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# IMMUNO-ONCOLOGY INSIGHTS

SPOTLIGHT ON:

## Optimizing clinical development strategy for the rapidly evolving I-O field

IMMUNO-ONCOLOGY INSIGHTS Volume 3, Issue 3

# Optimizing clinical development strategy for the rapidly evolving I-O field

SPOTLIGHT

FOREWORD

# Spotlight on optimizing clinical development strategy

**JILL O'DONNELL-TORMEY**, PhD is chief executive officer and director of scientific affairs of the Cancer Research Institute, a nonprofit organization founded in 1953 that is today the global leader in supporting and coordinating research aimed at harnessing the immune system's power to conquer all cancers. She joined the organization in 1987 and has been chief executive since 1993. Prior to joining Cancer Research Institute, she served as a research associate in the department of medicine at Cornell University Medical College and as a postdoctoral fellow in the laboratory of cellular physiology and immunology at The Rockefeller University.

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While immunotherapy has become the standard of care for several cancers and is now viewed as the fourth pillar of cancer treatment joining surgery, chemotherapy, and radiation, attainment of its full potential as an effective treatment for all cancers requires a continued commitment to research.

In this issue of *Immuno-Oncology Insights* our contributing authors provide opinion and review on the learnings, challenges, and requirements that clinical research in the immunotherapy space now demands. Repeated reference is made to the need for building better mechanistic studies and better biomarker identification into clinical trials. Fitzgerald et al emphasize the need for the bench to bedside mindset to evolve into a bench AND bedside interrogation. Mantle and Sabil expound on the optimization of a variety of factors that include tumor mutation burden, tumor-infiltrating lymphocytes, tumor-associated macrophages, the microbiome, and circulating tumor DNA, to serve as biomarkers to expand the effective use of immunotherapy in the metastatic

setting. Postow discusses checkpoint combinations and overcoming primary and acquired PD1 resistance in melanoma. While Brown et al discuss immunotherapy going beyond PD-1 by identifying predictive biomarkers in renal and urothelial cancers. Forde discusses the use of neoadjuvant studies to expedite drug development for lung and other cancers and points to moving biomarker-directed trials to the second-line setting. He additionally highlights the trend, beyond melanoma, to move immunotherapy treatments from the advanced cancer setting to earlier stages. Dancey summarizes the clinical trial considerations for the evaluation of cancer immunotherapies, noting among them a goal of using predictive biomarkers for the benefit of patients, while Allen discusses the need to expand clinical trial access by examining and changing eligibility criteria to benefit a larger and more diverse patient population.

Innovative clinical trial designs coupled with in-depth correlative science and harmonization of the vast amount of data currently being generated by the more than 5,600 current IO clinical trials worldwide (CRI IO Landscape October 2021) will provide the necessary scientific grist for future hypothesis-driven trials.

A concerted commitment to research—the entire continuum from lab to clinic—is required if we are to be successful in developing effective immunotherapies for all cancers. Research focused on the complex interplay of cancer and the immune system that takes place at the tumor microenvironment and throughout the body. Research that marries basic biology discovery and human clinical investigation. Research that focuses on the mechanisms and pathways that control the function of a diverse group of immune cells that together orchestrate the recognition and destruction of cancer. Research that unravels the ways a tumor suppresses an immune response. Research that identifies the biomarkers of innate and acquired resistance to the immune system's response to cancer. Research that identifies the extrinsic factors, such as genetics, metabolism, and the microbiome, that impact the cancer-immunity cycle and aid or abet a productive immune response against cancer. The questions are clear, and it will take the field-wide collaborative and complementary efforts of academia, biotech, and pharma with patients at the center to arrive at the answers.

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IMMUNO-ONCOLOGY INSIGHTS

OPTIMIZING CLINICAL DEVELOPMENT STRATEGY FOR THE RAPIDLY EVOLVING I-O FIELD

SPOTLIGHT

REVIEW

# Expanding the reach of immuno-oncology: considerations for optimizing treatment of solid malignancies in the future

Luke Mantle & Samuel D Saibil

The treatment of cancer has been rapidly changing with the emergence of highly effective immunotherapies. The majority of this success stems from the development of monoclonal antibodies targeting negative regulatory immune checkpoint molecules. Despite the efficacy of these immune checkpoint inhibitors across a range of tumor types, unfortunately about 70% of patients [1] either do not respond to treatment or subsequently develop resistance to checkpoint inhibitor therapy. Here, we will review the current landscape of immune-modifying treatments, ranging from chemotherapy and radiation to cellular therapies, which have the potential to further increase the clinical impact of immunotherapy. We will also highlight some of the current challenges in the field. These include the need for further mechanistic studies to better understand the complex biology of the anti-tumor response and to identify better biomarkers to rationally inform the selection of novel immunotherapy combinations. Further insights in the function of the immune system will allow the maximal leveraging of the growing number of immunotherapeutic modalities available in the clinic.

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## INTRODUCTION

In the past decade, immune therapy has revolutionized the treatment of cancer. Tremendous progress has been made in utilizing therapies which harness the immune system to fight cancer since the first observations in the late 19th century that Coleys cocktail of bacterial toxins could elicit regression of some tumors. Much of the recent success of immune therapy has been due to the introduction of antibodies blocking key regulatory molecules of the immune system, referred to as immune check point inhibitors (ICIs). Treatment with ICIs has resulted in durable tumor regression in multiple different solid tumors. Despite this success, sustained responses to treatment are not achieved by a significant number of patients treated with immune check point inhibitors [1]. This is particularly true of treatment with monotherapy targeting a single immune checkpoint molecule, such as programmed cell death-1 (PD-1/CD279), programmed cell death-ligand1 (PD-L1/CD274) or cytotoxic T-lymphocyte antigen-4 (CTLA-4/CD152).

This review will focus on highlighting the growing number of tools in the immunotherapeutic toolbox (Figure 1) and the current challenges presented in determining the ideal combination of these therapeutic modalities and approaches for each individual patient. Despite of all these emerging agents, the 'Holy Grail' of multimodal, personalized immunotherapy remains unrealized for most patients due to a lack of biomarkers to guide the integration of different immunotherapeutic agents.

## IMMUNE CHECKPOINT INHIBITORS

Within the immune system, the process of immunosurveillance assessing for foreign pathogens or malignant cells is finely balanced against the development of autoimmunity. This balance is partially maintained by immune checkpoints [2], an array of receptors on the immune cell surface which, in turn, promote activation or suppression of the immune response. Seminal work in murine models in the 1990s established that preventing the ligation of the inhibitory receptors CTLA4 or PD-1 by their cognate ligands could result in the activation of T cells and tumor clearance [2,3]. More than a decade later, unprecedented durable responses were observed in roughly 20% of patients with advanced melanoma treated with an anti-CTLA-4 agent [4]. Increased numbers of responses were subsequently observed with anti-PD1 therapy for patients with advance melanoma [5,6]. Moreover, further increases in response rates and patient survival were observed when a PD1 blockade was combined with anti-CTLA-4 therapy, albeit at the cost of increased toxicity [7]. The success of ICIs targeting PD-1, PD-L1 and CTLA-4 has now been duplicated in multiple other tumor types. Interestingly, the enhanced efficacy of combined ICI therapy with anti-PD1 and anti-CTLA-4 observed in patients with melanoma has also been seen in patients with other solid tumors [8–10]. The hope was that this success of combined anti-PD1 and anti-CTLA-4 therapy could be replicated, and perhaps enhanced, with novel agents targeting other key immune inhibitory or costimulatory molecules. As recently reviewed extensively by Esfahani *et al.*, there are a multitude of other potential immune checkpoint targeting agents currently under investigation in various stages of clinical trial [11]. Many of these novel agents target other inhibitory checkpoint molecules, such as lymphocyte-activation gene 3 (LAG-3/CD223), V-domain immunoglobulin suppressor of T cell activation (VISTA/B7-H5) or T-cell immunoreceptor with immunoglobulin and ITIM domain (TIGIT). Others are agonistic antibodies which target co-stimulatory molecules on T cells, such as tumor necrosis factor receptor superfamily member 9 (TNFRSF9/ 4-1BB/CD137), inducible T-cell costimulator (ICOS/CD278) or CD28. To date, many of the results of these trials with novel ICI/costimulatory


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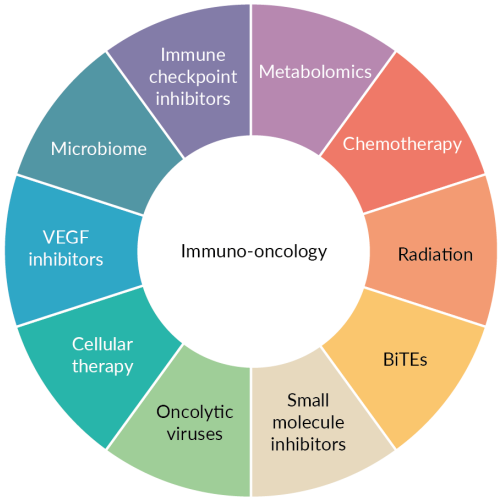
agonist combinations have been disappointing. Some agents, such as those early studies using an agonistic anti-CD28 antibody have displayed unacceptable toxicity [12]. On the other hand, many of the novel combinations of ICIs have not yet demonstrated significant clinical activity, although trials are ongoing [13]. A major limitation in the development of these novel ICIs/agonistic antibody combinations is lack of detailed mechanistic understanding of the underlying biology of many of these immune checkpoint molecules. The unique role of each immune checkpoint molecule in governing the anti-tumor immune response is unclear and unfortunately likely context-dependent. For example, recent studies have suggested that the inhibitory receptor V-domain immunoglobulin suppressor of T-cell activation (VISTA) is expressed on multiple tumor-infiltrating lymphocytes such as myeloid-derived suppressor cells (MDSCs) and T regulatory cells (Tregs). Interestingly, however, expression of VISTA is not sufficient for it to engage is cognate receptor and induce suppression of the anti-tumor immune response. For VISTA to be an active inhibitory receptor, it also requires an acidic environment [14]. Thus, measuring VISTA protein expression alone is not sufficient to predict the inhibitory activity of VISTA. Factors such as this have prevented the identification of robust biomarkers to predict the efficacy of many of these novel ICIs as well as hampering the rational selection of novel ICI combinations amongst the numerous possible combinations. As a result, no ICI combinations have yet demonstrated superior clinical efficacy than the original anti-PD1 and anti-CTLA-4 combination for most solid tumors. This failure to improve on the success of combination of anti-PD1 and anti-CTLA-4 also speaks to the complexity of immunoregulatory mechanisms in the tumor microenvironment (TME) and the need to target multiple different regulatory pathways in the TME beyond just immune checkpoint molecules to elicit an anti-tumor immune response in some patients.

FIGURE 1

**The expanding range of treatments in immuno-oncology.**




Immune therapy has expanded from the use of immune checkpoint inhibitors to include multiple other treatment modalities. These include the combination of immune therapy with chemotherapy and radiation, addition of VEGF inhibitors or small molecule inhibitors, cell therapy, bispecific antibodies, cytokines and tools to activate the innate immune system such as vaccines and oncolytic viruses. Immune-modifying therapies targeting manipulation of the microbiome and the metabolic composition of the tumor microenvironment are also being developed.

## TUMOR MICROENVIRONMENT: BEYOND ICIS

The approach to immunotherapy needs to be undertaken in the context of the TME which encompasses the cellular milieux of tumor cells, stromal cells as well as a diverse array of immune cells such as T lymphocytes, dendritic cells, macrophages, polymorphonuclear cells and natural killer cells. The TME can show a wide degree of heterogeneity from patient to patient depending on tumor type, anatomical location and molecular characteristics of the tumor. Tumors can be conceptually divided into immunologically 'hot' or 'cold' microenvironments. 'Hot' tumors typically display evidence of robust infiltration of $CD8^+$ T cells and expression of IFN-γ or PD-L1 with high PD-L1 expression in NSCLC shown to be predictive of clinical response to ICIs [15]. Conversely, 'cold' microenvironments demonstrate limited or no infiltration of immune cells. 'Cold' microenvironments are associated with poor response to immune checkpoint inhibitors [16]. A myriad of

factors can contribute to this 'cold' immune phenotype. These can include the recruitment of immune suppressive cells, such as Tregs and MDSCs, or the expression of immune suppressing chemokines and cytokines. The tumoral stroma itself can create a barrier to the infiltration of immune cells [17]. Additionally, the TME is a harsh environment depleted of many nutrients required by T cells and other immune effector cells to function properly [18]. Accordingly, understanding additional approaches to transform immunologically 'cold' tumors to 'hot' tumors has been an ongoing area of investigation. Below, we will discuss additional therapies that help stimulate an anti-tumor immune response.

## CHEMOTHERAPY

Chemotherapy was initially considered as a treatment modality which would potentially decrease or interfere with the use of immunotherapy due to its potential toxicity to myeloid cells and T-cell populations involved in immunotherapy responses. However, upon further investigation, it was discovered that chemotherapeutic agents given at specific doses and intervals could improve the response of immunotherapy. Chemotherapies, such as doxorubicin, mitoxantrone and cyclophosphamide, can induce immunogenic cell death via a number of cellular pathways. Immunogenic cell death leads to the activation of the innate immune system, and particularly antigen presenting cells such as dendritic cells (DCs), to support the activation of a tumor-specific adaptive immune response [19,20]. Pathways involved in immunogenic cell death include the activation of Toll-like receptors via post apoptotic release of nuclear chromatin binding protein HMGB1 [21,22]. Cytotoxic agents can also result in the release of ATP from lysosomal stores stimulating macrophage recruitment and maturation [23] and NK cell proliferation and IFNγ secretion [24]. Chemotherapy can also lead to tumor cell immunogenicity by inducing expression of MHC-I molecules and tumor specific antigens on the tumor cell surface [25]. Leveraging these effects, the use of chemoimmunotherapy combinations with standard chemotherapy regimens in combination with ICI has been studied in Phase 3 clinical trials and has been FDA approved for tumor tissue types including non-small-cell lung cancer, small cell lung cancer, triple negative breast cancer and head/neck cancer with evidence of clinical benefit [26–29].

In addition to standard-dose chemotherapy, continuous low-dose exposure to chemotherapy or 'metronomic' chemotherapy has been studied as a means to enhance the anti-tumor immune response. In clinical studies metronomic dosing of cyclophosphamide treatment of end-stage cancer patients (50 mg orally, b.i.d., 1 week on, and 1 week off, for 1 month or more) strongly curtailed immunosuppressive Treg cells, leading to a restoration of peripheral T-cell proliferation and innate immune cell killing activities [30]. Another study of metronomic cyclophosphamide in metastatic breast cancer showed a 40% reduction in T regulatory cells initially however these numbers recovered during the treatment course however the treatment induced a stable tumor specific T-cell response which correlated to improved clinically outcome [31]. Despite these promising results, metronomic chemotherapy has yet to show any synergistic activity with ICI or other immunotherapy modalities in prospective, randomized trials. Clearly, further research is warranted to be elaborate the ideal deliver of chemotherapeutic agents to optimize the activation of both the innate and the adaptive immune system and synergize with immunotherapy.

## RADIATION

The addition of radiation is another potential tool which can be used to alter the TME potentially changing a noninflamed environment into a more immune sensitive environment. There is ongoing discussion regarding the pro-inflammatory versus the immune suppressive effects of radiation on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anti-tumor immunity. The immune response to radiation is thought to depend on multiple factors including timing, dose, fractions, site radiated and also the tumor type. Low dose radiation at 2Gy has shown it can create an immunogenic environment via the innate immune system through macrophage stimulation [32]. In contrast high dose radiation has been thought to promote tumorigenic macrophages [33] and cause vascular damage limiting access of immune cells to the TME [34]. In a similar process to the effects of certain chemotherapeutic agents discussed above radiation treated cells undergo immunogenic cell death causing release of specific proteins which can activate Toll like receptors of the innate immune response [35]. DNA released from radiation damaged cells have also been shown to active the c-Gas-STING pathway causing increased type I interferon release by dendritic cells in the TME [36]. Radiation has also been shown to increase expression of major histocompatibility complex (MHC) I on tumor cells and increase T-cell activity [37]. In totality, these changes may be responsible for the 'abscopal effect' that has been described with radiation therapy; the abscopal effect is the observation of regression of non-irradiated metastatic lesions following the treatment with radiation of another site of disease, presumably due to the activation of the immune system. Unfortunately, to date, combinations of ICIs and radiation to induce the ascopal effect and enhance tumor immunity have proven difficult to demonstrate in clinical trials [38].

## ACTIVATION OF THE INNATE IMMUNE SYSTEM: DANGER SIGNALS, ONCOLYTIC VIRUSES & VACCINES

Failure to active the innate immune system, and particularly DCs, can allow tumors to circumvent the immune response and undoubtedly contributes to the 'cold' tumor phenotype. Both chemotherapy and radiation can initiate cellular pathways that promote DC activation and allow for the bridging of the innate to the adaptive immune response, as activated DCs increase antigen presentation and provide costimulatory signals and cytokines to promote T-cell activation [39]. The pattern recognition receptor (PRR) family play a pivotal role in DC activation. These PRRs recognize bacterial or viral molecules called pathogen associated molecular patterns (PAMPs) or endogenous molecules called damage associated molecular patterns (DAMPs). Rather than trying to induce the release of DAMPs with chemotherapy or radiation, another approach is to directly provide DAMPs or PAMPs into the TME. This approach has been quite successful with non-metastatic tumors. The treatment of non-invasive bladder tumors involves the use of the attenuated bacillus Calmette–Guerin (BCG) and some superficial basal cell carcinomas of the skin can be treated with the synthetic TLR agonist Imiquimod. For metastatic disease, intertumoral injection of DAMPs or PAMPs have been found to synergize with ICI therapy and enhance the tumor clearance both of the injected lesion as well as at distant sites of disease. This systemic effect of local injection has been observed both in mouse models [40,41] and early clinical trials [42]. Additionally, synthetic DAMPS activating the cGAS-STING pathway which can be administered systemically but still result in potent tumor regression in preclinical models have been described [43]. These agents hold the promise to allow for the activation of DCs and the innate immune response in patients for which intra-tumoral injections are not safe or feasible. Unfortunately, these new STING agonists, as well as the other DAMPs and PAMPs that have been tested, have yet to demonstrate efficacy in large, randomized trials.

