Journal for ImmunoTherapy of Cancer

SITC vision: Opportunities for deeper understanding of mechanisms of anti-tumor activity, toxicity, and resistance to optimize cancer immunotherapy

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To cite: Sullivan RJ, Cillo AR, Ferris RL, *et al*. SITC vision: Opportunities for deeper understanding of mechanisms of anti-tumor activity, toxicity, and resistance to optimize cancer immunotherapy. *Journal for ImmunoTherapy of Cancer* 2025;**13**:e011929. doi:10.1136/jitc-2025-011929

Accepted 01 June 2025

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ABSTRACT

Cancer immunotherapy has radically changed the management of several malignancies, and dozens of agents have been approved in the past 15 years. While these advances have changed the field, many challenges lie ahead and must be addressed if we are to optimize the management of cancer with these approaches. A more comprehensive understanding of the mechanisms of action, toxicity, and resistance is needed to guide the next decade of cancer immunotherapy development. To this end, members of the Society for Immunotherapy of Cancer met and identified challenges and opportunities to improve cancer immunotherapy by focusing on the mechanisms by which the specific agents work, the mechanisms of how they cause adverse effects, and the mechanisms of resistance that limit the effectiveness of these agents. The priorities of this effort were to (1) level set by describing the state of the field; (2) describe what is known about how these agents work, fail to work, and cause side effects as well as the key knowledge gaps in these areas and associated challenges for addressing them; (3) provide a patient perspective to highlight the importance of this work to the community most affected; (4) look ahead to the future by identifying and describing prioritized opportunities that the field may focus on to expand the knowledge base of the field and optimize the management of cancer with immunotherapy.

INTRODUCTION

The Society for Immunotherapy of Cancer (SITC) gathered a diverse group of experts across the cancer immunotherapy landscape to identify challenges and opportunities in cancer immunotherapy, which were outlined in the prefatory manuscript.[1] This manuscript serves to further address three of the outlined topics: mechanisms of anti-tumor activity and toxicity, mechanisms of drug resistance, and highlight additional opportunities to leverage our understanding to optimize advancements in cancer immunotherapy.

Prioritized opportunities

The developments over the past four decades have led to the approval of multiple immunotherapies across several therapeutic classes including cytokines, dendritic cell vaccine, inhibitors against four immune checkpoints (CTLA-4, PD-1, PD-L1, LAG-3), three distinct types of adoptive cell transfer therapies (ACT), infectious agents directed to the tumor (BCG, TVEC), and T-cell engagers (TCEs) targeting tumor-associated antigens (gp100, DLL3) (figure 1). These therapies span diverse solid tumor diseases as well as hematologic malignancies. However, most patients treated with immunotherapy will not benefit and many will have adverse events which may limit the delivery of therapy and/or impair quality of life. It is clear we need to do better, and it is believed that a greater understanding of the mechanisms of action (MoA), resistance (MoR), and toxicity will help to highlight opportunities to do so (table 1).

What is response? What is resistance?

A first opportunity which should be prioritized is the development of a deeper understanding of what is response and what is resistance. Drug developers have used standard measures of radiographic response (WHO, Response Evaluation Criteria in Solid Tumors (RECIST)) in clinical trials which have been well validated for chemotherapy and targeted therapies. Immunotherapy poses new challenges for these standard measures given the well-documented potential for late responses, tumor growth followed by shrinkage due to infiltration of immune cells, and residual necrotic and/or fibrotic masses

