = 843 and 768, [61,62], respectively) and have demonstrated hat tumor immune infiltrate patterns and subsets in colorectal cancer are significant prognostic biomarkers, even after adjusting for stage, lymph node count, and well-established prognostic tumor molecular biomarkers. The latter includes microsatellite instability (MSI), BRAF mutation, and LINE- hypomethylation. A potential clinical translation of these observations was the development of an Immunoscore, based on the numeration of two lymphocyte populations (CD3/CD45RO, CD3/CD8 or CD8/CD45RO), both in the CT and in the IM of tumors, [63], The Immunoscore (“I”) utilizes the enumeration of CD8 and CD45RO cells in the center of the tumor (CT) and the invasive margin (IM) of the tumoral nests of resected tumors to provide a score ranging from Immunoscore 0 (“I;”0), when low densities of both cell types are found in both regions, to Immunoscore 4 (“I”4), when high densities are found in both regions. This Immunoscore was incorporated into 2 large independent cohorts (n = 602). Of patients with a high “I”4, 4.8% relapsed after 5 years with the remaining patients alive. In comparison, 72% of patients with a low score (“I”0 and “I”1) experience tumor recurrence and 27.5% remained alive at five years. Based on these results, the “I”0 and “I”1 patients may have derived some benefit from adjuvant therapy [63]. It is suggested that the prevalence of immune infiltrates, may have prognostic significance superior to that of the AJCC/UICC TNM-classification system [63].

An international consortium of expert laboratories is currently testing the immunoscore in routine clinical settings for cancer classification. The authors [59,60] suggest that validation of the immunoscore as a standardized metric could result in the implementation into the working classification of cancer, to be designated at TNM-I (TNM-immune). However, its application to genitourinary malignancies may be more difficult as not all genitourinary cancers express high levels of TIL cells; immunoscores may need to be individualized for each cancer.

Conclusions:

Melanoma remains a unique model for testing immunologic strategies. Despite the successes seen, there is still a need for better trial design that incorporates viable immunologic endpoints able to demonstrate a correlation with an antitumor effect. There are many potential immune biomarkers that await validation in large clinical trials. Unfortunately, not all of them may be broadly applicable and may need to be custom-tailored not only to the disease but to the immune agent used. Their applicability may be also restricted to aspecific solid tumor. Efforts are underway in multiple solida tumors attempting to validate immune endpoints via rapid throughout approaches that interrogate both tumor and blood samples.

Future Directions:

Phase III clinical trials that evaluate novel immune strategies must include tissue and blood for interrogation of how immunologic populations can be translated into clinical response. Approaches with immunogenomics may further identify which patients may be have a greater likelihood of response to a particular therapy as recent observations have indicated suggesting that responses to specific immune agents may be seen in hypermutated tumors but the hypermutational status within an individual tumor may not reflect the norm. The future of immunotherapy remains brightly optimistic.

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Table 1

“Biomarkers” of disease activity

Known disease elated biomarkers: CEA, CA-125, CA-19-9, and PSA
Blood: circulating tumor cells (CTCs)
Serum: quantitation of soluble antigen Humoral or cellular responses, that is, T cell subpopulations
Tissue: tumor infiltrating lymphocytes (TILs) 5T4 (Renal, breast, GI, colon, prostate, and ovarian)
Tissue expression: PSA, ACP, PSMA, PSCA, STEAP, lewisy, TF, Tn, KSA, GM2, Globo H, chromogranin, synaptophysin, and neuron-specific enolase
Molecular imaging of specific targets: AR

ACP = acid phosphatase; AR = androgen receptor; GI = gastro-intestinal; PSA = prostate-specific antigen; PSCA = prostate stem cell antigen; PSMA = prostate-specific membrane antigen; STEAP = six transmembrane epithelial antigen of the prostate.

Table 2

Current paradigms to assess immune response to cancer

Radiographic assessment for decrease or absence of clinical response
Quantitate degree of immune recovery from immune suppression after “successful therapy” (normalization of antitumor immune responses)
Assessment of TILs and T cell subpopulations and scoring of T cells at tumor sites
T-cell signature: frequency, function, and immune and molecular phenotype of circulating T cells, ELISpot, cytokine flow cytometry, and tetramer binding
Apoptosis of $CD8^+$ cells
B-cell signature
Suppressor cells in tumor microenvironment:
Tregs or MDSC in human tumors:
Assessment of $CD4^+FOXP^+CD24^{high}$ Treg (associated with poor prognosis)
Correlation of Treg frequency among TILs with survival parameters
Correlation of tumor grade with survival (seen in breast and ovarian cancers and glioblastoma)
Cytokine gene or protein profiling well suited to evaluation of tumor microenvironment
Potential for capturing polarization in the cytokine repertoire or differences in patterns of their production by immune or tumor cells may be related to clinical response

ELISpot = enzyme-linked immunospot.

Table 3

Milieux for interrogation of immunologic integration

Neoadjuvant (early)	Metastatic (late)
Direct interrogation of tissue and stroma	High tumor burden
Effect on local disease to p0 or to MPR (<10% residual dz)	Biology of disease different at metastatic sites: bone vs. LN vs. visceral metastases
Questionable effect on systemic progression unless validated in high-risk population	Not every site of known disease is active
Hard to design trials owing to long natural history	Immune cells can be crowded out by increased tumor cells in LN or bone

Cancer: Th2-dominant disease either excess of IL-4, IL-5, IL-10, or TGF-β production with a therapeutically drive shift back toward the Th1 profile. LN = lymph node; TGF-β = transforming growth factor β.