Oncolytic viruses have also emerged as an immunotherapeutic modality whose mechanism of action relies heavily upon activation of DCs and the innate immune system. Early in their development, oncolytic viruses (OV) were envisioned as engineered therapeutic that would selectively infect and lyse tumor

cells. Further research, however, has indicated that the dominant mechanism of action of these viruses is to induce an anti-tumor immune response [44]. To date, only one OV has been licensed for clinical use in the USA, Europe and Australia. Talimogene laherparepvec (T-VEC) is a modified herpes simplex virus type I that results in the expression of the human granulocyte-macrophage colony stimulating factor (GM-CSF) in infected cells. The combination of the expression of GM-CSF to attract immature DCs and the natural PAMPs in the T-VEC virus and DAMPS released by virally-lysed cells, co-ordinate to boost DC activation in the TME and promote anti-tumor immunity. Indeed, T-VEC treatment was demonstrated to induce durable clinical response in patients with advance melanoma when used as monotherapy [45]. Combination trials of T-VEC, as well as other OVs, with ICIs are ongoing with some encouraging early results being reported, albeit in small number of patients [46,47].

Finally, tumor-specific vaccines are another potential tool to provide both tumor-specific antigen as well as molecular signals to activate innate immune cells. Historically, tumor vaccines do not have a tremendous track record of success for the treatment of advanced cancer, even when used in combination with ICIs. For example, the seminal trial that established the potential of ipilimumab to induce durable responses in patients with melanoma also included treatment with a therapeutic vaccine targeting the gp100 melanoma peptide which did not display any added therapeutic benefit [48]. Since then, however, it has been discovered that tumors contain multiple mutated proteins that can give rise to novel 'neo-antigens' that can be recognized by the immune system. Vaccine strategies utilizing neo-antigen targets have shown promise in early clinical trials [49,50]. Moreover, novel vaccine platforms utilizing mRNA technology have improved the antigen expression and immunogenicity of the vaccine antigen [51]. There is now reason for growing enthusiasm that personalized, mRNA vaccines targeting tumor antigens or neo-antigens will be a key component of immune therapy combinations in the future.

## CYTOKINE THERAPY

Early in the immunotherapy era stimulation of an immune response with provision of cytokine therapy was attempted in melanoma and renal cell carcinoma with high dose interleukin (IL)-2 or interferon alpha [52]. Unfortunately, these treatments had very high levels of toxicity with not very good efficacy. Subsequently, alternative cytokine therapies have been tested in early clinical trials with the aim of improving T cell and NK cell function, including Il-12, Il-15 and Il-21 [53]. Unfortunately, all of these treatments were also associated with high rates of toxicity. This has led to the development of modified cytokine agents, including bempegaldesleukin a polyethylene glycol–conjugated recombinant IL-2. These modified IL-2 agents have demonstrated anti-tumor activity but acceptable toxicity in animal models [54] and multiple clinical trials are ongoing. To date, however, no modified cytokine agents are approved for clinical use.

## SMALL MOLECULE INHIBITORS: TARGETING VEGF & BEYOND

In parallel with the immune therapy revolution, there has also been an explosion of the number of tyrosine kinase inhibitors (TKIs) and other targeted therapies aimed at blocking key aspects of oncogenesis, from cell growth to angiogenesis [55]. For many malignancies harboring driver mutations, such as epidermal growth factor receptor (EGFR)-mutated non-small-cell lung cancer (NSCL), these targeted therapies are the standard first line therapy and have demonstrated impressive clinical activity [56]. There is now increasing interest in combining many of these targeted therapies with immune therapies, as there is emerging evidence that many of these targeted therapies may also aid in enhancing the anti-tumor immune response. This is


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particularly true of agents targeting the vascular endothelial growth factor (VEGF) receptor signaling pathway. The VEGF family of growth factors bind to VEGF receptor tyrosine kinase triggering their signal transduction pathways. VEGF stimulates formation of new blood vessels to help supply growing tumors. Signaling via VEGF also potentially suppresses immunity through effecting accumulation of immature dendritic cells, myeloid derived suppressor cells and inhibiting of T cell migration to tumors [57]. This pathway has been targeted clinically via the use of bevacizumab, a VEGF-A blocking antibody, in several solid malignancies and has shown varying degrees of clinical response [58,59]. Studies have shown that VEGF inhibition allows for dendritic cell maturation and treatment with bevacizumab have shown increased dendritic cell maturation [60]. Blockade of VEGF signaling with bevacizumab has also been used in combination of with ICI therapy. A combination of the anti–PD-L1 monoclonal antibody atezolizumab with bevacizumab and chemotherapy was assessed in first line treatment of metastatic nonsquamous cell lung cancers. This triplet regimen resulted in improved median overall survival compared with patients treated with bevacizumab and chemotherapy alone. Predictably, however, patients treated with the triple therapy also had higher rate of serious toxicity compared to the control group [61].

Echoing these data with bevazicumab, are the results of pre-clinical and clinical trials which combined ICIs with Lenvatinib. Lenvatinib is an oral small-molecule inhibitor of VEGFRs, as well as other receptors such as FGFRs, PDGFRα, KIT and RET proto-oncogene. Lenvatinib was previously used as monotherapy of several malignancies including hepatocellular carcinoma, renal cell carcinoma and thyroid cancer [62–64]. In murine models, Lenvatinib in combination with anti PD-1 blockade was shown to enhance anti-tumor immunity by reducing tumor associated macrophages and increasing the percentage of activated $CD8^+$ T cells secreting interferon IFN)-γ+ and granzyme B in the TME [65]. In trials with patients with various different cancers including, urothelial cancer, head and neck squamous cell carcinoma, melanoma, non-small cell lung cancer, renal cell carcinoma, colorectal cancer and endometrial cancer, combination Lenvatinib with anti-PD1 therapy displayed impressive rates of response ranging from 25–55% [63,66]. Again, these data clearly indicate the blockade of VEGF signaling is active in combination with ICI therapy. The challenge still remains as how best select patients for this combination, as the trials have yet to offer definite insight into this important question.

In addition to agents blocking the VEGF receptor signaling pathway, inhibitors targeting other signaling pathways have been suggested to have significant immuno-modulatory properties. For instance, combined BRAF and MEK inhibition as well as CDK4/6 inhibitors have been demonstrated to enhance the T cell response in pre-clinical models [67]. Evidence of these synergies are also emerging in the clinic as patients with BRAF-mutant melanoma treated with a triplicate regimen of a BRAF inhibitor, a MEK inhibitor and an anti-PD-L1 agent displayed increased progression free survival compared to patients treated with targeted therapy alone [68]. Similarly, in hormone receptor-positive breast cancer a triplicate regimen of anti-PD1, hormonal therapy and a CDK4/6 inhibitor displayed encouraging results in a Phase 1/2 trial [69]. Obviously, further trials are required, but these early data are notable as previously hormone receptor-positive breast cancer was considered an immunologically 'cold' tumor, refractory to ICI treatment. These data provide early clinical data that targeted therapies could hold the potential to expand the reach of immune therapy.

## CELLULAR THERAPY

Adoptive cellular therapy (ACT) is a form of immunotherapy which uses *ex vivo* expanded T cells to generate an anti-tumor response. There are three major ACT modalities used

in treatment of solid malignancy: autologous tumor infiltrating lymphocytes (TIL), genetically engineered T cell receptors (TCRs) and chimeric antigen receptors (CAR) T cells. CAR T cells have demonstrated unprecedented results in the treatment of hematological malignancies but have yet to have the same level of success for solid tumor malignancy [70]. To date, ACT using TILs has displayed some of the best clinical responses in patients with certain solid tumors. Treatment protocols for ACT using TILs require the harvest of autologous $CD8^+$ and $CD4^+$ T from a tumor lesion via surgical resection. These TILs are then massively expanded *ex vivo* and reinfused back into the patients following preparative treatment with lymphodepleting chemotherapy. Engraftment and expansion of the infused TILs is then supported by interleukin-2 treatments. There has been promising clinical responses observed in patients treated with ACT using TILs, particularly for melanoma [71]. Importantly, significant clinical response have been observed in patients whose disease previously progressed on treatment with ICI therapy. For instance, a recent study assessed treatment with TIL product lifileucel in patients with unresectable melanoma after progression on either ICI or targeted therapy. The overall response rate was 36.4% and disease control rate of 80% [72]. Moreover, the reach of TIL therapy is now being studied beyond the treatment of melanoma. TIL therapy in combination with ICI treatment has shown promise in patients with NSCLC. Amongst 20 NSCLC patients treated with TILs and ICI after prior progression on anti PD-(L)1 inhibitors, 2 patients achieved durable complete response [73]. These data speak to the potential of TIL therapy to treat tumors that are refractory to ICI therapy alone.

Genetically engineered T-cell receptor therapy modifies naïve lymphocytes to recognize tumor antigen via the expression of T-cell receptor specific for a tumor antigen expressed in the context of the major histocompatibility complex (MHC). One of the major challenges of TCR therapy is identifying a tumor antigen which is specific to the tumor therefore avoiding activation of cells towards tissue other than the tumor. For example TCRs specific against the MART1 antigen [74] in melanoma and NY-ESO-1 and MAGE A3/6 antigens [75] have been used in clinical trials due to the high levels of expression of these antigens on certain tumor types. A drawback of TCR based therapies is the dependence of antigen presentation on MHC. CAR T cell lymphocytes are genetically modified to have specificity for tumor antigens however the engineered construct can recognize surface antigen that is not restricted based on MHC presentation. Several different tumor antigens have been targeted in CAR T cells for solid malignancy including IL-13 receptor α 2 (IL13Rα2) in a patient with multifocal glioblastoma multiforme [76]. Other targets have included mesothelin [77] and [78] HER2 both of which showed limited response to date. The next generation of engineered T cell products, T cells redirected for antigen-unrestricted cytokine- initiated killing' (TRUCKs) aim to combine CAR T-cells with inducible release of a transgenic protein, typically a cytokine at the time of activation to stimulate a wider immune response. These next generation cell therapies offer the potential for 'build in' combination immune therapy with ACT in addition to other therapeutics being expressed by the modified T cells. These approaches may help overcome some of the current issues of antigen-targeting currently impeding the development of gene-engineered T cells and CAR T cells and allow the engineered T cells to deliver agents to the TME that will support the reinvigoration of an endogenous, polyclonal anti-tumor T cell response.

The next generation of ACT may enroll the aid of gene editing technologies such as cluster regulatory interspaced short palindromic repeat/CRISPR-associated protein [9]. The addition of gene editing to CAR T cells has the ability to help enhance potency and safety of treatment via for example knocking out of inhibitory molecules such as PD-1 and TGF-beta which has shown increased tumor


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elimination in patient derived xenograft solid tumor models [79,80].

## T-CELL ENGAGERS

T-cell engager are molecules that induce anti-tumor immunity by inducing the targeting and activation of polyclonal T lymphocytes to tumor-expressed antigens. Bispecifc T-cell engagers (BiTEs) are recombinant proteins made of scFv regions from two different antibodies. One scFV targets a specific tumor antigen and the other targets and activates T cells independent of antigen specificity, typically via engaging the CD3 complex. These molecules are able to reorient T cells that do not express a T-cell receptor specific for tumor antigens and thereby allow for an amplification of the anti-tumor response by recruiting 'bystander' T cells. As recently reviewed elsewhere [77], there currently are a range of target antigens expressed by tumors that are being tested as targets for BiTE therapy. These include HER2, EGFRvIII, mesothelin, GD2, CEA, PSMA, EpCAM and AFP. Similar to the challenge faced in the development of CAR T cell therapy for solid tumors, expression of the target antigen by tumor must be weighed against expression in healthy tissue. High expression of the target antigen in normal tissue can result in significant toxicity in what is terms an 'on target off tumor' effect. Despite this caveat, a modified T cell engager has recently demonstrated significant activity in the treatment of uveal melanoma.

Tebentafusp is classed as an immune-mobilizing monoclonal T-cell receptor against cancer (ImmTAC). The molecule differs from a classic BiTE as the tumor targeting is achieved via the use of a soluble, affinity-enhanced HLA-A*02:01–restricted T-cell receptor that is specific for a peptide from the glycoprotein 100 (gp100) protein. This soluble TCR is fused to an anti-CD3 single-chain variable fragment that induces activation of the recruited T cells. In a recent trial, first-line treatment tebentafusp was found to increase overall survival versus ICI monotherapy for patients with metastatic uveal melanoma [81]. These data establish that T cell engager therapies have the potential to be clinical benefit for solid tumors. Moreover, they reinforce that tumors thought of as immunologically 'cold', such as uveal melanoma, due to poor response to ICI therapy still can be amenable to treatment with a different modality of immunotherapy.

## MICROBIOME

There has been growing interest in modulating the microbiome, the billions of bacterial that colonize the human skin, respiratory and digestive tracts, to enhance the outcomes of immunotherapy treatments. This interest stems from the multiple pre-clinical studies that have demonstrated the profound impact of the composition of the intestinal microbial flora has on the efficacy of immune therapy treatments, particularly ICIs [82,83]. Encouragingly, these results have been mirrored in cohorts of cancer patients, with different species of microbiota being found to be enriched in responders versus non-responders to ICI therapy. For example, patients with metastatic melanoma who were responders to anti–PD-1 therapy were shown to have enrichment of *Bifidobacterium longum*, *Collinsella aerofaciens*, and *Enterococcus faecium* in pre-treatment stool samples [84]. Additionally, it has been found in cancer patients treated with ICI there exists a correlation between the microbiome and the toxicities experienced. A protective effect of a Bacteroidetes-rich phylotype against CTLA-4 blockade–induced colitis was observed in patients with melanoma [85]. Further studies have also correlated the microbiome with adverse events experienced by patients treated with combination PD-1 and CTLA-4 blockade [86]. Collectively, these data verify that the microbiome is an attractive target for modulation to enhance the efficacy and potentially lessen the toxicity of immune therapy.

One challenge has been to understand the best ways to modulate the microbiome to

improve immune therapy. It appears that a favorable microbiota contains a vast diversity of microbial species. Accordingly, promoting a diverse microbiome seems to be a key principle to guide potential therapeutic interventions. Avoiding concurrent therapies, such as antibiotics, that lessen microbial diversity has been suggested to improve outcomes with ICIs in retrospective studies. Wilson *et al.* reviewed 766 studies assessing the effects of antibiotic use in immune checkpoint blockade looking at the outcome of 2889 patients and showed an increased overall survival in patients that were not exposed to antibiotics during treatment [87]. Aside from preventing damage to microbial diversity, other treatment approaches have demonstrated promise to promote a varied intestinal ecosystem. A diet enriched for dietary fiber has recently been found to promote the diversity of the colonic microbiome and correlate with superior outcomes to ICI therapy [88]. Fecal microbiota transplant has also been utilized to attempt to repopulate the microbiome with flora supportive of response to immune therapy. To date, in early phase trials this approach has been able to rescue the response to anti-PD1 therapy in some patients whose disease initially progressed on treatment [89]. Collectively, all of these studies indicate that the microbiome has the potential not only to be an important biomarker for treatment selection but also an important therapeutic target for future immunotherapy regimens.

## METABOLISM

Within the TME there are numerous suppressive factors that can blunt the anti-tumor immune response. In addition to the presence of many negatively regulatory cells such as Tregs and MDSC, there are multiple metabolic factors within the TME that can constrain T-cell activation and immunity. These include hypoxia, altered pH as well as the depletion of many key nutrients required for immune cell function [18]. In particular, the amino acids tryptophan and arginine are depleted in the TME via their catabolism by the enzymes indoleamine 2,3 dioxygenase (IDO) 1 and arginase 1 (Arg1) respectively, which are expressed by multiple cell types present in the TME [90]. Accordingly, agents that inhibit the enzymatic activity of IDO and ARG1 and prevent the depletion of tryptophan or arginine in the TME have the potential to help enhance anti-tumor immunity. Unfortunately, the first IDO inhibitor, epacadostat, to be trialed in combination with anti-PD1 agent in a randomized Phase 3 trial failed to demonstrate clinical benefit [91]. There remains, however, many questions as to the reason for this observed lack of benefit, ranging from the dosing regimen used to the trial design [92]. Further trials with novel, more potent IDO inhibitors that also inhibit the IDO2 enzymes [93] or ARG1 inhibitors are still required to fully evaluate this treatment strategy in the context of immune therapy.

Aside from enhancing amino acid levels in the TME, reducing hypoxia is another therapeutic approach that has shown promise in pre-clinical models. In mouse studies the commonly used diabetes drug metformin was shown to reprogram tumor metabolism, reducing oxygen consumption by the tumor cells and thereby increasing the oxygen available to immune cells in the TME. Treatment of mice bearing murine melanoma and colon cancers with metformin and anti-PD1 blockade demonstrated reduced hypoxia in the TME and increased efficacy of anti-PD1 therapy [94]. In patients, a retrospective cohort study that included patients diagnosed with metastatic malignant melanoma and treated immune checkpoint inhibitors plus metformin showed the overall response rate was higher in the combination group at 68% versus 54%, however this difference did not reach the threshold of statistical significance. The study did show a decrease in the mean number of new metastatic sites which appeared during in the combination group [95]. Again, prospective studies are indicated, but this shows proof in principle that medications with existing indications to treat metabolic diseases have the potential to be leveraged to


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alter the metabolic composition of TME and enhance the efficacy of immunotherapy.

## CONCLUSION

Immunotherapy has caused a paradigm shift in the treatment of solid malignancy over the past decade providing the potential for durable long-term control of a broad range of malignancies. Unfortunately, however, treatment failure is still common. To overcome these failures, multiple different immunotherapeutic modalities have been developed. There exists solid evidence that combining these different modalities can over-come resistance and improve outcomes for patients. The challenge, however, is the emerging complexity; with each additional treatment modality the number of potential combinatorial treatments increases exponentially. This increasing complexity stands in stark contrast to the binary 'hot' and 'cold' tumor paradigm that has dominated the literature. To help resolve this conflict, studies integrating multiple different large-scale 'omics' approaches are needed. Integrated analysis of gene expression signatures, immune cell infiltrates and metabolic milieux within TME in conjunction with other patient factors, such as the microbiome, will hopefully aid in identifying novel biomarkers and global patient phenotypes to inform novel combination treatment regimens. These types of analysis, however, will likely have to leverage machine-learning approaches due the size and the complexity of the datasets. Artificial intelligence and machine learning technologies are being developed to aid in interpreting imaging studies, assessment of TME, prediction of immunotherapy side effects and treatment response [96]. To this end, a recent study utilized a transcriptomic-based analytics platform to characterize the TME of multiple different tumor types. This analysis allowed them to refine the 'hot' and 'cold' tumor model and define four different subtypes of TME, each conserved across multiple tumor types and each displaying a different responsiveness to immune therapy [97]. Importantly, these analyses also predicted the immune therapy approaches which would be potentially most efficacious for each of the novel TME subset defined. It will be of great interest to see if these predications can be validated in prospective trials. If these predicted treatment regimens prove to be effective, this would enable the use of TME characteristics, rather than just tumor histological type, to guide the choice of immunotherapeutic regimen. Encouragingly, studies like these suggest that the existing arsenal of different immune therapy modalities may already be large enough to greatly increase the clinical benefit for many patients; the challenge for the next decade of immune therapy is to match agents in the current treatment arsenal to the correct patients for maximal effect.