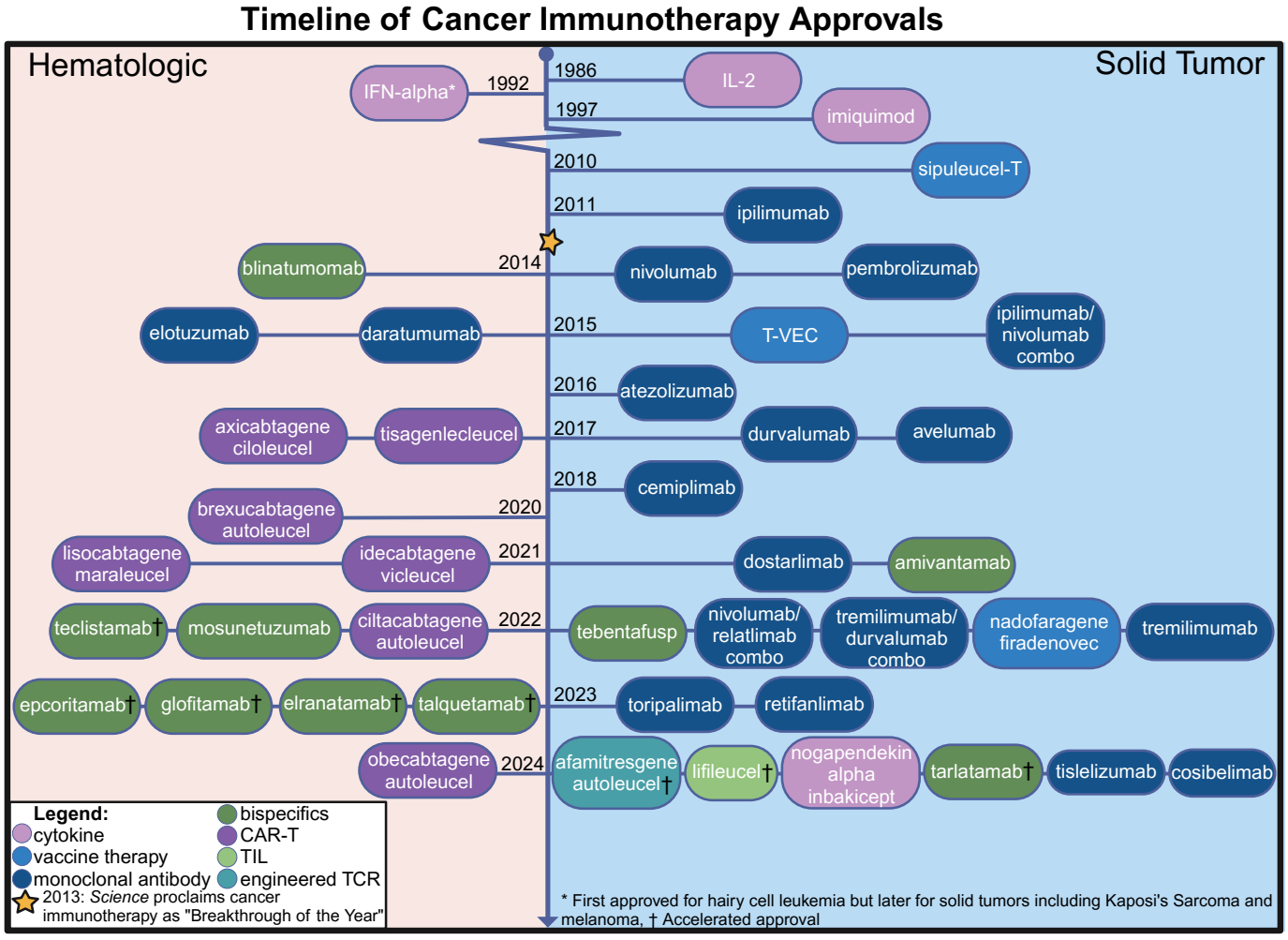

Figure 1 Timeline of cancer immunotherapy approvals. The cancer immunotherapy treatment landscape began in 1986 with the approval of IL-2 cytokine therapy, and has expanded to a robust number of approvals in a variety of drug classes, including cytokines, vaccines, monoclonal antibodies, bispecific antibodies, chimeric antigen receptor T cells, tumor-infiltrating lymphocytes and engineered TCRs. Following the first monoclonal antibody approval, ipilimumab, with others following close behind, cancer immunotherapy was proclaimed the "breakthrough of the year" in 2013 by the Journal of *Science*. While the majority of cancer immunotherapies target solid tumors, there is a significant number of approvals in hematologic malignancies as well. As not all patients are cured of disease with existing therapies, continued research to develop new cancer immunotherapies and immunotherapy combinations is ongoing. Created in BioRender. Staff, S (2025) https://BioRender.com/b48h063. combo, combination; IFN, interferon; IL, interleukin; TCR, T cell receptor.