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IMMUNO-ONCOLOGY INSIGHTS

OPTIMIZING CLINICAL DEVELOPMENT STRATEGY FOR THE RAPIDLY EVOLVING I-O FIELD

SPOTLIGHT

COMMENTARY

# Cancer immunotherapy & clinical trial design: issues & practice

Janet E Dancey

Immunotherapies for the treatment of cancer have become standard of practice and improved outcomes for many patients. Rapid development of these agents has been facilitated by first-in human- multiple expansion cohort trials which used a single protocol with an initial dose-escalation Phase followed by multiple additional cohorts with cohort-specific objectives to seamlessly progress through early Phase development. Despite successful development and adoption of these therapies, there have been challenges: rapid development has resulted in limited evaluation of drug dose/schedule/duration. Attempts to develop predictive biomarkers for benefit or harm have had limited success. Evaluating agents with standard cancer treatments and other immunotherapies requires careful consideration of the rationale for the combination, dosing and scheduling, safety and tolerability. This review covers the clinical trial considerations for evaluation of immunotherapies, and recommendations for future directions.

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The clinical progress of cancer immunotherapies has been unprecedented in its rapidity and its impact on patient outcomes. The breadth of activity of the immune checkpoint inhibitors (ICI), and resultant interest of clinicians, patients, and companies has led to a broad portfolio of clinical trials across cancer settings [1]. Many trials have been designed to seamlessly progress from Phase I to Phase III, leading to early conditional approvals of agents for patients with advanced/refractory disease [2]. While the rapidity of clinical testing of ICIs has been unprecedented and largely successful, there have been challenges.

For example, rapid development has precluded the definition of optimal dose, schedule, and duration of therapy of single agents and combination regimens. Eligibility criteria for Phase III trials remained as restrictive as early Phase trials so that benefits for patients with poorer performance status and comorbid conditions are uncertain. Prediction of risk and management of immune mediated toxicities remains challenging. Thus, rapid development of these promising agents must balance efficient clinical trial design and execution with scientific and regulatory oversight to ensure safety of trial participants and scientific as well as the clinical needs of future patients who may receive the agents as standard of care. This article summarizes the innovations and limitations in clinical trial approaches of ICI therapy.

## TRENDS IN CANCER IMMUNOTHERAPY TRIAL DESIGN

### Master protocols & seamless designs

Traditionally, cancer drug development has evaluated dose, safety, activity, and benefit in discrete trial phases with specific designs and endpoints. More recently, 'seamless' trial designs have been used that consolidate the phases of trials from first in human Phase I single agent through to Phase II in multiple cancer settings within a single protocol so that various aspects of the new drug can be evaluated in a single seamless clinical trial [3–5]. The 'FIH multiple expansion cohort trial' has a single protocol with an initial dose-escalation Phase followed by multiple additional cohorts with cohort-specific objectives [6]. The objectives of these expansion cohorts can include assessment of antitumor activity in specific cancers; dose/safety specific populations (e.g., pediatric, elderly, organ impairment); alternative doses or schedules; combinations with other cancer therapies; or the predictive value of potential biomarkers. Thus, expansion cohorts enable further assessment of tolerability, pharmacology, and biomarkers, and obtain additional data to assess therapeutic activity across tumor subtypes [3–6]. Seamless trials are generally designed around early Phase development of a drug. In contrast, 'Master Protocol Trials' test multiple drugs and/or multiple cancer subpopulations in parallel under a single protocol and include umbrella, basket, or platform designs [7]. These trials have expanded and evolved from adaptive trial design principles [8] (see Table 1 for definitions and examples).

Among the many trials that have used seamless designs, several trials have accrued

TABLE 1

**Trial designs and definitions.**

| Term | Definition | Examples | Reference |
|---|---|---|---|
| Master Protocol | A trial to designed to test multiple drugs and/or multiple cancer subpopulations in parallel under a single protocol. Includes umbrella, basket, or platform designs. | SWOG S1609/NCT02834013 – Dual Anti-CTLA-4 and Anti-PD-1 Blockade in Rare Tumors (DART) | [41] |
| First in Human Multiple Expansion Cohort Trial | FIH trial with a single protocol with an initial dose-escalation Phase followed by three or more additional subject cohorts with cohort-specific objectives. | KEYNOTE-001/NCT01295827 – Study of Pembrolizumab (MK-3475) in Participants with Progressive Locally Advanced or Metastatic Carcinoma, Melanoma, or Non-small Cell Lung Carcinoma | [2] |
| Adaptive Trial Design | A clinical trial design that allows for prospectively planned modifications to one or more aspects of the design based on accumulating data from subjects in the trial. | A082002/NCT04929041 A Randomized Phase II/III Trial of Modern Immunotherapy Based Systemic Therapy with or Without SBRT for PD-L1-Negative, Advanced Non-Small Cell Lung Cancer | [42] |


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more than a thousand patients, and a few have enrolled nearly 2,000 [3,4,6,9]. Merck's FIH trial of pembrolizumab (KEYNOTE-001) opened to accrual in 2011 [2]. Initially designed as a conventional 3 + 3 dose-escalation study to explore the safety and preliminarily assess antitumor activity of pembrolizumab in patients with advanced cancers, serial amendments to the protocol resulted in 1235 patients enrolled in 24 cohorts at its completion in 2014. The melanoma- and NSCLC-specific expansion cohorts included three dose-finding, randomized experiments with pre-specified statistical analyses. The evaluation of pembrolizumab in the trial in patients with metastatic NSCLC led to breakthrough therapy designation in that indication in 2014 and accelerated FDA approval in 2015 for the treatment of patients with PD-L1-expressing metastatic NSCLC with disease progression following platinum combination regimen [10] The duration of clinical development from the initiation of FIH evaluation to accelerated approval occurred in under 4 years.

Despite the remarkably short time to market approval of ICIs like pembrolizumab, there have been notable challenges. Most seamless designs begin with dose selection to identify the maximum tolerated dose (MTD) or a recommended dose for future study. The typical early Phase I 3+3 dose-escalation design using the initial one or two cycles as a dose-limiting toxicity (DLT) assessment period. For immunotherapies, dose-response and dose-toxicity relationships often fail to adhere to assumptions that underly the escalation designs for cytotoxic drugs. Dose-limiting toxicities (DLTs) are often not observed at all or within the observation period prior to dose escalation with these agents. In a pooled analysis of 576 patients with advanced melanoma receiving nivolumab, the median time to onset for treatment-related adverse events (AEs) of any grade ranged from 5.0 to 15 weeks [11]. Therefore, an MTD may not be determined at the end of dose escalation.

Given the infrequency and variable onset of serious AEs of immune-related AEs (irAEs) to guide the determination of recommended Phase II dose (RP2D), additional time and effort may be required to define preferred dose and schedules before proceeding through efficacy assessments. Several drug doses can be evaluated in randomized trials early in development to inform dose selection for further development and, ultimately, registration, based on the assessments of pharmacokinetics, pharmacodynamic features, safety and efficacy [12]. For example, the initial Phase I trial of nivolumab did not identify a MTD among doses 1, 3, and 10 mg/kg using the 3 + 3 design [13]. The RP2D of pembrolizumab was based on results showing PD-1 target engagement was fully saturated at doses ≥1 mg/kg q3 weeks, mouse to human translational studies predicted maximal responses at doses of ≥2 mg/kg q3 weeks [14], and clinical randomized comparisons of pembrolizumab 2 mg/kg and 10 mg/kg q3 week dosing demonstrated equivalence [15]. Dose and response data from these trials and models led the selection of lower doses for clinical evaluation. Questions remain however, regarding the optimal dose, schedule and duration of therapy [16].

To support the rapid progress from Phase I first in human testing (FIH) through multiple subsequent cohorts requires expanding the numbers of participating investigators, patients and statistical, operational and logistical supports to ensure appropriate design, execution and oversight [17]. Limiting patient eligibility criteria to those with excellent performance status, limited co-morbidities or risk factors for significant drug related adverse events is a means to mitigate safety concerns. While these criteria may be appropriate for initial FIH evaluations, they are often carried forward through to Phase III trial trials. Restrictive eligibility criteria not only limits recruitment but also limits the assessment efficacy and safety in these groups of patients prior to market approval and subsequence use. Thus, at time of initial approval of ICI, there was limited data supporting safety and activity among patients who were elderly, with poorer performance

status, comorbidities including chronic viral hepatitis, autoimmune disorders, and major organ dysfunctions.

Clinical trials, whether seamless or traditional in design, should follow similar approaches to ensure scientific rigor, safety, data quality, operational efficiency, and regulatory compliance. For each Phase and cohort, the background information should support its scientific rationale. Eligibility criteria should be appropriately considered based on knowledge of safety and characteristics of the patient population for whom the agent is intended to be used. The specifics should be provided for the trial interventions including drug administration, safety, and efficacy assessments. Statistical design should include the justification for patient sample size based on cohort objectives and prespecified stopping rules for that cohort based on insufficient antitumor activity or unacceptable level of toxicity for that population. Timely data submission, review and communication across investigators, sites, patients, and regulators are required to ensure appropriate regulatory and safety oversight of trials as they progress through their phases and cohorts.

## Endpoints

Traditional oncology trial safety and efficacy endpoints have been used for ICI trials with notable modifications to capture irAEs and progression events. Both Common Terminology Criteria for Adverse Events (CTCAE) [18] and Medical Dictionary for Regulatory Activities (MedDRA) [19] have been used to assess irAEs in clinical trials of ICIs. CTCAE 5.0 has 837 AE terms aligned with the Medical Dictionary for Regulatory Activities (MedDRA) codes and System Organ Class (SOC). AE terms have a standard five-grade severity scale and descriptions. Although CTCAE includes terms and grades for most adverse events caused by cancer therapies, not all irAEs are included in the current version of CTCAE. Thus, MedDRA, which is a standardized medical terminology with more than 70,000 terms widely used in the drug safety surveillance, is used with CTCAE to capture irAEs.

The most common primary efficacy endpoints for expansion cohorts and other Phase II trials have been objective response rate (ORR) per Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST v1.1) [20, 21], disease control rate (DCR) per RECIST v1.1; and for randomized trials, progression-free survival (PFS) and overall survival (OS). In most clinical trials, initial response assessments generally occur at 8 to 12 weeks; however, immunologic responses can be delayed and pseudo progression followed by objective response or stability of disease may occur [21, 22].

The onset of response and the incidence of pseudo progression may vary across tumor types. It is more common in melanoma where immune modulation has a substantial therapeutic effect and rarer in NSCLC where response rates are lower. Pseudo progression is often difficult to distinguish from true progression. For clinical trials, the need for standardized documentation of progression events and patient management led to the modifications of response criteria and inclusion of patient management recommendations to allow for continuation of therapy beyond RECIST progression in otherwise clinically stable patients in trials [21, 22]. However, treatment beyond RECIST progression confers limited benefit. A recent FDA analysis of a completed trial of nivolumab in advanced NSCLC found that 5%of patients who received treatment after progression per RECIST had subsequent tumor response and this comprised 2% of the overall trial population [23].

All clinical trials must have defined efficacy and toxicity endpoints for patient cohorts under evaluation. The timing and decision thresholds for any interim and final analyses should be specified and guide subsequent design modifications for both safety and efficacy endpoints. Safety endpoints should consider potentially dose limiting, late or delayed AEs when selecting the most appropriate dose for future study. As noted previously, expanding


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accrual to evaluate several doses and the duration of observation periods may provide more detailed safety, pharmacokinetic and pharmacodynamic assessments of drug combinations. In general, direct comparison of efficacy between cohorts in a multi-cohort trial is only done when the protocol has a prespecified randomization and analysis plan [24]. However, integrating information, particularly safety data, across cohorts is efficient and Bayesian multivariate modeling and sequential monitoring strategies may be used to enable cohort analyses while controlling the probability of falsely declaring superiority of a treatment which increases with multiple comparisons [25–27].

## PREDICTIVE & PROGNOSTIC BIOMARKERS

Biomarkers that improve the selection of patients who will respond to therapy or are at risk for severe toxicity, help tailor treatment to patients most likely to benefit and mitigate treatment costs [28–30]. Soluble, cellular, and genomic biomarkers under evaluation include serum proteins, tumor-specific receptor expression patterns, factors in the tumor microenvironment, circulating host immune and tumor cells, and genomic DNA. FDA approved biomarkers for anti-PD-1 therapies include PD-L1 expression within the tumor microenvironment, high tumor mutational burden (TMB) or mismatch repair deficiency [31]. Antitumor immunity is complex and context-dependent on host, cancer histology and stage of disease and treatment intervention. These currently approved biomarkers for patient selection are imperfect and considerable research is underway to identify and validate better biomarkers that correlate with patient benefit to ICIs and other immunotherapies.

Better biomarkers predictive of benefit will emerge with further understanding of host immune and tumor cell biology. Such understanding will arise from preclinical models, assay technologies to accurately measure these biomarkers in patient samples, and well-designed clinical trials that validate the biomarker strategy and patient benefit. Well-characterized in vivo and in vitro models such as patient-derived organoids and patient-derived xenografts may reveal additional insights into host-tumor interactions, mechanisms of action of immunotherapies as they modify the complex immune response to cancer, tumor microenvironment and immune milieu [32]. These studies may identify phenotypic and genomic features that correlate with sensitivity or resistance to the investigational drug or drug combinations. Serial sampling of tumor and blood for circulating cells and DNA from patients on clinical trials may identify markers of drug resistance that can be subsequently used to select subsequent therapy and to develop rational combinations.

With advances in assay technologies, more complex signatures of relevant tumor, immune and stromal cell features might effectively be used as clinical biomarkers. Newer assay technologies and methods may better characterize tumor and immune cells, and their microenvironment. Larger panel of gene signatures, chemokines, and other factors that correlate with response have been proposed to provide a more comprehensive profile [32]. Candidate predictive biomarkers include genome–based mutation signatures, [33] profiling of proteins or RNA in the tumor immune microenvironment, [34] and radiomic analysis of quantitative features extracted from routine imaging [35].

Inclusion of candidate biomarkers into clinical trials requires standardization in sample collection, measurement, and interpretation of the assay within an appropriately designed trial. The selection of biomarker and assay is complex as different technologies for the same target may utilize different cut-offs, or other features that impact the patient populations they identify and, consequently, the likelihood of a biomarker positive patient to respond to a given therapy. Trial protocols should include the background rationale supporting the use of the biomarker and detail

the type and timing of sample collections. When a biomarker is used to assign a patient to a treatment arm, the protocol should specify and justify how subjects with more than one biomarker of interest will be assigned. Assignment may be by predefined list of priorities, or by randomization. Biomarker assays should be adequately analytically validated to allow interpretation of the results. Procedures for tumor sample acquisition, handling, and the testing and analysis plans need to be defined in the protocol and/or laboratory manual.

Successful execution of a biomarker strategy in a clinical trial requires that sample collection be feasible to implement across participating centers, and analyses must be robust to yield interpretable results. Biomarkers for patient eligibility or stratification for a clinical trial require not only standardized, reproducible assays that are feasible to use but also that analysis be rapidly completed with clearly defined cutoff values and with high sensitivity and specificity. Finally, biomarkers will have to be clinically validated within defined patient populations based on histology, regimen, and clinical setting. The biomarker correlation with benefit may be modified when used in early versus more advanced disease settings, when used in combinations or for treatment of patients with a particular cancer.

## APPROACHES TO COMBINATION TREATMENT REGIMENS

While predicative biomarker strategies aim to select potential responders and/or exclude potential non-responders to ICI treatments to maximize potential for benefit and minimize risk of toxicity, the evaluation of combinations of treatments aims to increase the numbers of patients that may benefit by using agents with different mechanisms of action and circumvent mechanisms of drug resistance.

Currently approved ICIs target the immune cell priming and activation (anti-CTLA4 antibody) or the final negative regulation of T effector cells (anti PD-1/PD-L1 antibodies). Given this limited targeting within the complex immune system, a minority of patients experience benefit from single agents. Slightly higher response rates have been observed with anti-CTLA4 and anti-PD1/PD-L1 combination treatments, at the cost of higher immune-mediated toxicities [36, 37]. Combination therapies are being extensively explored to target multiple defects along the immunity cycle and cancer intrinsic alterations and improve the anti-cancer efficacy [1]. These combinations of drugs will however require some means of specific targeting of immune response to tumor cells relative to normal tissues to mitigate rising rates of immune-mediated toxicity.

Cytotoxic chemotherapy and radiation regimens were developed based on identifying a maximum tolerable dose and schedule for anti-cancer effect. Despite initial concerns that lymphodepletion and immunosuppressive effects of chemotherapy and radiation would attenuate the effectiveness of ICIs, preclinical and clinical studies of chemotherapy and/or radiation and immunotherapy have shown that these combinations can improve tumor responses and patient benefit [38–40]. In addition to traditional cytotoxic chemotherapy and radiation, agents targeting the VEGF-VEGFR pathway, which plays critical roles in almost every subpopulation of immune cells, have been shown to benefit patients when combined with ICIs. Similar rationale and preclinical data support combinations of ICIs with cell cycle inhibitors and signal transduction pathway inhibitors [38]. Among the multiple mechanisms that cytotoxic therapy may improve immune cell mediated tumor cytotoxicity are tumor debulking, which may increase immune cell distribution within the tumor; improved tumor antigen presentation to immune cells through tumor cell death and release of neoantigens; depletion of immunosuppressive cells; and epigenetic modifications to improve immune cell recognition and cancer cytotoxicity [38].


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The challenges for the identification of optimal doses and schedules for single agent immunotherapies also apply to combinations of immune cell targeting agents with other anticancer therapies. New drugs are generally added to existing regimens without considering whether all drugs are need, maximal tolerable doses are optimal, or the duration of therapy for optimal efficacy and cost effectiveness. If toxicity occurs that requires dose modifications for continuation of treatment, there may be little prior understanding of the contribution of each drug, and the preferred dose modifications to safely continue treatment. Addressing these challenges may require novel designs, expanded cohorts for dose/schedule explorations and strategies for managing acute and delayed toxicities especially when the optimal doses may not be known of each agent.

Whether assessed within an expansion cohort in a seamless trial, or separate Phase IB/II trial, the evaluation of an investigational drug administered with an approved drug or another investigational drug should be initiated after the safety profile is established for the investigational drug as a single agent at the recommended Phase II dose. The trial protocol should include the justification and scientific rationale for the safety and potential activity supporting combining these drugs. Key trial design considerations are the recommended starting doses/schedules, the toxicity management including safety monitoring activities, and the sequence and magnitude of dose modifications including discontinuation of drugs for specific toxicities, particularly for overlapping and/or potential synergistic toxicities. The protocol may include a dose-finding component for novel combinations when the RP2D of the combination regimen has not been established previously. Generally, safety data from a minimum of six subjects treated at the proposed dosage for the drug combination regimen is assessed before proceeding with subsequent enrolment for the efficacy evaluation. Expanding accrual and extending the observation periods may facilitate more detailed assessment of safety and tolerability of combinations and randomized designs with a control arm may facilitate evaluation of efficacy.

## REGULATORY EXPECTATIONS

The overarching aim for investigators, patients, regulators, and ethics board members is that clinical trials be well-designed and well-conducted to ensure participant safety and efficiently generate high quality data demonstrating safety and effectiveness of the therapeutic intervention. Successful execution begins with a comprehensive protocol and statistical analysis plan. Early trials should be designs to ensure safety and identify clinically important and rule out clinically unimportant efficacy signals and include appropriately designed interim analyses to limit exposure to an ineffective and unsafe drug. Oversight of trial conduct should ensure compliance with protocol and regulatory requirements, safety of patients, and high quality data. Data collection, review and analyses should be of sufficient quality to be 'fit for purpose' whether to inform the academic research community or support marketing application. There should be rapid communication of serious safety issues and planned changes to trial protocol and conduct to relevant parties.

Because of the complexities of seamless trials and master protocols, the FDA has published guidance documents with recommendations for their design and conduct [6,7]. In general, FDA recommends each master protocol or FIH multicohort study is part of a new IND to FDA. Timely communication points include pre-IND filing, with emerging safety signals, before submitting any protocol amendment that substantively affects the safety or scope of the protocol (e.g., interim analyses for safety such as end of dose escalation, efficacy) or to modify the development program (e.g., discuss potential for breakthrough therapy designation). Early discussion with the review division of biomarker development plans is also recommended when

there are plans to use biomarkers to inform the selection of patients for trials. For larger trials, an independent data safety monitory committee (IDMC) or other appropriate independent entity should be responsible for assessing efficacy and to recommend protocol modifications or other actions as appropriate based on findings.

## SUMMARY & CONCLUSIONS

Immune checkpoint inhibitors have revolutionized the management and improved outcomes of patients with advanced cancers. The rapid development and adoption into practice of these agents has been aided by efficient trial designs that employ multiple, concurrently accruing patient cohorts where individual cohorts assess different aspects of the safety pharmacokinetics and anti-tumor activity of the drug. Early efficacy, safety, and exposure-response data collected through systematic evaluation early in development can support more informed dose selection in later Phase trials and clinical practice. As trials progress in development, broader eligibility to ensure a fuller information range of patients who will receive a drug in clinical practice, such as the elderly and patients with multiple illnesses, should be implemented.

The identification and validation of predictive biomarkers remains challenging due to the complexity of host-immune system and tumor cells and micro-environments but may be added with advances in scientific knowledge and technologies that allow broad, comprehensive, and accurate measurement of molecular and phenotypic features. Such approaches remain a higher priority to pursue in clinical trials as they have the potential to enrich for patients most likely to benefit, limiting their exposure to ineffective, toxic, and expensive therapies.

Trials will continue to evaluate promising new immune and tumor cell targeting therapies as single agents and combinations across cancer histologies and stages. The assessments of new single agents and combinations that address novel mechanisms of action and resistance will continue the advances and improve patient outcomes.

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OPTIMIZING CLINICAL DEVELOPMENT STRATEGY FOR THE RAPIDLY EVOLVING I-O FIELD

SPOTLIGHT

VIEWPOINT

# Critical collaboration: Efforts to expand clinical trial eligibility

**JEFF ALLEN**, PhD serves as the President and CEO of Friends of Cancer Research (Friends). For over 25 years, Friends has created unique scientific partnerships, accelerated policy change, and supported groundbreaking research to deliver new therapies to patients quickly and safely. As a key thought leader on issues related to the U.S. Food and Drug Administration, healthcare, and regulatory policy, he is regularly published in prestigious medical journals and policy publications and has contributed his expertise to the legislative process on multiple occasions. Recent Friends initiatives include the establishment of the Breakthrough Therapies designation, innovative research consortia to enhance biomarker development, and the launch of a unique cross-sector partnership to accelerate clinical trial conduct and rapidly assess if a patient's treatment is working. Jeff received his Ph.D. in cell and molecular biology from Georgetown University and holds a Bachelor of Science in Biology from Bowling Green State University.

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For over 25 years, Friends of Cancer Research (*Friends*) has worked to design innovative research partnerships and evidence-based policy aimed to improve the lives of people with cancer. As cancer science continues to evolve, policy needs should follow suit to develop and deliver new medicines as safely and efficiently as possible.

Barriers to the conduct of clinical trials presents a perennial challenge to the development of new medical products. For several decades, the average enrollment of adults with

cancer into clinical trials has hovered around 2–8% [1]. These participation rates have been accompanied by numerous efforts to raise awareness about trials and increase educational outreach to patients and medical providers. While such efforts have likely identified many prospective participants, the trials themselves have largely remained unchanged in terms of the criteria used for determining if patients are able to be included in a trial. Overly restrictive eligibility criteria can limit individual access to clinical trials as part of cancer care, impede enrollment by reducing the number of eligible patients, and cause trials to be less reflective of the patient population that eventually use new medicines [2].

To explore how eligibility criteria could be modified to enable greater patient access to clinical trials we partnered with the American Society of Clinical Oncology (ASCO) to launch a collaborative effort alongside expert advisors from clinical research, industry, the Food and Drug Administration (FDA), the National Cancer Institute (NCI), and other advocacy organizations. The objective was to establish multiple subject-specific working groups to develop recommendations for ways in which eligibility criteria could be expanded in cancer clinical trials [3,4].

Eligibility criteria play an important role in protecting patients and when attempting to isolate the effect of a drug, a relatively homogenous population can be beneficial. However, it was frequently noted that trial eligibility criteria are often established based on past trials, rather than evaluating and prospectively designing optimal eligibility criteria based on the properties of the drug and the patient population that is being sought.

When trial protocols are developed, there may be a tendency to rely upon trial parameters and inclusion criteria that have made it through various regulatory hurdles in the past. This "cut and paste" approach to subsequent protocols may contribute to unnecessary exclusion of patients. It may also be a barrier to inclusion of more diverse patient populations in clinical trials. For example, routine lab tests are used to identify potential confounding conditions, such as abnormal kidney or liver function, that may make trial participation potentially unsuitable for a patient. The acceptable reference ranges for normal values have been based on averages, and over the years, these averages have come from the most frequent trial participants – similarly aged Caucasian males [5]. This can effectively exclude a more diverse and representative population, whose metabolic factors may not match those historic participants, but still represent normal biologic functioning as able clinical trial participants.

Over time, excessively restrictive eligibility criteria have limited the number of patients able to participate in clinical trials, and yield trials with a somewhat limited understanding of the applicability of a product to the broader population. Addressing this challenge requires all stakeholders in the oncology clinical trial space to implement changes and increase opportunities for trial participation – and progress is being made. Examples of this include several recently finalized FDA guidance documents on how eligibility criteria might be broadened [6–9], and updated NCI trial templates to help ensure that eligibility criteria routinely start broad and narrow as appropriate, rather than carrying over previous trial parameters [10]. These efforts can help engage trial sponsors within the pharmaceutical industry and academic research centers to expand eligibility and take steps to not exclude potential patients who could benefit from their products.

When looking more broadly at ways to make clinical research more accessible, eligibility criteria is just one opportunity. However, expanding the criteria for trial entry will allow more people to have access to clinical research as part of their care, allow drug developers, medical practitioners, and patients to gain insights into a more representative population, and increase the number of patients who can participate in trials. Tangible and collaborative steps to modify clinical trial constructs can help raise historic rates for trial accrual and speed up trial enrollment – ultimately resulting in new medicines making it to patients sooner.


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IMMUNO-ONCOLOGY INSIGHTS

OPTIMIZING CLINICAL DEVELOPMENT STRATEGY FOR THE RAPIDLY EVOLVING I-O FIELD

SPOTLIGHT

COMMENTARY/OPINION

# The cutting edge: leveraging pre-surgical immunotherapy trials to understand therapeutic mechanisms.

Bailey G Fitzgerald, Matthew D Galsky & Thomas U Marron

One of the most persistent sources of consternation in the pathway for immuno-oncology drug development today is the high failure rate of early phase trials. Promising treatments, with excellent rationale, and similarly excellent outcomes in model systems are often discarded after failing to demonstrate similar benefit in patients. The difficult translation from preclinical to clinical testing may be in part attributable to heterogeneity in human immunobiology and carcinogenesis which is not recapitulated by animal models. Nonetheless, the contemporary clinical drug development paradigm was designed for traditional cytotoxic drugs and the complexity of modern anticancer therapeutic approaches warrants a rethinking of the most efficient path to transition novel strategies from the laboratory to the clinic. Examples of the shortcomings of current clinical drug development strategies in the context of immunomodulatory therapies include dose escalation designs with drugs that exhibit non-dose dependent effects and often delayed adverse events [1,2]. Furthermore, there is an enormous unmet need to better understand the mechanisms of action of immuno-oncology drugs, and the underlying mechanisms of resistance that can explain heterogeneity of response and identify biomarkers to help enrich clinical trials for those patients most likely to benefit. To address this need, the platforms on which trials are designed will have to evolve, more seamlessly integrating translational science into studies with clinical endpoints, and changing a traditional 'bench to bedside' approach to one of contemporaneous 'bench *and* bedside' interrogation.

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Several such platforms which have largely emerged in the era of 'targeted' and immune-oncology agents are neoadjuvant and so-called Window-of-Opportunity trials in the perioperative setting. Although the terms are related, the terminologies have subtle distinctions. A window trial is one in which a new approach with limited data is applied in a relatively brief exposure capitalizing on the 'window of opportunity' between diagnosis and surgery. A neoadjuvant trial rather refers to the use of a treatment or combination that has at least some track record of clinical benefit and may be administered with the aim of cytoreduction and/or changing operative approach, alongside the ultimate aim of limiting the chances of post-operative recurrence. Both types of trials can serve as an exemplary tool for cancer drug discovery and innovation due to the large biospecimens obtained following therapy, rather than meager blind biopsies of heterogeneous metastatic tumors. In these perioperative trials patients receive a short duration of therapeutic intervention prior to surgical resection of cancer tissues. A neoadjuvant trial can effectively test a clinical hypothesis and demonstrate a drug's anti-tumor effect. However through optimal biospecimen collection— requiring deliberate multidisciplinary collaborations—high resolution characterization of post-treatment, often in comparison to pre-treatment, tissue can help inform a drug's mechanism of action [3]. Institutional consortiums, such as The neoAdjuvant Research Group to Evaluate Therapeutics (TARGET) at Mount Sinai are needed to support these platforms and maximize the prospect of insight into the mechanisms and vulnerabilities of novel anti-cancer agents.

## PERIOPERATIVE & ADJUVANT TREATMENTS ARE ALREADY PRODUCING CLINICAL BENEFITS.

Coupling surgeries with systemic therapy is not new, and increases the likelihood of cure across multiple cancer types. Adjuvant immunotherapy has become standard practice; perhaps the best established is melanoma, where Phase III trials have demonstrated improvements in recurrence free survival with adjuvant immunotherapy for resected disease [4,5]. Other disease fields have caught up significantly in recent years, with adjuvant approvals following for genitourinary cancers [6,7], esophageal cancer [8], and lung cancer [9]. Perioperative treatments, given in the brief period immediately before and after tumor resections may be uniquely useful. The stress of surgery on the body as well as the medications given for pain and anesthesia have well characterized immunosuppressive effects [10–12], and these conditions are hypothesized to predispose to proliferation of micrometastatic disease [13,14]. Several groups have effectively explored perioperative immunomodulation as a strategy to combat this effect, both in preclinical models and in-human clinical trials [15–20].

## NEOADJUVANT IMMUNE CHECKPOINT BLOCKADE MAY BE BETTER THAN ADJUVANT

Despite the remarkable successes in the adjuvant space, there are many reasons to believe that neoadjuvant immunotherapy may be even more clinically beneficial, and not require prolonged periods of this expensive therapy without any pathological or clinical indication of efficacy. Robust preclinical data supports the hypothesis that the increased tumor antigen burden available in the neoadjuvant approach acts as more effective fodder for systemic T cell priming. In murine cancer models immunotherapy produces more potent anti-tumor activity against metastatic disease compared to adjuvant treatments [21]. These studies suggest that induced systemic anticancer surveillance is responsible for the improved efficacy, with increase in circulating tumor-specific CD8+ T cells corresponding to this more robust response [21,22]. In addition, neoadjuvant immunotherapy has been explored as an effective primer of *de novo* T


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cell clones when compared to adjuvant treatment [22–29]. In situ pathologic architecture may enhance this effect in tumor draining lymph nodes prior to surgical removal. Dendritic cells, activated by PD-L1 blockade and exposed to tumor antigens in the tumor, travel to tumor draining lymph nodes. There, in the presence of PD-L1 blockage they may educate naïve T-cells with their tumor-specific antigen [22,27–29].

Furthermore, the efficacy of immune checkpoint blockade may be stymied in the post-operative setting. Alongside a paucity of neoantigen *in vivo* in this setting where there may only remain micrometastatic disease, as discussed briefly above, previous work has raised concern that the inflammatory stress responses provoked by surgery may suppress cellular immunity, impairing the efficacy of immune checkpoint blockade and T cell priming in this environment [10,30,31]. Given the long half-live of current immune-checkpoint inhibiting monoclonal antibodies [32], pre-surgical immune therapy has the potential to extend benefits of pre-operative treatment throughout the critical peri-operative period.

## THE NEOADJUVANT MODEL FOR CLINICALLY MEANINGFUL ENDPOINTS

Outside of the immunotherapy space, neoadjuvant trials for breast cancers commonly use pathological complete response (pCR), which can be used to screen the activity of novel therapies and in many cases correlates with survival outcomes [33]. Recent studies have extended this benefit into the immunotherapy sphere, with the I-SPY 2 and Keynote-522 trials demonstrating improved pCR most impressively in triple-negative cohorts with the addition of pembrolizumab to neoadjuvant chemotherapies [34,35]. While prior pCR validation was from chemotherapy alone trials, immunotherapy has a very different mechanism of action through which it may decrease the likelihood of recurrence; importantly this improved pCR seen in TNBC studies was confirmed to correlate with subsequent DFS and OS endpoints [36]. Ongoing trials are attempting to define benefit in this space when combined with adjuvant endocrine therapy include CheckMate 7FL which is adding nivolumab to neoadjuvant chemotherapy in patients with high risk ER+ breast cancers [37], as well as KEYNOTE-756 which will add pembrolizumab to both neoadjuvant chemotherapy and adjuvant endocrine therapy [38].

To date, pCR has not been accepted by the FDA as an endpoint in most other cancer types, and even in breast cancer its use as a regulatory endpoint is accepted only under specific circumstances. However, in many solid tumors, work examining the association between pathological response and time-to-event outcomes is underway, and the use of pathological response as rapid measure of the activity of novel therapeutic regimens is expanding.

In melanoma, success of neoadjuvant targeted therapies for BRAF mutated melanomas has led to the evaluation of neoadjuvant combination ipilimumab and nivolumab, where dual checkpoint blockade produced 73% overall response rate (ORR) by RECIST and a 45% pCR rate [39,40]. Pooled analysis of 6 neoadjuvant targeted and immunotherapy trials demonstrated improved RFS in patients who achieved pCR or near pCR after neoadjuvant treatment [41]. Notably, robust translational science has been performed alongside neoadjuvant immunotherapy trials in melanoma. Using bulk RNA sequencing researchers found that B cell markers were the most differentially expressed genes in the tumors of responders versus non-responders, providing useful practical information for design of future trials and clinical biomarker development, as well as generating a number of insights into the role of B cells and tertiary lymphoid structures in response to immune checkpoint inhibition [42]. Similar advances are occurring in non-small cell lung cancer where prior standards of care include perioperative chemotherapy with equipoise as to the

benefits of the neoadjuvant versus the adjuvant setting. Phase I and II trials combining limited courses of immunotherapy alone or chemoimmunotherapy have demonstrated clinical activity along with generating mechanistic insights [43–47]. The Phase III CheckMate 816 was reported at AACR 2021, in which nivolumab was added to neoadjuvant platinum-based chemotherapy, with resulting increases in major pathological response rates compared to chemotherapy alone (36.9% and 8.9%, respectively) and improvement in pCR rates to 24% compared with only 2.2% with neoadjuvant chemotherapy alone [48,49].

Similarly, neoadjuvant chemotherapy remains the standard of care for muscle-invasive bladder cancer [50–55], where pCR has correlated at the trial level with improved survival. However investigations into neoadjuvant immunotherapy have already demonstrated induction of pCR in Phase II studies of both cisplatin-fit and 'unfit' populations [56,57] and many trials investigating immunotherapy combinations are ongoing, including the large Phase III KEYNOTE-905 trial which is randomizing patients to neoadjuvant and adjuvant pembrolizumab versus neoadjuvant and adjuvant combination enfotumab vedotin plus pembrolizumab versus surgery alone [58].

Recent data in hepatocellular carcinoma, where the recurrence rate following surgery is over 50% but no agents are regularly used in the perioperative setting due to lack of survival benefit, two recent studies reported roughly a third of patients achieving significant pathological responses after brief treatments with either cemiplimab or nivolumab with or without ipilimumab [59,60]. Multiple trials of neoadjuvant immunotherapy for HCC are currently ongoing – CA209–956 (NCT03222076), AURORA (NCT03337841), PRIME-HCC (NCT03682276) – with results expected in the next few years.