that lack clear viable cancer cells. Moreover, the immune response can continue substantially past the presence of active drug, raising questions about the timing of scans and what defines resistance after stopping therapy and whether tumor regrowth, or even new tumor sites, long after treatment discontinuation truly reflects resistance rather than insufficient stimulation of immune cells prior to treatment cessation. This is important in both the metastatic and adjuvant settings, with key differences between the two. Therefore, accurate definitions of response and resistance to immunotherapies are necessary to understand the true benefit of these therapies and the development of post-resistance treatment regimens to ensure enrollment of patients with disease that is truly resistant to the last regimen. To that end, SITC convened immunotherapy resistance task forces in 2019 to generate clinical definitions of resistance for PD-(L)1 monotherapy and combinations with other immune checkpoint inhibitors (ICIs), targeted therapies, or chemotherapies.[1–4] For each of these four treatment categories, there was an attempt to define primary resistance, secondary resistance, and resistance after stopping therapy in the adjuvant setting, the neoadjuvant setting, and the metastatic setting. These definitions are summarized in box 1.

While these definitions are being adapted into clinical trials in the post-anti-PD-(L)1 setting, there remain challenges including a clear need for validation (box 2). To address these challenges, SITC is currently working with cooperative groups and large pharmaceutical companies to obtain data from clinical trials that are agnostic to tumor type and specific ICIs, particularly PD-(L)1 inhibitors. This effort and others like it require sharing of de-identified data, particularly data from older trials that were conducted with meticulous tumor assessment

Table 1 Challenges and opportunities ahead for a deeper understanding of MoA, MoR, and toxicity

Challenges	Future opportunities
Definition of clinical response and relapse	► Integrate radiomics parameters in clinical evaluation. ► Use circulating assays that reflect activated immune status or tumor burden (eg, cell-free DNA). ► Consider distinct response categories based on differential therapeutic MoA (ICI versus CAR T/TIL versus vaccines), indication, and long-term durability of responses. ► Identification of more precise biomarkers that accurately capture the biology of patients sensitive to drug MoA to inform treatment decisions.
Link immunotherapy mechanism of resistance to clinical presentation	► Curated analysis of available studies to identify the clinical presentation of specific resistance mechanisms to optimize patient treatment and clinical trial enrollment. ► On-treatment samples, at the time of resistance, will likely be most informative and though less easily obtained from tumor need to be rigorously assessed to define MoR. ► Identification of novel biomarkers that accurately capture major resistance mechanisms to inform treatment decisions.
Maximize immunotherapy efficacy limiting immune-related side effects	► Pre-emptive therapies to mitigate side effects. ► Collaboration with autoimmune experts to identify targets that uncouple antitumor immunity from irAEs.
Tailor immunotherapy intensity regimen to patient needs and risk of toxicity	► Use of predictive biomarkers of response and toxicity to tailor immunotherapies regimen intensity (eg, combination of immunotherapy versus monotherapy). ► Targeted tumor drug delivery.
Development of preclinical models of immunotherapy efficacy and toxicity	► Multicellular compartments in vitro models (eg, tumor-on-a-chip). ► Prospective integration of patient-derived models in clinical trials. ► Combination of omics datasets with ex vivo experiments.

CAR T, chimeric antigen receptor T cell; ICI, immune checkpoint inhibitor; irAEs, immune-related adverse events; MoA, mechanisms of action; MoR, mechanisms of resistance; TIL, tumor-infiltrating lymphocyte.

at well-defined time points. Additionally, validation in non-clinical trial settings will be critical, particularly as real-world data includes patients at risk for immune-related adverse events (irAEs) who might receive fewer cycles of treatment or less frequent treatment and might be imaged at variable time points, and single treatments, such as cell therapies, will be increasingly used. As treatments that are partnered with PD-(L)–1 inhibitors improve, responses might be seen at different times during treatment depending on whether the added therapy exhibits a complementary MoA to PD-(L)1 inhibition or overcoming resistance to it. In particular, the underlying biology associated with response and resistance to combination therapies will be distinct from that previously defined for PD-(L)1 inhibitor monotherapies. These changes will require constant assessment and refinement of the SITC definitions (box 1) as well as the generation and maintenance of large datasets containing clinical trial level and real-world data that can serve as a shared resource to ask and answer questions such as "what is response?" and "what is resistance?".