Within this pre-surgical space, window trials represent an exciting opportunity to capture this clinical benefit in smaller trials. Small sample sizes mean that accrual happens quickly, and the pre-operative intervention periods are kept intentionally brief, usually less than two months, to both limit delay of surgery and the potential for toxicity that may further delay surgery. Surgical interventions after this brief window of time mean that pathological results can be obtained in a relatively short period of time. The clinical outcomes can therefore be assessed quickly and correlated with the myriad hypothesis generating results which can be derived quickly from post-operative tissue analysis.

## UNDERSTANDING THE MECHANISMS

A window approach may be a boon for both patients and physician scientists, as it also allows for a robust scientific exploration of what is actually happening when we give drugs to patients. Pre-surgical immunotherapy in particular offers a unique opportunity to understand the mechanism of action of these drugs, and window trials are emerging as an important platform for mechanistic investigations.

Recent advances in technologies available for tissue analysis have made single-cell phenotyping possible. Single cell sequencing platforms (e.g., CITEseq [61], PICseq [62], and ATACseq [63]) enable transcriptomic, proteomic and epigenetic data on individual and physically interacting cells. Multiplex and imaging and spatial transcriptomics platforms describe the interplay between immune cells, stroma, and cancer cells [64–70]. Analysis at this level of both pre- and post-treatment samples allows for an extremely precise look at the dynamics of immune cell subsets critical to inducing anti-tumor response.

While immunotherapy trials employing standard post-treatment biopsies can provide tissue for limited analysis such as focus on a limited panel of biomarkers, neoadjuvant and window approaches offer the benefit of large surgical tissue specimens that can be compared to treatment-naïve tumor biopsies, amplifying the effects of technological innovations. This wealth of substrate allows


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for both deeper and more comprehensive characterization of the effects of checkpoint blockade on tumor cells, the immune infiltrate, intratumoral stroma, and the spatial interface between these compartments. In one example, a recent small (15 patients) Phase I study examined neoadjuvant cabozantinib and nivolumab for hepatocellular carcinoma, including patients outside of traditional resection criteria. In addition to allowing 12/15 of the patients to achieve negative margin resections, the biospecimen analysis allowed for insight into the mechanisms of this effect; in-depth profiling demonstrated an enrichment in effector T cells, as well as tertiary lymphoid structures, $CD138^{+}$ plasma cells, and a distinct spatial arrangement of B cells in responders compared to non-responders [71]. These findings, specifically differentiating responders and non-responders, have been recapitulated in subsequent neoadjuvant studies in HCC [59,60]. This effectiveness of biospecimen analysis in pre-surgical trials may even be amplified by the fact that traditional surgical candidates may be more likely to have smaller, less-heterogeneous tumors and more intact immune systems than patients with metastatic disease [72]. In this (relatively) simplified setting, dynamic changes induced by therapy and mechanisms of action may be less confounded and more amenable to characterization.

Furthermore, the tissues available for analysis after surgical resection are not limited to a single location within a sampled tumor. In addition to multiple tumor regions, resected materials often also include associated non-tumor stromal tissues and draining lymph nodes in addition to peripheral blood samples. This breadth of biospecimens available for analysis enables a more comprehensive understanding of the tumor immune macro and microenvironment. Head and neck cancer trials examining pre-surgical immunotherapy have also highlighted the opportunities for correlation of dynamic tumor tissue findings with circulating tumor-DNA and cell free DNA in window trials. A recent study examining preoperative sitravatinib and nivolumab in patients with oral cavity squamous cell carcinoma demonstrated that cell-free DNA dynamics correlated with clinical and pathological response, and concurrent tumor immunophenotyping and scRNA-seq analyses revealed differential changes in the expression of immune cell populations between the treatment groups [73]. Further exciting immunotherapy window trials are ongoing in not only head and neck cancers (NCT03238365, NCT03618654, NCT03784066, NCT03906526), but also in breast cancers (NCT03594396), cervical cancers (NCT04630353), and even malignant pleural mesothelioma (NCT02707666), as well as many others not yet reported.

## PUTTING PATIENTS FIRST

This deep dive into tissue analysis facilitates maximizing knowledge generation from each patient exposed to immunotherapy on a clinical trial. Neoadjuvant trials (and to an even greater extent window trials) employ a short, targeted intervention. Although registrational Phase III trials require large numbers even in the neoadjuvant space, early phase neoadjuvant trials offer the opportunity to restrict interventions to a small number of patients with maximal biospecimen analysis. This contrasts with classical Phase II trials with clinical efficacy endpoints which require large numbers of patients, and without the robust pharmacokinetic and mechanistic data generated by in depth tissue analysis, it is unlikely that the bulk of these patients will derive any clinical benefit from participation.

Erlotinib, a targeted tyrosine kinase inhibitor now used to treat patients whose advanced lung cancers harbor an EGFR mutation, may be used as a case study in this concept. The drug was initially developed with the intuitively logical but unfortunately incorrect rationale that it would be effective in patients with lung cancers harboring high levels of EGFR expression. Following exciting early data during which no predictive biomarker

was defined, the drug was originally approved by the FDA in 2004 for all advanced lung cancer patients with a randomized clinical trial demonstrating response rates less than 10% [74,75]. It was only after approval, when in 2016 the indication was modified to include only those patients in whom an EGFR mutation is present. This modification was based on analysis of Phase III trials demonstrating no benefit of the drug in hundreds of patients without the mutation, and only after patients without the activating mutation had been treated with the drug for over 10 years [76]. This is not to criticize these Phase III trials; they were well designed with the best available information at the time and there is no guarantee that testing in small patient numbers in the neoadjuvant setting would have identified the molecular basis for sensitivity to EGFR inhibition. However, a better mechanistic understanding from rigorous translational science in small populations may have allowed these trials to be designed with more advantageous inclusion criteria. Of note, this paradigm would require a reframing about the ways in which we determine activity in early phase clinical trials, as the temptation to overlay long term clinical outcomes without close attention to correlative science has the potential to lead to the dismissal of drugs with great activity whose benefit will not eventually be the most robust in the neoadjuvant setting. Here again erlotinib serves as an apt example, as despite the drug's clear utility in the advanced disease setting, a Phase II trial of neoadjuvant erlotinib failed to demonstrate distinct survival benefit when compared to neoadjuvant chemotherapy. Still, a better elucidated target population increases the yield for the large amount of financial and institutional resources necessary to run large scale clinical trials. It also prevents patients from wasting time they cannot spare in trials from which they cannot hope to benefit.

The nimbleness of design in neoadjuvant and window of opportunity trials by virtue of small sample sizes and rapidly attained pathologic endpoints may enable other innovations. In these platforms, drug repurposing is far more feasible as mechanistic data generated by analysis of surgical specimens can generate rationale for antineoplastic activity in a drug previously intended for another indication. This can then be turned around for testing in a small population of patients in whom the mechanistic insights generated in preliminary or preclinical data suggest the highest probability of benefit, either alone or in combination with immunotherapy.

## PITFALLS & PERILS

To be sure, neoadjuvant and window trials are not an unequivocal panacea. The correlative science involved in maximizing the utility of these studies is expensive, and current funding mechanisms are not always set up to allow for this robust analysis. Additionally, although analysis of pre- and post-surgical samples can give us a great deal of information about a drugs mechanisms, it does not obviate the need for larger trials examining clinical outcomes in different patient populations, since the pharmacodynamics of a drug or its in vivo clinical activity may be different across different disease stages with inherent differences in clonal diversity or vascularization. Moreover, many clinicians may be concerned about the risk of disease progression prior to surgery or theoretical possibilities of increased inflammation at the time of surgery. Thankfully, large neoadjuvant trials have demonstrated this approach does not result in a large percentage of patients missing the chance at curative intent surgery – indeed this brief pause may also represent a biological window to reveal micrometastatic disease that would save a patient with metastatic disease the burden of surgery—and multiple large trials have reported no significant increase in surgical complications [49,56,57,77,78].

## THE ROAD AHEAD

The implementation of a platform for conducting neoadjuvant and window trials


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allows for rich collaboration across disciplines within the cancer institute and enables all team members to investigate the dialectic relationship between therapeutics, cancer cells and a patient's immune micro and microenvironment. Several crucial barriers to success in optimal neoadjuvant platforms exist and must be addressed in order to get the most out of these trials.

The technologies for single-cell phenotyping generally require high-volume samples of fresh tissue to be available. Addressing this need starts even before the pre-treatment biopsy, when multidisciplinary teams must coordinate, with radiologists to identify optimally accessible sites, and interventionalists from teams as varied as GI endoscopists, pulmonologists, and interventional radiologists available to sample from the agreed-upon tumor location. Large core biopsies must be obtained, and commonly performed fine-needle aspirations are unlikely to be sufficient. At the time of resections, trial leaders from the surgical teams must coordinate procedural scheduling and sample delivery closely with the institutional pathology team and members of the research lab, to avoid processing delays which might limit sample quality. Given limitations in tissue preservation, this factor currently prohibits many smaller institutions without access to on-site analysis from participating in this paradigm.

In addition, the traditional designs used for chemotherapy will have to be adjusted. Alongside the scientific and pathologic endpoints described above, clinical endpoints will need to be creatively assessed. RECIST criteria for disease response, while traditionally used for chemotherapy and targeted therapy trials, does not always describe immune response well, and immunotherapy specific iRECIST criteria will need to be correlated with pathologic outcomes if imaging assessment is to be included [59,60,79].

Although the resources and efforts required are substantial, the rewards are unparalleled; if platforms for robust neoadjuvant and window trials can be established, the benefit will be a system which allows for a precision approach to the development and testing of novel therapeutics. By looking as closely as possible at the effects of each intervention, we more rapidly advance our understanding of the diseases we treat and maximize the prospect of benefit to patients enrolled in clinical trials.

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INTERVIEW

# Immunotherapy questions in melanoma

**David McCall,** Editor, *BioInsights*, **speaks to Michael Postow,** Chief of the Melanoma Service, Associate Attending Physician, Memorial Sloan Kettering Cancer Center.

**MICHAEL POSTOW** is the Chief of the Melanoma Service and an Associate Attending Physician at Memorial Sloan Kettering Cancer Center. He was involved in the clinical development of the nivolumab + ipilimumab combination and led the first randomized trial of combination immune checkpoint inhibition, resulting in its FDA approval in 2015. He has written papers on immunotherapy toxicities and combining systemic immunotherapies with other treatments such as radiotherapy. He enjoys skiing, outdoor activities, and trying to keep up with his two toddlers.

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**Q** What are you working on right now?

**MP:** My group and I are tackling a few major questions in melanoma. Personally, I have been working on answering the question of how much ipilimumab in combination with nivolumab we have to give patients. We have recently completed a study in which we gave

patients two doses instead of the usual four doses of treatment, which was published in the *Journal of Clinical Oncology.*

In addition, we are running a new neoadjuvant study prior to surgery in which we are testing a single dose of combination immunotherapy. One of the major questions we are addressing is how much combination immunotherapy patients need.

The other area of interest is understanding how to better image patients' immune systems whilst they are undergoing immunotherapy treatment. Hopefully, in the future we could use imaging modalities such as novel PET scan techniques to better modify and adapt our treatments in patients with advanced cancers.

**Q** What are the potential mechanisms of resistance for PD-1 checkpoint inhibitors in melanoma, and what are the possible routes around them?

**MP:** For the immune response to work, you need to have an activated T cell enter a tumor and kill it. Every step along the way of a T cell entering a tumor could pose a problem where the immune response goes awry. At each of these nodes, there are studies looking at why that step of an immune response does not work and why that patient might be resistant.

For example, one potential mechanism of resistance is that the T cells might be unable to get into the tumor. It could also be that the T cells cannot kill the tumor, either because the T cells themselves are dysfunctional and unable to execute the killing processes, or the tumors themselves are more resistant and inherently able to block the effects of otherwise adequate T cells. It may be that the tumor cells are not recognized by the T cells, if they can hide from an immune response.

In an effective immune response against a cancer, so many things have to go right. There are many steps along this cascade of recognizing tumor cells as something that needs to be killed and then actually killing them that can go wrong and cause resistance. New drugs are being tested in multiple different nodes of this immune response to see if the efficacy of existing immunotherapy can be improved and reverse resistance to immunotherapy.

**Q** What would a clinical trial designed to answer questions of resistance in melanoma patients look like?

**MP:** When designing studies to address mechanisms of resistance, it is important to understand what kind of resistance we are talking about. There is

"New drugs are being tested in multiple different nodes of this immune response to see if the efficacy of existing immunotherapy can be improved and reverse resistance to immunotherapy."


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primary resistance, where the tumors never respond at all to treatment, and there is secondary resistance, where initially there is a benefit to treatment but the tumors subsequently get worse. There is a published piece from the Society for Immunotherapy of Cancer describing the different forms of resistance as they may pertain to clinical trial design of new agents in immunotherapy-resistant melanoma. For example, a test of a novel agent in primary refractory melanoma may resemble a situation where a patient with growing or new tumors on a single agent checkpoint inhibitor then receives a novel agent added to PD-1 to see if the growing tumors would ultimately stabilize, or ideally, shrink.

To sum up, the open questions that are relevant for clinical trial design are: how do you define the tumor growth, and how long do you wait for your treatment with PD-1/PD-L1 before you declare progression?

**Q** Is what we learn in melanoma applicable to other cancers? If so, how, and where?

**MP:** Melanoma has been the poster child for the development of immunotherapy for many reasons. The first insights into the fact that the number of mutations is relevant for immunotherapy responsiveness arose within melanoma, and this has led to multiple other cancers with high tumor mutation burden responding better to checkpoint inhibition. We are also developing cellular therapy in melanoma – ways in which cells can be administered to patients with solid tumors that can lead to responses were discovered in melanoma, and are now being extended to other solid tumors.

Melanoma is a type of cancer where immunotherapy often works well, so we have learned a lot of scientific principles that we have been able to carry forward to help patients with other types of cancer. Moving forward, we, as a melanoma field, plan to continue that type of leadership for immuno-oncology.

**Q** What does data obtained so far tell us about future I-O applications, specifically in the neoadjuvant and adjuvant settings in melanoma?

**MP:** Neoadjuvant and adjuvant are quite different, but their principle is the same. We want to treat patients that have earlier stages of melanoma to prevent recurrences and stage 4 disease, whether it is in the neoadjuvant setting prior to surgical resection of high-risk disease, or in the adjuvant setting administering checkpoint blockade after initial surgery to prevent recurrence.

In terms of data, the main themes emerging are that with neoadjuvant immunotherapy, whether it is one dose of single agent PD-1 or more commonly, nivolumab plus ipilimumab, there is a very high rate of complete pathologic response in patients after the tumors are resected. The rate of complete pathologic response (or any immune therapy response) in tumors when they are resected from neoadjuvant studies is correlated with longer term relapse-free survival.

In neoadjuvant trials, we can give very effective immune therapy treatment pre-surgery and remove the tumor. What happens in the tumor gives a good indication of the ultimate likelihood of recurrence in the patient. It is the future in terms of how to modify: a few doses of treatment are given, the tumor is removed, and based on what happens in the tumor, one could think about future ways for modifying treatment in the adjuvant setting. We have not got data on this yet, but it is where the neoadjuvant trial landscape is heading.

In terms of adjuvant treatments, immune checkpoint-blocking antibodies, namely nivolumab and pembrolizumab, have both shown a recurrence-free survival benefit. We await longer term data to see if an overall survival benefit is seen.

It is encouraging that recurrence-free survival has been improved with checkpoint inhibition. Very recently, data for adjuvant treatment with pembrolizumab in lymph node-negative, high-risk stage 2 melanoma has also shown recurrence-free survival. We are seeing this migration of immunotherapy earlier in disease. Trying to weigh the pros and cons of treating patients in those settings is an active area of research, especially as we develop new biomarkers to see who really needs this treatment and when.

**Q** Where do you see potential in emerging immunotherapy modalities beyond PD-1 in melanoma?

**MP:** One important area that started in melanoma but is being tested in many different cancers beyond checkpoint inhibition are T cell redirecting antibodies. In melanoma, there is a new drug called tebentafusp, which redirects T cells to gp100-expressing tissue. There have been great results recently for patients with advanced uveal melanoma as tebentafusp improved overall survival in a Phase 3 study and recently was FDA approved as the first FDA approval ever for advanced uveal melanoma. This general strategy of T-cell redirection will hopefully be highly effective in the long-term not only in uveal melanoma patients, but targeting many different antigens. This may be a way to circumvent the problem of primary resistance in patients to checkpoint inhibitors, where you do not have T cells in the tumor microenvironment. You could use an agent like this, for example, to bring T cells into the tumor microenvironment, before disinhibiting those T cells with checkpoint inhibition.

Cellular therapy is another huge field, which includes tumor infiltrating lymphocyte therapy where you harvest the tumor, extract the lymphocytes, expand them, and administer them with lympho-depleting chemotherapy and IL-2. Other types of autologous T cell engineering include taking T cells from peripheral blood and genetically engineering them to be better melanoma fighting cells before reinfusing them back into patients. We are not quite at the stage where CAR T cells are ready for primetime in melanoma. However, there are clinical trials testing different CAR-T cells in this setting.

**Q** What future trends do you expect to see in combination therapy development within your field?


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**MP:** We have done many combinations of checkpoint inhibitors thus far, such as CTLA-4 and PD-1. In particular, there have been a lot of combinations with novel agents with PD-1 and PD-L1 in refractory melanoma. I am very impressed with recent data for upfront combination of LAG-3 inhibition (relatlimab) with PD-1 inhibition (nivolumab). Relatlimab and nivolumab have set a new standard in treatment with PD-1 inhibition of treatment-naïve metastatic melanoma with a positive Phase 3 study.

One questions whether PD-1 monotherapy will remain the backbone for future combinations, or if we are considering PD-1 plus LAG-3 as a backbone, and now building triplets. Ipilimumab-PD-1 combination in melanoma has many side effects, so you would have to be very careful in considering dosing in triplets and beyond in that context.

So a big trend is rethinking the PD-1 monotherapy backbone, and another is combining immunotherapy drugs with completely different mechanisms of action. I gave the example previously of T cell redirecting antibodies plus checkpoint blockade or T cell redirecting antibodies plus other inhibitors of immunosuppressive cells within the tumor microenvironment – myeloid cells, regulatory T cells, and others.

**Q** Where specifically is innovation in clinical trial design most required in IO for you?

**MP:** We need better understanding of what we are looking at on patient scans when we give I-O treatment. We are still stuck observing tumors and seeing if they are growing or shrinking on scans. We need better imaging modalities for those patients who have what appear to be stable tumors, or who have tumors that are growing only a small amount, in order to know if immunotherapy is working. We have learned from neoadjuvant clinical trials that when we give checkpoint inhibition, tumors can grow and still have a pathological response. We need new imaging modalities to know whether we should change treatment, if should we escalate or de-escalate treatment. It is not feasible to do this through biopsies, because it is very difficult to biopsy patients in treatment sequentially. The biopsies are limited to only being one specific location of one tumor at one time.

I am hopeful that we can get imaging to the point where we will be able to see what is happening based on our interventions in a pharmacodynamic way, such that we can adopt combinations of treatment in an ongoing fashion, or switch treatment earlier.

"I am hopeful that we can get imaging to the point where we will be able to see what is happening based on our interventions in a pharmacodynamic way, such that we can adopt combinations of treatment in an ongoing fashion, or switch treatment earlier."

## Q What are your major goals and priorities in your work over the next few years?

**MP:** Personally, I want to characterize the safety and efficacy of one dose of combination checkpoint blockade with nivolumab and ipilimumab. We are currently doing a neoadjuvant study for this, and I would love to find a solution. If we can make some inroads into the PD-1 resistant melanoma, then we will also have come a long way. We do have studies testing novel drugs in combination with various PD-1 combinations in PD-1 refractory melanoma.

The other big area of interest in the field is how to better risk stratify patients in adjuvant or neoadjuvant settings for various degrees of combination therapies, so that we know who needs treatment in those spaces, and who we can observe safely. We need better understanding as to whether it is circulating tumor DNA or other characteristics in tumor microenvironments that affect responsiveness to adjuvant or neoadjuvant treatments.

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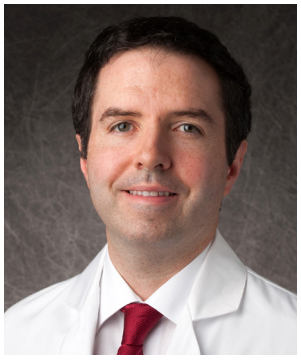
OPTIMIZING CLINICAL DEVELOPMENT STRATEGY FOR THE RAPIDLY EVOLVING I-O FIELD

SPOTLIGHT

INTERVIEW

# Expediting drug development & targeting early-stage disease: how is clinical trial design in immuno-oncology evolving?

**Roisin McGuian**, Commissioning Editor, Bioinsights, **speaks to Patrick Forde**, Director, Thoracic Oncology Clinical Research Program, Associate Professor of Oncology, Johns Hopkins



**DR PATRICK FORDE** is currently Co-Director of the Division of Upper Aerodigestive Malignancies at Johns Hopkins and also directs the multidisciplinary Thoracic Oncology Clinical Research Program. He has led development of a clinical-translational research program focused on the immuno-oncology of upper aerodigestive malignancies. Dr Forde's research examines the role of immunotherapy for mesothelioma and lung cancer and his work has led to the development of several ongoing Phase 3 trials. He leads international Phase 2 and 3 clinical trials of novel immunotherapy approaches for lung cancer and mesothelioma that are currently active in Europe, Asia and North America while also serving as principal investigator for the thoracic cancer immunobiology biospecimen repository at Johns Hopkins. He is focused on providing compassionate, state of the art care for his patients in conjunction with a team of oncology specialist nurses, nurse practitioners and dedicated staff.

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## Q Can you tell me a bit about your own background and current work?

**PF:** I originally trained as a medical oncologist in Ireland, moved to the US back in 2011, and have been at Johns Hopkins since then. I am currently director of our thoracic oncology research program, and co-director of our upper area digestive malignancies division, which encompasses head and neck cancer, esophageal cancer, lung cancer and other rarer chest malignancies.

We have a group of about 10 faculty members and 20 to 30 nurses, research team members, and other staff members involved in developing both clinical, translational, and basic laboratory research focused on those malignancies.

## Q What are your biggest priorities at the moment?

**PF:** To some extent it is a transitional time in terms of the next big set of clinical trials for patients with lung cancer. When I started training as an oncologist in 2007, there was a very standard treatment pathway for lung cancer. We had first-line platinum double chemotherapy, and then single-agent chemotherapy in the second-line setting, and not a lot else.

In the past 15 years there have been a lot of developments. We have seen this across tumor types as well – in melanoma we went from having little or no therapies to multiple approved novel immunotherapies, and some targeted therapies too. The same has happened in breast cancer and in other common tumors.

In the immunotherapy space we now have a backbone of PD-1 or PD-L1 antibodies which are very effective for a minority of patients, particularly with lung cancer, kidney cancer, or melanoma. But for the majority of patients they unfortunately still don't work as a single agent, although they may have additional efficacy in combination with chemo. We are looking at new ways to improve outcomes for those patients, either by adding novel immunotherapy agents to PD-1 as a backbone, or by looking at new forms of therapy, such as cellular therapy for solid tumors.

What I have been most involved in over the last few years is in moving therapies from metastatic or advanced cancer to early-stage disease, particularly in the neoadjuvant setting. Here at Hopkins we have been doing that in mesothelioma, lung cancer, and esophageal cancer, with some success.

I've been involved in developing PD-1 inhibitors – particularly nivolumab – in the pre-operative setting for lung cancer. I served as principal investigator for the CheckMate 816 study, which looked at combining chemotherapy with nivolumab and comparing that to standard chemotherapy prior to surgery for resectable stage 2 and 3 non-small cell lung cancers. The study was successful in increasing the pathological complete response rate from 2% to 24%. More recently the study has also shown positive results for event-free survival leading to FDA approval of neoadjuvant nivolumab with chemotherapy in March 2022.

We are starting to see these therapies move successfully from metastatic disease to earlier-stage disease, which is very welcome.


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## Q How is clinical trial design for novel immunotherapies evolving?

**PF:** We have seen a move from the traditional Phase 1 study with a 3+3 design, where we're looking for maximum tolerated dose, to a much more fluid design – particularly in the early Phase 1 and 2 studies.

One of the reasons for this is that with immune-based therapies we often don't reach a maximum tolerated dose, so we don't have cumulative toxicity by dose level. We have to make a choice based on pharmacodynamics, pharmacokinetics and efficacy signals as to whether we have reached the optimal dose before moving into a Phase 2 or 3 study. This has changed things significantly.

In Phase 2 trials, for example in lung cancer, we have relatively effective first-line therapies with either immunotherapy, targeted therapy, or chemotherapy. However, after that first-line space we don't really have a lot of effective therapies, and we have seen the development of platform trials in the second-line setting. These have had varying levels of success, there have been attempts to do platform trials where you have multiple different immunotherapy combination arms and the patients are randomized between the arms without selecting the patients by biomarker. Those trials have not been as successful.

More recently we are seeing biomarker-directed trials in the second-line setting. A good example is a study I've been involved in called HUDSON, which is led by AstraZeneca and looks at triaging patients who have had disease progression on PD-1 or PD-L1 antibodies and chemotherapy, and performing next-generation sequencing of their tumor as well as some immunohistochemical assays. Depending on whether the patient has a positive biomarker, they are moved into an appropriate arm of the trial. If there is no biomarker found then they are moved into a control arm. I think these types of design are more likely to yield clear signals as to whether these drugs should move into Phase 3 or not.

## Q What innovations in clinical trial design are most exciting you right now?

**PF:** In lung cancer we have been selecting patients for targeted therapy for several years based on next-generation sequencing, mostly in the first-line setting. We haven't been doing that as much in terms of immunotherapy. Moving towards doing that in advanced disease makes sense.

The other relatively novel designs – although they are not novel for all tumor types – are in the neoadjuvant space, where you have the option to give six to 12 weeks of neoadjuvant therapy, and that can be immunotherapy, targeted therapy, or even

"We have seen a move from the traditional Phase 1 study with a 3+3 design, where we're looking for maximum tolerated dose, to a much more fluid design – particularly in the early Phase 1 and 2 studies.."

chemotherapy. You have that early readout in terms of pathological response at the time of surgery when the tumor is removed. That can then be used to decide on post-operative therapy, and perhaps even incorporating s circulating tumor DNA (ctDNA) dynamics either during neoadjuvant therapy or post-operatively as a composite biomarker with pathological response.

These are two things that are potentially very helpful in terms of expediting drug development, both in advanced disease with the platform trials, and in early-stage disease where I think neoadjuvant designs have a lot of potential benefits.

## Q Where are you incorporating these novel designs into your own work?

**PF:** The areas where I have found these useful are in terms of developing novel concepts in earlier-stage disease. Particularly early adaptive designs based on pathological response – this is an area we have been looking into for many years. We have been focused mainly on early-phase trials, but we are starting to look at Phase 3 trials in this setting where we can triage patients to different arms post-operatively depending on pathological response, or even ctDNA changes.

One of my colleagues here at Hopkins, Valsamo Anagnostou, currently has a trial ongoing in lung cancer which is essentially a molecular response adaptive trial. In the first-line setting for lung cancer we have two choices for PD-L1-positive tumors: pembrolizumab monotherapy, or pembrolizumab plus chemotherapy. The particular design Dr Anagnostou has developed in conjunction with the Cancer Research Institute and the Canadian Cancer Trials Group (CCTG) is looking at that first couple of months of therapy. If the patient clears ctDNA on pembrolizumab monotherapy, they can potentially be randomized to either continue on pembrolizumab or add chemotherapy. This is a potential way of intelligently addressing the question of which patients need chemotherapy with immunotherapy and which patients can benefit from immunotherapy alone, using that clearance of ctDNA.

## Q What are currently the biggest challenges when designing I-O clinical trials?

**PF:** One of the challenges – and this is partly affected by the pandemic as well – is that the regulatory burden on academic health centers and clinical trials is significant. It seems to keep growing in terms of the number of case report forms to be completed; the number of documents involved in trying to get a trial open. This becomes more complex every year, and that is partly driven by regulatory authorities, and partly by companies and sponsors as well.

The other challenges are more general and not specific to clinical trials. Here in the US in particular, and indeed worldwide, retaining staff to be able to conduct the trials is a problem. This is something we need to address in our budgets, our salaries, and by exploring other options which may help, such as perhaps remote working.


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**Q** Turning specifically to patient selection and biomarker-driven studies, can you tell us about the current state of play?

**PF:** We are starting to see biomarker-driven studies for immunotherapy. We have seen them for many years for targeted therapies, but the use of biomarkers to select patients for immunotherapy is happening in the second and third-line settings, and we haven't seen it as much in the first-line setting for most tumor types. This is because the signals from the second line so far have been somewhat equivocal. However, I think it is potentially helpful.

One of the challenges in lung cancer, and probably across tumor types, is that when patients experience tumor progression on PD-1 or PD-L1 therapy, most clinical trials in the relapsed/refractory setting are not showing response rates of more than about 20%. Despite all the agents in development we have yet to discover the next PD-1/PD-L1-like molecule. But we are seeing a small number of patients in each trial showing a response. Trying to enrich for those patients, and trying to see which characteristics of the patient and the tumor are most likely to predict a response with a particular agent is very important.

I mentioned platform trials earlier, and I think we also have that opportunity in earlier stage disease with the neoadjuvant trials where we can do in-depth correlative analysis, because you obtain the tissue post-treatment as the time of resection.

In terms of the unique challenges that biomarker-driven trials pose, one of the biggest is actually the pre-screening and screening process for getting the patient on the trial. Generally these sorts of trials involve obtaining a tumor sample and analyzing that sample using either next-generation sequencing or other assays, before the patient can even be enrolled. That process can take anything from two to six weeks. Particularly in the relapsed/refractory setting where patients have had progression and previous treatment, that is a relatively long period of time.

"In terms of the unique challenges that biomarker-driven trials pose, one of the biggest is actually the pre-screening and screening process for getting the patient on the trial. Generally these sorts of trials involve obtaining a tumor sample and analyzing that sample using either next-generation sequencing or other assays, before the patient can even be enrolled"

Turning to the benefits they provide, one example is that in lung cancer docetaxel is the standard of care chemotherapy after prior treatment with platinum doublet chemotherapy and immunotherapy. It is very modestly effective, but relatively toxic, and that is a concern for patients in that setting. Biomarker-driven studies are attractive to patients because there is some indication that the treatment given in the trial is not just a generic treatment, but is being given based on a specific rationale for that patient and their tumor.

While they probably require more investment upfront in terms of testing and biopsies, in the longer run they can potentially expedite drug development in two ways – both by allowing good candidate drugs to

move forward to Phase 3, and also by providing an early negative signal. If the drug is really not showing activity even in the biomarker-driven cohort, I think that is a situation where that specific drug development program should be shut down early.

**Q** What further developments do you expect to see in the next five years? And what would be at the top of your own "wish list" for this space?

**PF:** It will be interesting to see how the trial design goes. I suspect controls, particularly in Phase 3 trials, are going to have to change. Up until very recently chemotherapy was still accepted as a control in the first-line setting in lung cancer. At least here in the US, recent suggestions from regulatory authorities are that chemotherapy will no longer be an accepted control in that first-line setting for Phase 3 trials. The new comparison would presumably be a PD-1 antibody or PD-1 plus chemotherapy. This is a relevant issue for anyone designing trials right now, as the bar for the control is going to be higher.

Another thing I can see towards the end of that five-year period is more widespread use of cellular therapies for solid tumors. At the moment they are confined to relatively few academic centers who have the capabilities to do those trials. If we are going to realistically move towards bringing these drugs to regulatory approval, then they are going to have to be more broadly available in the trial setting as well. That infrastructure will have to be built at more centers.

It is going to be a relatively highly selected population of patients that can do these trials, and the question for that population is whether they will be randomized trials in some way. For example, cellular therapy versus docetaxel – those are two radically different treatments. How do you realistically compare them? Will they be single-arm trials with a response rate as primary endpoint, which is what we've seen in targeted therapy? Those questions are still to be determined, but I can foresee that there will be a lot of discussion about that. For example, if you choose response rate as your primary endpoint for a second-line lung cancer trial with cellular therapy, what does that response rate need to be? One might imagine it would need to be 50% or more. Additionally, how durable does it need to be, and what will toxicity bar have to be to make it practical, both financially and physically?

In terms of things I personally would be interested in, I would like to see more therapies moving into early-stage cancers across tumor types. We have been relatively slow to do that in lung cancer, but we are finally starting to see it happen. The melanoma community have done that already several years ago with adjuvant and neoadjuvant immune checkpoint inhibitors, and we are also starting to see these now.

In lung cancer we have a whole host of targeted therapies which are approved in metastatic disease, but we don't really have data for them in the early stages. There are also novel immunotherapies such as TIGIT and other agents which may soon become a standard in advanced disease, and we have to look at how we move them quickly into early-stage disease. I would like to see a focused effort on improving outcomes for our earlier-stage disease patients.

**Q** Looking again at that five-year timeline, what will you personally be focused on?


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**PF:** I will be working on developing our programs at Hopkins, and also our collaboration with centers both nationally and internationally.

Another priority for me, and anyone involved in oncology, is making sure the next generation of investigators are getting training and being made enthusiastic about a career in oncology and clinical investigation. In some ways it is a good time for oncology – because of all the developments in terms of immunotherapy and targeted therapy over the past 10–15 years, the science and the clinical developments have been in both the science news and the popular media. For medical students and science graduates it is a very topical area to get involved in. I think we will have the cream of the crop in terms of investigators and scientists who are interested in going into cancer medicine and science. Providing an appropriate mechanism for them to be trained and a welcoming environment with a diverse group of team members is very important.

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## AUTHORSHIP & CONFLICT OF INTEREST

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IMMUNO-ONCOLOGY INSIGHTS

OPTIMIZING CLINICAL DEVELOPMENT STRATEGY FOR THE RAPIDLY EVOLVING I-O FIELD

SPOTLIGHT

COMMENTARY

# Beyond PD-L1: novel predictive biomarkers for adjuvant immunotherapy in renal cell and urothelial carcinoma

Jason R Brown, Aarthi Rajkumar & Jorge A Garcia

Within the past year, immune checkpoint inhibitors have been approved by the Food and Drug Administration for adjuvant treatment of both renal cell carcinoma and urothelial carcinoma following definitive surgery. The landmark clinical trials on which these approvals were based stratified patients by PD-L1 status. Unfortunately, this biomarker inadequately distinguished the patients who would receive benefit, given PD-L1 negative responders. Combining additional novel biomarkers with PD-L1 could potentially enhance its predictive power. Such potential biomarkers that have been studied include tumor mutation burden, neoantigens, immune microenvironment components, microbes inhabiting the gastrointestinal and urinary system, metabolic byproducts, gene expression signatures, and non-invasively detected circulating tumor DNA. Machine learning can synthesize these biomarkers with histopathologic, radiographic, and clinical data to optimize prediction of response to immunotherapy. This commentary reviews recent scientific advances in developing predictive biomarkers and suggests potential applications to adjuvant immunotherapy in renal cell and urothelial cancer.

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## INTRODUCTION

Immune checkpoint inhibitors have revolutionized treatment of several cancers [1]. For renal cell carcinoma and urothelial carcinoma, initial approvals were in the metastatic setting. In metastatic renal cell carcinoma, combinations of immunotherapy with tyrosine kinase inhibitors and combinations of anti-CTLA4 and anti-PD-1 immune checkpoint inhibitors are approved treatments [2]. Meanwhile, in metastatic urothelial carcinoma, single agent immunotherapy has been approved as first-line therapy for platinum ineligible patients and as maintenance or second-line therapy following platinum-based chemotherapy. Combinations between immune checkpoint inhibition and conventional cytotoxic chemotherapy did not significantly improve urothelial carcinoma outcomes in clinical trials [3].

In 2021, immune checkpoint inhibitors were approved by the United States Food and Drug Administration for the first time for use as adjuvant therapy in renal cell and urothelial carcinoma [4,5], although previously, adjuvant pembrolizumab had been in melanoma [6]. The KEYNOTE-564 Phase 3 study evaluated the use of pembrolizumab for one year following nephrectomy in renal cell carcinoma with a clear cell component and high recurrence risk. This study reported a 32% risk reduction in recurrence or death with pembrolizumab compared to placebo (HR 0.68, 95% CI 0.53–0.87, p = 0.002) [7]. Similarly, the CheckMate 274 Phase 3 clinical trial evaluated 1 year of nivolumab following radical surgery for urothelial carcinoma, including bladder cancer and upper tract disease. This study found a 30% risk reduction in recurrence or death with adjuvant nivolumab compared to placebo (HR 0.70, 98.22% CI 0.55–0.90, p < 0.001) [8]. While these are the first adjuvant trials to report positive results, several ongoing trials including PROSPER, CheckMate-914, RAMPART, IMmotion-010 in renal cell carcinoma and AMBASSADOR in urothelial cancer, are also evaluating the benefit of adjuvant immune checkpoint inhibition.