Box 1 First version of the Society for Immunotherapy of Cancer clinical resistance definitions

Primary resistance

⇒ Disease progression after receiving at least 6 weeks of exposure to PD-(L)1 inhibitors, typically aligning with two complete therapy cycles.

⇒ Ideally confirmed by a follow-up scan at least 4 weeks later.

Secondary resistance

⇒ Disease progression after complete or partial response or stable disease beyond the expected duration for a specific tumor type, preferably confirmed on a follow-up scan.

Resistance after stopping or adjuvant therapy

⇒ Progression or regrowth occurs within 12 weeks of treatment cessation (primary resistance).

⇒ Tumor growth or regrowth within 12 weeks after stopping therapy for metastatic disease would be considered primary resistance if no response was observed prior to discontinuation and secondary resistance if response was seen prior to discontinuation (eg, non-response to neoadjuvant therapy).

OPTIMIZING PATIENT BIOSPECIMENS

The advancement of new radiomic approaches and circulating assays that reflect either activated immune status or tumor burden (eg, circulating tumor DNA (ctDNA)) is in progress and should be adopted rapidly into our clinical practice based on careful retrospective analysis of clinical trial samples, or well-curated prospectively collected samples from standard of care patients and new agent clinical trial patients. This can be aided by improvements in the collection and assessment of longitudinal clinical samples. However, samples may be difficult to collect due

Box 2 The Society for Immunotherapy of Cancer resistance definitions need validation

The definitions require empiric validation as they are based on assumptions that need to be verified. These include:

- ⇒ The rate of pseudoprogression of 5–10% for immunotherapy and response after rapid progression at first set of scans occurs is ≤5%.
- ⇒ Uniform definitions should be used for all solid tumors, other than those not amenable to standard radiographic assessment, such as prostate cancer, glioblastoma and ovarian cancer.
- ⇒ Early toxicity requiring steroids and cessation of immunotherapy is difficult to assess for resistance.
- ⇒ Time frames for determining primary versus secondary resistance and resistance after stopping treatment are arbitrary.
- ⇒ Minimal exposure for immune checkpoint inhibitors for the purpose of determining resistance is two cycles over 6 weeks.
- ⇒ Six months of stable disease for most solid tumors suggests tumor sensitivity.
- ⇒ Definitions have no more than a 5% error rate.
- ⇒ Clinical definitions of primary versus secondary resistance are associated with different biology.
- ⇒ Validation in the second line setting is challenging given long-term effects of immune modulation.
- ⇒ Definitions are based on standard cross-sectional imaging, but as alternative modalities are used to assess response, such as positron emission tomography tracers and cell-free DNA, these definitions might need to be refined, particularly as they pertain to time points for determining primary versus secondary resistance.

to burdens for the patient, cost, and lack of sufficient hospital infrastructure (eg, staff). Moreover, protocols for collection (methods and timing) and processing of samples are not standardized across institutions. While many studies rely on the translational analyses of archived material collected sometimes years before the initiation of immunotherapy, recent work suggests that using biopsies taken right before the start of immunotherapy reveals more predictive information compared with archived material.[5] Of note, the timing of on-treatment biopsies is relevant as not every timepoint is suitable to address every question (see figure 2). For instance, major pathological responses have been observed after only one cycle of ICI treatment, and biopsies to study the MoA of ICI should, therefore, be taken early on-treatment in these patients. Vice versa, to investigate MoR (ie, secondary), biopsies at later time points may be relevant.