Because nivolumab and pembrolizumab target the PD-1/PD-L1 pathway, PD-L1 positivity by immunohistochemistry has been considered as a potential biomarker of response but remains controversial in renal cell and urothelial carcinoma. In subgroup analyses of landmark trials in metastatic disease, PD-L1 expression has been unable to select all responders to immune checkpoint inhibitors [9,10]. Potential issues with PD-L1 biomarker assays include intratumoral heterogeneity of PD-L1 expression and differences in how tumoral and stromal cellular populations are measured [10–12]. Therefore, results can be dependent on the specific assay used (Figure 1). Characteristics intrinsic to the tissue specimens being analyzed may also affect PD-L1 scoring. Older formalin fixed paraffin embedded specimens have been correlated with lower PD-L1 scores compared to more recently obtained samples. Specimens with fewer than 100 viable cells also were more likely to have a lower PD-L1 score [13].

FDA approvals for adjuvant pembrolizumab in renal cell carcinoma and adjuvant nivolumab in urothelial carcinoma do not restrict usage based on PD-L1 status, as many PD-L1 negative patients appear to derive benefit as well [4,5]. PD-L1 expression was studied in subgroup analyses of both trials that led to the FDA approvals. In KEYNOTE-564, amongst PD-L1 positive (Combined Positive Score (CPS) ≥ 1) patients, hazard ratio for recurrence or death significantly favored pembrolizumab over placebo (HR 0.67, 95% CI 0.51–0.88). Amongst PD-L1 negative patients (CPS < 1), a trend toward improved survival was also observed between pembrolizumab and placebo (HR 0.83, 95% CI 0.45–1.51) [7]. The CheckMate 274 trial defined positive PD-L1 expression as expression level of 1% or more tumor cells using the Dako PD-L1 IHC 28-8 pharmDx immunohistochemical assay. According to the study, 39.4% of patients were PD-L1 positive, and a subsequent analysis measuring expression by CPS found that 88.6% of patients were PD-L1 positive [14]. While a significantly higher proportion of PD-L1 positive patients


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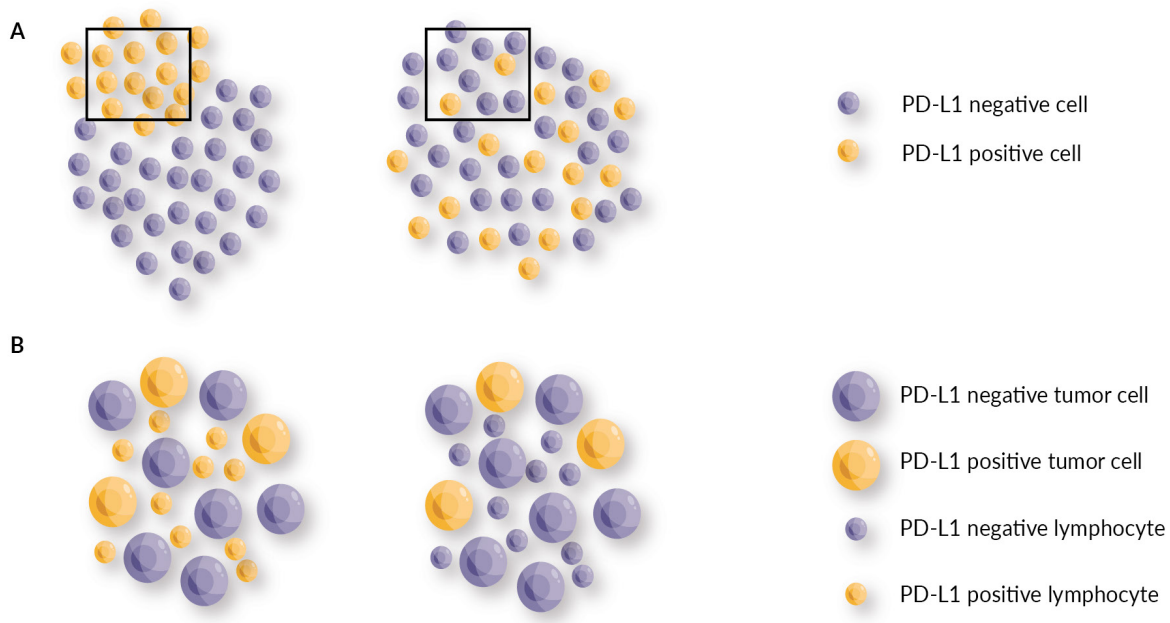
FIGURE 1

**Variability in PD-L1 scoring.**




(A) While the left and right diagrams have equal numbers of PD-L1 negative (purple) and PD-L1 positive (yellow) cells, within the selected field of view (black box), there is a stark difference in PD-L1 positivity due to the unequal distribution of PD-L1 positive cells on the left. (B) These two diagrams show equal tumor proportion scores (TPS), as calculated by number of PD-L1 positive tumor cells divided by total number of viable tumor cells. The combined positive score (CPS), as calculated by total number of PD-L1 positive cells, including immune cells, divided by total number of viable tumor cells is higher on the left than the right. Therefore, based on scoring system used, PD-L1 scoring would be remarkably different.

exhibited disease-free survival at 6 months with nivolumab compared to placebo (HR 0.55, 98.72% CI 0.35–0.85, $p < 0.001$), subgroup analysis shows improved survival with nivolumab regardless of PD-L1 status when measured by the initial assay [8].

Since PD-L1 expression is an imperfect predictive biomarker, additional biomarkers are needed to better determine the subset of patients that would benefit from immunotherapy. In the adjuvant setting, both prognostic and predictive biomarkers are important. Prognostic biomarkers can select the patients at highest risk of recurrence following definitive surgery and therefore would most benefit from adjuvant therapy. Defining this high-risk population could prevent unnecessary exposure to potential immune related adverse events in patients who would not benefit. This would also ease the financial toxicity of unnecessarily administering expensive drugs like immune checkpoint inhibitors. Moreover, a comprehensive predictive biomarker would improve overall response to treatment by selecting patients most likely to respond to therapy. This would also protect likely non-responders from toxicity and allow for other potentially more effective treatments for this population. Often, predictive biomarker studies conducted in the metastatic setting can be extrapolated to the understand adjuvant treatment response. One potential pitfall of this strategy is biological differences between primary and metastatic disease, and therefore further validation is necessary in the adjuvant setting. Studies in the neoadjuvant setting and window of opportunity studies can also be used to better understand the science behind the disease and identify biomarkers in a shorter period of time, although translation to the adjuvant setting may be challenging as well. The science behind immunotherapy resistance is complex and not entirely understood, however, this field is rapidly evolving. In this commentary, we review recent scientific developments in novel

biomarkers that could enhance prediction of response to immune checkpoint inhibition in the adjuvant setting for renal cell and urothelial carcinoma.

## TUMOR MUTATION & NEOANTIGEN BURDEN

High tumor mutation burden (TMB-H), defined by greater than or equal to 10 mutations per megabase (mut/Mb), is an FDA-approved indication for use of pembrolizumab in treatment of metastatic or unresectable solid tumors [15,16]. This approval is based on the KEYNOTE-158 trial, which demonstrated relatively improved survival in patients who exhibited this biomarker [17]. Initial studies of TMB were conducted using whole exome sequencing on tumor and matching normal DNA [18–20]. More commonly, targeted sequencing is used in clinical practice due to its relative cost effectiveness and quicker turnaround for results [21–23]. Initial correlative studies that found benefit of immunotherapy in TMB-H patients used various thresholds for TMB-H, including greater than 100 total mutations [24,25], 200 total mutations [20], 9.9 mut/Mb [23], and 9.65 mut/Mb [26]. The latter two values, both near the approved threshold of 10 mut/Mb, correspond to median TMB measured using targeted sequencing in non-small cell lung cancer and urothelial cancer, respectively, in two studies [23,26]. TMB-H has also been associated with environmental factors, including smoking, a known risk factor for renal cell and urothelial carcinoma, and ultraviolet light exposure [27,28]. At present, TMB-H is not an approved indication for use of immunotherapy in the adjuvant setting. One real world study found a response in all eight TMB-H patients treated with adjuvant immunotherapy [29], however adjuvant data remains limited.

Contrary to expectation given response to immunotherapy, renal cell carcinoma notoriously harbors a low tumor mutational load. One study of mutational burden across multiple primary cancers, including 214 clear cell renal cell carcinoma cases, found that average TMB in renal cell carcinoma was 1.1 mut/Mb. Only one specimen from this study exhibited more than 10 mut/Mb [30]. Analysis of the most common mutations found that single nucleotide polymorphism occurred more frequently than insertions and deletions (indels), with C>T being most common [31]. Another study showed that renal cell carcinoma had higher rate of indel mutations and deletions than any other cancer, with more than double the overall median. This study also showed that indels were more likely to produce high-binding affinity neoantigens due to possibility of creating a novel open reading frame [32]. Although this data on neoantigens appears promising, neoantigen production has not yet proven a reliable predictive biomarker of response to immunotherapy. In exploratory analysis of IMmotion-150, a Phase 2 study of atezolizumab and bevacizumab in metastatic clear cell renal cell carcinoma, tumor neoantigen burden, indel load, and TMB were measured but none of these variables correlated with improved response to atezolizumab [33]. Similarly, correlative analyses of other landmark clinical trials in renal cell carcinoma have not found an association between high TMB and response to immune checkpoint inhibition [34].

Contrary to renal cell carcinoma, TMB is relatively high in urothelial carcinoma compared to most other cancers, with a reported median of 7.2 mut/Mb [27,30,35,36]. Moreover, TMB-H has correlated with enhanced response to immune checkpoint inhibition in exploratory analyses from clinical trials in metastatic urothelial carcinoma [37–39]. In non-muscle invasive bladder cancer, TMB-H also associated with response to BCG immunotherapy [40]. Nonetheless, whether these findings translate to predicting response to adjuvant immunotherapy remains unclear. In exploratory analysis of the Phase 2 ABACUS trial of neoadjuvant atezolizumab in bladder cancer, TMB-H was not significantly predictive [41]. Another study measured mutational and neoantigen load following chemotherapy in specimens from patients with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resistant muscle-invasive bladder cancer but found no enhancement in post-chemotherapy bladder cancer compared to pre-chemotherapy baseline [42]. Neoantigen load has also been evaluated in muscle-invasive bladder cancer. In one study, increased numbers of neoepitopes significantly inversely correlated with recurrence at two years following cystectomy [43]. Therefore, these patients may not derive expected benefit from adjuvant immunotherapy due to better outcomes at baseline. Further studies are necessary to determine the value of TMB and neoantigen load in predicting response, as well as potentially combining these markers with PD-L1. While TMB-H and PD-L1 are not significantly correlated in most cancers [44], both TMB-H and high PD-L1 expression often independently correlate with benefit to immune checkpoint inhibition, indicating an additive benefit to incorporating both biomarkers [22].

## IMMUNE MICROENVIRONMENT

The immune milieu that surrounds a tumor, including lymphocytes and macrophages, plays an important role in response to immunotherapy. Immune checkpoint inhibitors recruit CD8 positive cytotoxic T cells, and infiltration is often associated with better prognosis [45,46]. One study showed relatively higher levels of T-cell infiltration in kidney cancer compared to other cancers [47]. In renal cell carcinoma, however, infiltration of CD8-positive T cells often paradoxically correlated with worse prognosis [48,49]. Conversely, other lymphocyte markers, including CD3 and CD20, may portend a favorable prognosis [50]. In addition to their prognostic value, tumor infiltrating lymphocytes location and tumor penetration may also predict response to immune checkpoint inhibition. Density of CD3-positive T cells at the tumor center and CD8-positive T cells at the invasive margin has correlated with response to nivolumab [50]. Another important measure of TILs is their contribution to tumor development, differentiating bystander TILs from 'exhausted', or dysfunctional TILs. Markers of exhausted TILs include Tim-3, Lag-3, and TIGIT [51]. Analysis of the Phase 2 CheckMate-010 study of nivolumab found that more T cells with a $CD8^+PD\text{-}1^+Tim\text{-}3^-Lag\text{-}3^-$ phenotype predicted immune progression-free survival and overall response following nivolumab treatment [52]. Exploratory analysis of IMmotion-150 discovered a T-effector gene signature associated with CD8-positive T-cell infiltration, which positively correlated with response to immunotherapy [33]. Whether this positive correlation between tumor infiltrating lymphocyte infiltration and response to immunotherapy carries over to the adjuvant setting remains to be seen. Higher levels of CD8-positive tumor infiltrating lymphocytes were observed in primary compared to paired metastatic renal cell carcinoma [53], indicating great potential for this predictive biomarker. In primary renal cell carcinoma, morphologic assessment of tumor infiltrating lymphocytes has been associated with higher rates of recurrence [54]. A population of $CD8^+PD\text{-}1^+Tim\text{-}3^+Lag\text{-}3^+$ tumor infiltrating T cells on nephrectomy specimens was also found to be poorly prognostic and could determine a subset of patients who may benefit from adjuvant immunotherapy [55].

In addition to T cells, other immune cells have been identified in the renal cell carcinoma microenvironment. An immune atlas of the clear cell renal cell carcinoma microenvironment found that while T cells were the most common immune cell population, there was a sizeable proportion of myeloid cells (31%), with 17 distinct macrophage populations identified [56]. Tumor-associated macrophages (TAMs) in clear cell renal cell carcinoma are predominantly of the M2 anti-inflammatory phenotype, which is associated with recruiting regulatory T cells and poor prognosis [57–59]. In two metastatic clear cell renal cell carcinoma studies, higher density of M2 TAMs in the microenvironment correlated with improved response to immune checkpoint inhibition [60,61].

Single-cell sequencing of renal cell carcinoma tumors from immune checkpoint naïve and treated patients also associated tumor associated macrophages with immunotherapy response [62]. Tertiary lymphoid structures, which are organized lymphoid formations consisting primarily of B cells, T cells, and dendritic cells, have also been identified in renal cell carcinoma. In the metastatic pre-operative setting, presence of these structures correlated with improved response to immunotherapy [63]. Future studies of adjuvant immunotherapy for renal cell carcinoma would therefore benefit to include all immune cell populations, rather than just CD8 positive T lymphocytes. In addition to the presence and quantity of various immune cells, spatial arrangement and density are also important to fully characterize the immune microenvironment [64].

In urothelial cancer, the presence of tumor infiltrating lymphocytes (TILs) generally carries a positive prognosis. In muscle-invasive bladder cancer, patients with intratumor CD8 positive as well as CD3 positive T lymphocytes have improved survival rates [65–67]. High stromal TIL levels have also been correlated to better response in upper tract urothelial carcinoma [68]. TIL infiltration also predicts favorable response to immunotherapy [69]. In analysis of the ABACUS trial, in which muscle-invasive bladder cancer patients were treated with neoadjuvant atezolizumab, tumors with higher proportions of CD8-positive lymphocyte infiltration exhibited higher rates of pathologic complete response and excellent one-year relapse free survival rates. Tumors that responded to immunotherapy also displayed increased intraepithelial levels of CD8 [41]. A $CD8^+$ T-effector immune signature also predicted response to immune checkpoint inhibition in analysis of the IMvigor 210 study [70]. In another study of muscle-invasive bladder cancer, expression of immune checkpoint genes including *PD-1*, *PD-L1*, and *CTLA-4* was enriched with high CD8-positive T-cell populations, which indicates that these may be complementary markers [67]. Therefore, the effect of residual TILs following neoadjuvant chemotherapy needs to be further studied and may play an important role in selecting optimal patients for adjuvant immune checkpoint therapy [71].

Other immune cell populations have also been studied in urothelial cancer. In urothelial carcinoma, fewer tumor associated macrophages were identified in TURBT specimens compared to radical cystectomy specimens, and specific macrophage populations were associated with worse outcomes [72,73]. Similarly, TAMs have been associated with worse response to immunotherapy. Correlative analysis of the IMvigor210 and CheckMate275 studies in metastatic urothelial cancer using single-cell RNA sequencing also implicated myeloid cells in resistance to immune checkpoint inhibition, although this finding was unrelated to M1 and M2 polarization [74]. Additionally, tertiary lymphoid structures have been identified in urothelial cancer and are enriched in muscle-invasive compared to non-muscle-invasive bladder cancer [75]. These structures have been identified in upper tract urothelial carcinoma as well [76]. The role of these tertiary lymphoid structures in urothelial cancer has been hypothesized to enhance adaptive immunity following persistent tumor-related antigen stimulation. Therefore, these antigens are more readily recognized and more effectively neutralized, however some studies have indicated that these structures may merely be bystanders of an immune response [75]. Regarding tertiary lymphoid structures as predictive markers of response to immunotherapy, one study found tertiary lymphoid structures in muscle-invasive bladder cancer to predict response to atezolizumab in analysis of specimens from the IMvigor210 study [77]. Moreover, *CXCL13* expression correlated with the presence of tertiary lymphoid structures in muscle-invasive bladder cancer. Expression was predictive of favorable response to immune checkpoint inhibition and therefore may provide a useful biomarker for adjuvant immune checkpoint inhibition [78].


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## MICROBIOME

Microorganisms that inhabit the gastrointestinal system have been shown to affect response to immune checkpoint inhibition in multiple cancers [79]. In renal cell carcinoma, a translational substudy of GETUG-AFU-26 NIVOREN analyzed microbiota from renal cell carcinoma patients treated with Nivolumab. Using whole genome sequencing, response to immune checkpoint inhibitors was predicted by analyzing diversity of stool composition [80]. Increased bacterial diversity also positively predicted immunotherapy response in a study of serial stool collection of patients on either nivolumab or combination nivolumab and ipilimumab [81]. Specific bacterial species, including *Akkermansia muciniphila*, *Prevotella copri*, *Bacteroides salyersiae*, *Eubacterium siraeum* and *Bifidobacterium adolescentis*, have also correlated with improved immunotherapy response in renal carcinoma [81–83]. Other bacterial species, including *Clostridium hathewayi*, *Clostridium clostridioforme*, and *Erysipelotrichaceae bacterium_2_2_44A* were enhanced in nonresponders [80]. Differences have been reported in the literature amongst species associated with response, for example one study correlating *Akkermansia muciniphila* with improved response to immunotherapy [82] while another finding no association with response [81]. Potential differences amongst these findings could be explained by variability in specimen collection, treatment received, and concomitant medications. Prior medications, including antibiotics, influence the gut microbiota role in evoking immunotherapy resistance [80]. In the adjuvant setting, this finding is especially relevant as patients receive antibiotics perioperative as well as for potential infectious complications. More needs to be understood regarding the mechanism by which these bacteria affect response to immunotherapy, especially in the adjuvant setting.

In addition to being a biomarker of response to immunotherapy, administration of bacterial species can enhance the antitumor effects of immune checkpoint inhibitors. One clinical trial evaluated the combination of CBM588, a strain of *Clostridium butyricum*, with nivolumab and ipilimumab for metastatic renal cell carcinoma. Thirty patients were enrolled, and this combination of immunotherapy and CBM588 improved overall response rate and progression free survival without increased toxicity [81,84,85]. Additionally, the ongoing TACITO trial is evaluating the benefit of adding fecal microbiota transplant to standard of care immunotherapy in renal cell carcinoma [86].

The gut and urinary microbiome have both been studied in urothelial carcinoma. Analysis of stool microbiota from patients in the neoadjuvant pembrolizumab PURE-01 study found bacteria enriched in immunotherapy responders, such as *Sutterella* species, and in non-responders, such as *Ruminococcus bromii* [87]. Like renal cell carcinoma, perioperative use of certain medications could limit the effects of adjuvant immunotherapy in urothelial carcinoma. Analysis of the IMvigor 210 and 211 trials of atezolizumab for urothelial carcinoma determined that alterations of the gastrointestinal microbiome by antibiotics and proton pump inhibitors limit the effectiveness of immunotherapy [88,89].

While typically more sterile than the gut microbiome, urinary microbiome could also provide biomarkers of response and resistance to immune checkpoint inhibition in urothelial carcinoma. Enrichment of bacterial species, including *Streptococcus*, *Firmicutes*, and *Acinetobacter*, have been identified in bladder cancer patients compared to healthy volunteers [90,91]. In non-muscle invasive bladder cancer, urinary microbiome analysis identified microbes that were more commonly detected in patients who recurred after Bacillus Calmette–Guerin immunotherapy [92]. An ongoing clinical trial is evaluating the combination of GEN-001, a microbial product, and avelumab in patients who progressed on immune checkpoint therapy for urothelial carcinoma [93]. Despite these advances, further studies are necessary to fully ascertain the impact of the gastrointestinal and urinary microbiome and specific bacterial species

that influence response to adjuvant immune checkpoint inhibition.

## METABOLOMICS

Metabolism plays an important role in the immune microenvironment, and crosstalk between the gut microbiome and metabolome has been described [94,95]. Microbial metabolites, such as short-chain fatty acids and tryptophan catabolites may induce immunosuppressive regulatory T cells [96]. Given metabolic alterations in renal cell carcinoma to meet the demands of hypoxia, byproducts of metabolism could predict response to immune checkpoint inhibition. In renal cell carcinoma resistant to immune checkpoint inhibitors, *UGT1A6*, which is involved in glucuronidation and lipid detoxification, was overexpressed [97]. A study of tryptophan metabolism in renal cell carcinoma patients treated with nivolumab found that elevated kynurenine to tryptophan ratio correlated with worse overall survival [98]. Higher adenosine levels have also correlated with decreased response to nivolumab in renal cell carcinoma [99].

Metabolomic profiling has been used to discover biomarkers from diverse metabolic pathways for detection of urothelial cancer [100]. In muscle-invasive bladder cancer, elevated prostaglandin and thromboxane levels were observed, as well as increased tryptophan metabolites [101]. Similar methods may be used in the future to ascertain metabolically relevant predictive biomarkers of response to immunotherapy.

## GENOMIC SIGNATURES

Gene expression profiling has yielded potential predictive biomarkers for response to immune checkpoint inhibitors. *PBRM1*, a tumor suppressor gene that is a member of the PBAF complex which belongs to the mammalian SWItch/Sucrose Non-Fermentable Complex (mSWI/SNF) chromatin remodeling complex, is the second most commonly mutated gene in clear cell renal cell carcinoma patients [102,103]. The role of *PBRM1* as a predictive biomarker for immune checkpoint inhibition, remains unclear. Loss of function mutations in *PBRM1* have correlated with response to nivolumab monotherapy [104]. Conversely, a retrospective cohort found that *PBRM1* mutation was not associated with prolonged overall survival with immunotherapy [105], and another study associated *PBRM1* loss with resistance to immune checkpoint inhibitors [106]. Other potential genes that predict response to immunotherapy include *ARID1A*, a tumor suppressor and member of the mSWI/SNF complex and *KMT2C*, a histone methyltransferase. In analysis of the IMmotion 151 clinical trial, loss of function mutations demonstrated favorable outcomes with atezolizumab plus bevacizumab compared to sunitinib [107]. Alternatively, a retrospective cohort found no association between *ARID1A* deficiency and response to immune checkpoint inhibitors in renal cell carcinoma [108]. Alterations in DNA damage repair pathways have also correlated with improved response to immunotherapy. Mutations in DNA damage repair genes, in particular homologous recombination repair, have been associated with improved response [109]. DNA damage repair alterations have been associated with neoantigen generation and adaptive immune markers, which are potential mechanisms by which these mutations effect a response to immune checkpoint inhibition [110].

In addition to single gene alterations, several gene expression signatures have been characterized to predict response to immunotherapy in renal cell carcinoma. A published analysis of IMmotion 151 proposed an integrated molecular classification of metastatic clear cell RCC into seven distinct types based on bulk RNA sequencing. Metastatic renal cell cancers were classified into angiogenic and proliferative phenotypes. The proliferative clusters, enriched in T effector and cell cycle genes, demonstrated improved response to atezolizumab compared to sunitinib [107].


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Similarly, in correlative studies of the JAVELIN Renal 101 trial, a 26-gene immune signature was generated that included NK cell, chemokine and cytotoxic T-cell related elements. This signature predicted a favorable outcome in patients treated with avelumab plus axitinib [111]. A distinct 18-gene expression T-cell inflamed signature, which was associated with overall response to pembrolizumab, was proposed in analysis of KEYNOTE-427 [112].

Prospective clinical trials in renal cell carcinoma are also utilizing genomic classifications upfront. The prospective phase 2 BIONIKK trial incorporated gene expression signatures and transcriptomic analysis to identify four clear cell renal cell carcinoma subtypes and to enhance response to front-line therapy, including immunotherapy, in metastatic renal cell carcinoma [113,114]. Another prospective clinical trial, REMEDY (NCT04005183), proposes to utilize single cell RNA sequencing, including nuclear (NUCseq) and epitope sequencing (CITEseq). This analysis plans to identify mutations and abnormal expression patterns within individual renal cancer cells in order to infer targetable vulnerabilities and biomarker signatures [115].

Gene signatures have also been proposed in the adjuvant setting. For example, a 16-gene recurrence score assay was generated to predict recurrence and validated on the S-TRAC study of adjuvant sunitinib [116,117]. A novel relevant gene signature for response to adjuvant immunotherapy may need to combine elements from existing gene signatures of both response to immunotherapy and recurrence following surgery.

In urothelial carcinoma, gene expression profiling has revealed predictive biomarkers of response to immune checkpoint inhibitor therapy. Gene expression signatures involving the transforming growth factor-beta (*TGF-β*) pathway have correlated with decreased efficacy of immunotherapy in metastatic bladder cancer [118,119]. Analysis of the ABACUS neoadjuvant atezolizumab trial also found poorer outcomes with *TGF-β* expression in urothelial carcinoma [41]. Other expressed genes correspond to favorable treatment outcomes with immunotherapy in urothelial carcinoma. In correlative analysis of the IMvigor 130 study, *APOBEC* mutagenesis was associated with improved survival with atezolizumab treatments [120]. Mutations in DNA damage repair genes have also been associated with clinical benefits with immunotherapy [121,122]. An *IFN-γ* mRNA immune signature has also been developed using the NanoString nCounter platform that could distinguish responders from non-responders to anti-PD-1 immunotherapy [123]. An *IFN-γ* signature also correlated with improved response to nivolumab in analysis of the CheckMate275 trial [124]. Germline variants have also been shown to exert some influence on immune cell regulation and cytokine production. Germline polymorphism affecting *ERAP2* results in lower expression and is associated with enhanced immunogenicity, thereby improving survival with atezolizumab [125]. Despite these correlations between genetic changes and response to immunotherapy, the mechanism by which mutations impact treatment response remains to be determined. Further optimization is necessary prior to widespread use as a prognostic or predictive biomarker.

Molecular subtyping of bladder cancer has also yielded potential predictive biomarkers of response to immune checkpoint inhibition. Gene expression data from TCGA identified intrinsic molecular characteristics of high-grade bladder cancers, which were classified into basal or luminal subtypes [126,127]. Of these, the highest response rate to atezolizumab treatment was observed in luminal cluster II subtype, which had increased presence of activated effector T cells. ORR was 34% for luminal cluster II compared to less than 20% in all other luminal and basal subtypes [39]. The impact of molecular subtyping on immunotherapy response has also been evaluated in localized bladder cancers. Correlative analysis of the neoadjuvant PURE-01 trial found that while molecular subtypes were not intrinsically predictive of response to immunotherapy, basal subtype with a high

Immune190 score had improved progression free survival [128]. Another study in early stage bladder cancer found that non-luminal tumors with higher immune infiltration benefit more from immunotherapy [129].

## NON-INVASIVE BIOMARKERS

Non-invasive biomarkers are readily obtained and have multiple applications, including serial monitoring of treatment response. Neutrophil to lymphocyte ratio (NLR) is one non-invasive validated predictive biomarker of immunotherapy response, and increased pretreatment NLR predicted worse outcomes in multiple cancers treated with immune checkpoint inhibitors, including renal cell and urothelial cancer [130]. Lower pretreatment NLR was also associated with better progression free and overall survival following immune checkpoint inhibitor treatment for renal cell carcinoma [131–133]. Change in NLR during immunotherapy treatment can also predict response. In renal cell carcinoma patients treated with immunotherapy, lower NLR following six weeks of therapy better predicted response and survival than the baseline ratio [134]. Decrease in NLR during treatment has also been associated with improved outcomes [134,135]. In the adjuvant renal cell carcinoma setting, low baseline NLR and decreased ratio following four weeks of treatment predicted better response to adjuvant sunitinib [136]. Based on these findings in adjuvant therapy and immunotherapy, NLR measurements at baseline and during treatment with adjuvant immunotherapy could play a valuable role in determining who benefits from treatment.

Cancers can be characterized non-invasively through analyzing circulating tumor DNA (ctDNA) in bodily fluids, such as blood, urine, or saliva. In renal cell carcinoma, ctDNA detection rate has been variable, ranging from as low as 30–40% [137,138] to as high as 70–80% of patients [139,140]. Concordance between genomic alterations in tissue and ctDNA has been reported, where in one study 34.2% genomic alterations in tissue were found in ctDNA. In this study, 28.2% of alterations seen in ctDNA were found in tissue [140]. Changes in ctDNA mutant allele frequency during serial measurements has also been successfully tracked, and ctDNA has been used to detect minimal residual disease earlier than imaging would confirm recurrent disease [137,138]. CtDNA has also been reported as a potential early predictor of response to immune checkpoint inhibition in metastatic renal cell carcinoma, however a larger cohort will be necessary to confirm this finding [141]. A comparison of response to adjuvant pembrolizumab between ctDNA positive and negative cases following nephrectomy would further solidify its value as a predictive biomarker. Measuring changes in ctDNA during treatment with adjuvant immunotherapy, including the emergence of minimal residual disease, would provide greater insight into treatment efficacy.

In urothelial carcinoma, ctDNA has been identified in plasma and urine from patients with non-muscle invasive, muscle invasive, and metastatic bladder cancer [142,143]. Analysis of 68 patients who received neoadjuvant chemotherapy for muscle invasive bladder cancer found that plasma ctDNA level following cystectomy was highly prognostic. Of the seventeen patients who exhibited detectable genomic alterations in the ctDNA at that timepoint, thirteen (76%) developed recurrent disease, whereas none of the 47 patients who were labeled as ctDNA negative recurred [144]. In the adjuvant setting, ctDNA was evaluated in patients who received adjuvant atezolizumab in the negative IMvigor 010 study. At the start of adjuvant immunotherapy, 37% of patients were ctDNA positive. Amongst these patients, there was significant disease-free survival benefit with atezolizumab compared to observation, whereas no such benefit was evident in ctDNA negative patients [145].

Prospective clinical trials are evaluating ctDNA as a biomarker to guide immune checkpoint inhibition in the recurrent and adjuvant settings. The TOMBOLA trial (NCT


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04138628) will treat patients with atezolizumab at the time of ctDNA positivity following radical cystectomy [146]. The IMvigor 011 trial (NCT 04660344) will treat patients with adjuvant atezolizumab who are ctDNA positive within 20 weeks of cystectomy for high-risk bladder cancer [147]. These studies will further define the utility of non-invasive ctDNA monitoring to determine which patients will benefit from immunotherapy.

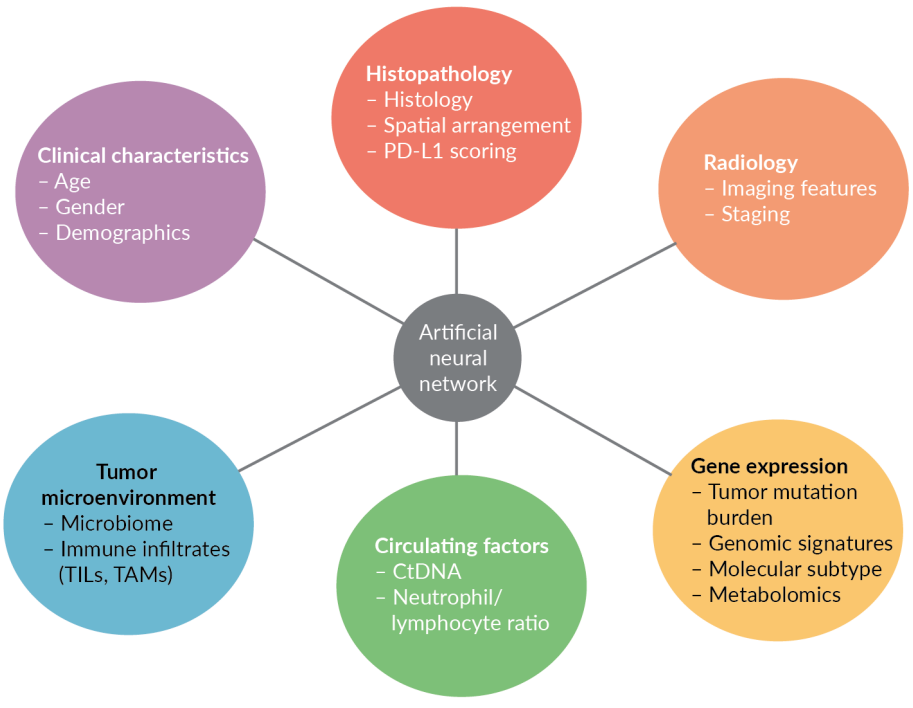
## ARTIFICIAL NEURAL NETWORKS

Artificial intelligence has utilized the power of machine learning to integrate multiple clinicopathologic characteristics into models that can improve upon prediction of cancer outcomes. This reduces subjective variability, for example in interpretation of tumor infiltrating lymphocytes by histology [64]. In some cancers, artificial neural networks have successfully predicted patient outcomes from tissue characteristics alone [148,149]. Artificial neural networks have also been used to predict response to immunotherapy. In melanoma, one such algorithm accurately stratified patients into high and low risk of progression on immunotherapy using hematoxylin and eosin-stained slides of metastatic lymph node and subcutaneous tissue [150]. Another study used radiographic features in melanoma and non-small cell lung cancer to determine which patients would respond to immunotherapy and found that these features corresponded with gene expression of markers involved in cell cycle progression and mitosis [151].

By combining multiple data types, including histopathology, sequencing data, and cross-sectional imaging, these networks can provide a more powerful predictive biomarker of response to immunotherapy than any of these variables alone. Analysis of contrast-enhanced CT images and RNA sequencing data determined a CD8 positive TIL infiltration signature across multiple cancers, including kidney and urothelial, developed a radiomic signature that predicted response to immunotherapy [152]. In renal

FIGURE 2

**Components of an Artificial Neural Network for Prediction of Response to Immune Checkpoint Inhibitors.**



cell carcinoma, machine learning was used to better characterize the immune microenvironment to assess prognosis and could potentially be valuable in understanding benefit of immunotherapy [153]. In localized cancers, artificial neural networks have been used to predict recurrence. Machine learning on histopathology features and clinical data used to predict recurrence of non-muscle invasive bladder cancer in multiple studies [154,155]. A similar approach may be used to combine information about the tumor microenvironment, genomics, and radiology to predict recurrence and to determine patients that would benefit from adjuvant immunotherapy (Figure 2). While the development of artificial neural networks is complex, a strong, reproducible algorithm could simplify patient selection by focusing on only the variables with the greatest contribution to prediction of immunotherapy response.

## CONCLUSIONS

While pembrolizumab and nivolumab have been approved as adjuvant therapy in renal cell carcinoma and urothelial carcinoma, respectively, the patient populations that derive treatment benefit are insufficiently characterized by assessment of PD-L1 alone. Several novel biomarkers of response to immunotherapy have been proposed, and the science behind these markers is still evolving. Many of these were identified in metastatic disease, however translation from the metastatic to adjuvant setting may present a new challenge. Nonetheless, tumor mutation burden, neoantigen load, tumor infiltrating lymphocytes, tumor associated macrophages, gastrointestinal and urinary microbiome, metabolomics, gene expression profiling, and circulating tumor DNA, should be considered as possible ways to optimize patient selection in the adjuvant treatment setting. Even more importantly, combinations of these biomarkers along with PD-L1 and patient characteristics would provide valuable information and could best optimize response to treatment. Given the complexity of assessing multiple predictive biomarkers, generation of artificial neural networks will enhance the capability of this analysis.

Despite the excitement of scientific advances in this field, several considerations must be taken in development of clinically relevant prognostic and predictive biomarkers. These biomarkers must undergo rigorous validation on independent datasets to fulfill regulatory requirements. Moreover, these biomarkers must be practical, readily interpreted, and affordable. A biomarker that requires complex procedures, has a long turnaround time, or is very expensive would not be able to be widely applied. Finally, these biomarkers must have low false negative rates, so that patients would not be denied treatment that could potentially prevent metastatic disease. With additional ongoing trials of adjuvant immunotherapy, correlative biomarker studies will be paramount to contextualize findings, to resolve potential differences, and to better stratify patients in order to maximize the efficacy of this treatment approach.

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