Based on recent guidance from the Food and Drug Administration (FDA), the inclusion of mandatory biopsies may be "reasonable" under two conditions: (1) Data is required to determine the eligibility of the patient for the treatment (eg, oncogenic mutation detection for targeted therapy or other biomarker selection strategy); (2) Tissue analysis is necessary to evaluate the clinical trial's primary or key secondary endpoint(s). The ramifications of this guidance will be the development of clinical trials in the USA that are less likely to include mandatory biopsies, and more likely to include optional biopsies, which ultimately will lead to less biospecimen collection. Alternatively, biomarkers with sufficient mechanistic evidence, and appropriately validated assays, will need to be incorporated into trials as primary or secondary endpoints and tested prospectively. The field will need to partner with US patient advocacy groups to rethink how clinical trial endpoints could include more tissue-based primary and key secondary endpoints as needed, and to develop

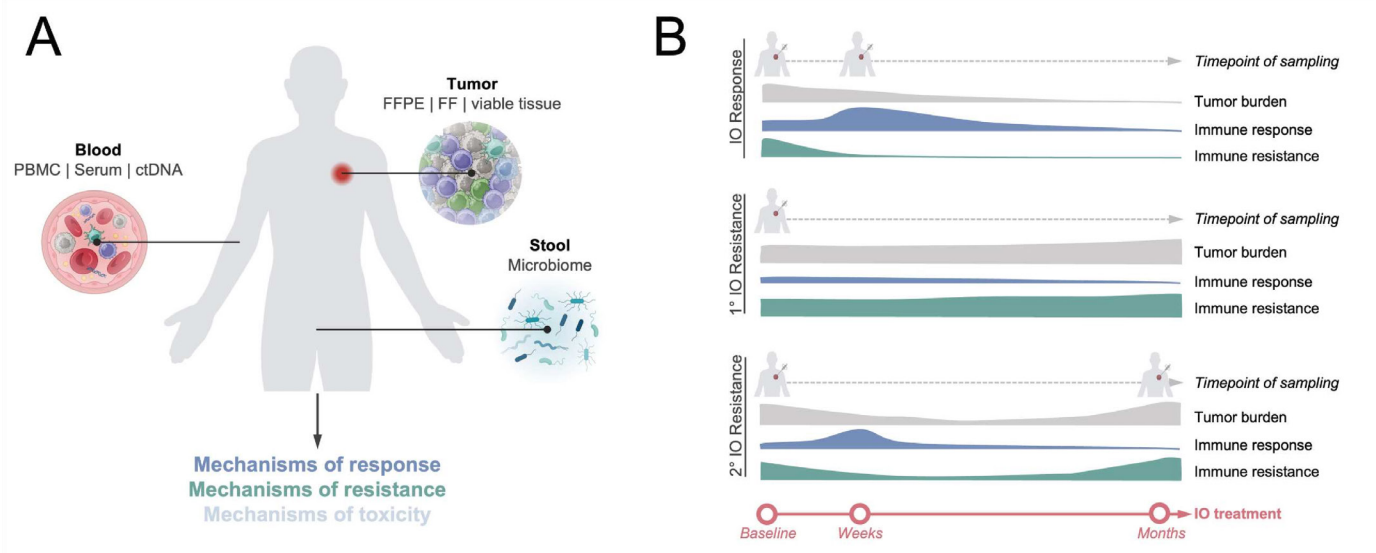

Figure 2 Considerations for patient sample collection. (A) Overview of distinct types of clinical samples including blood, tumor tissue, and stool, that can be collected for translational research, and different types of analyses that can be used for investigation of mechanisms driving response, resistance or toxicity. (B) Individual sampling schedules may be considered to investigate mechanisms of IO response or of primary and secondary resistance, respectively. The figure indicates an assumed time course of clinical burden during treatment and underlying dynamics of immune response or resistance for distinct clinical outcomes. ctDNA, circulating tumor DNA; FF; fresh frozen; FFPE, Ffrmalin-fixed paraffin-embedded; IO, immunotherapy; PBMC, peripheral blood mononuclear cells.

messaging that encourages, when appropriate and safe, inclusion of optional biopsies. As most trials are run globally, leveraging partnerships with ex-US institutes that have a focus on tissue collection as a priority to advance mechanistic insights and translational research progress will also be critical.

The availability of matched, longitudinal biospecimens (baseline, on-treatment, post-treatment) acquired from patients being treated with immunotherapy is essential to improve our understanding of why some tumors respond to immunotherapy and others do not, as well as why patients with initial prolonged responses ultimately develop acquired resistance.[6] Not only can on-treatment biopsies result in dynamic biomarkers, but they also provide insight into which immune cells mediate or block the immunotherapy response which may extend beyond T cells.[7] Whether sequential blood samples or biopsies could be useful to predict IR-toxicity is currently an open question. With emerging single-cell technologies activation status of these cells can be assessed which can provide a basis to develop novel immunomodulatory approaches.[8 9] Beyond tumor biopsies, sampling of peripheral blood is a more accessible compartment that can also offer significant insight into immune states prior to and following immunotherapy.[10 11] Longitudinal samples of patients treated with immunotherapy are essential to help refine response and resistance categories as well as improving our ability to personalize immunotherapy for an individual patient.

WHY DOES IMMUNOTHERAPY WORK?

The use of cancer immunotherapy has radically changed the standard of care of solid tumors and hematologic malignancies, but a deeper understanding of the MoA and MoR will help optimize therapy allowing more patients to have durable immunotherapy responses. Immune evasion is a core hallmark of cancer development, highlighting the natural role of antitumor immunity in preventing and controlling cancer development.[12] In the third of individuals that will develop cancer in their lifetime, there is a breakdown in the immunosurveillance process[13] primarily linked to key features of successful antitumor immunity (figure 3). The MoA of currently approved immunotherapies seeks to enhance four key features of naturally occurring processes across the cancer immunity cycle that involve (1) increasing tumor antigenicity and adjuvanticity for T cell activation and priming, (2) stimulating robust T cell expansion, (3) promoting sufficient T-cell trafficking and infiltration into the tumor, and (4) reversing escape from immune-suppressive signals and suppressive immune cells in the tumor microenvironment (TME). Granular understanding of the MoA associated with therapeutic efficacy across diverse cancer immunotherapy approaches has been leveraged to try and provide insights for patient selection to enrich for those most likely to benefit. This highlights the critical need to continue to dissect and refine our understanding

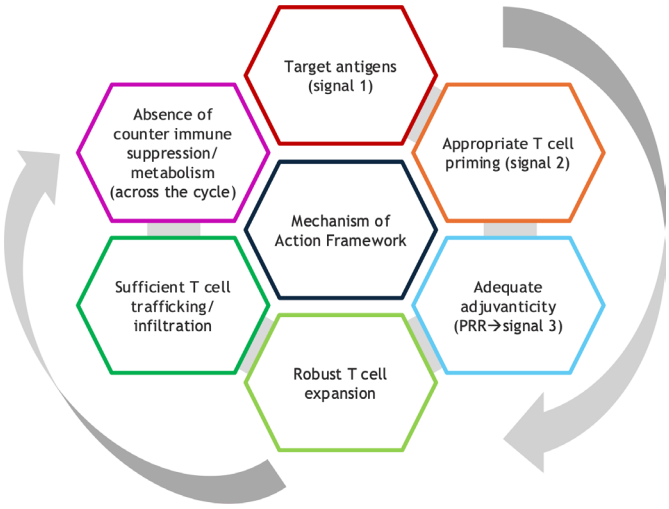

Figure 3 Antitumor immunity. Successful antitumor immunity involves six key features that begin with immunogenic antigens being presented (signal 1) to T cells in the proper context including co-stimulation (signal 2) and danger/damage signals to stimulate APC maturation. Properly matured APCs can then provide the appropriate cytokine signaling (signal 3) to activate helper T cells to stimulate robust antitumor CD8 T cell expansion. Expanded T cells subsequently traffic to tumor sites and attack, provided that they are not inhibited by immune suppressive factors or cells. *MoA of cancer immunotherapies*: (1) Strategies that increase tumor *antigenicity* (increased neoantigen burden, tumor antigen release) and/or tumor *adjuvanticity* (eg, PRR engagement, DC maturation) for T cell activation. (2) Strategies that enhance T cell *priming* and *expansion.* (3) Strategies that promote T cell *trafficking* and *infiltration* into tumors. (4) Strategies that ablate *immunosuppressive pathways* and/or suppressive immune cell subsets in TME. APC, antigen presenting cell; DC, dendritic cell; MoA, mechanisms of action; PRR, pattern recognition receptor; TME, tumor microenvironment.

of MoA specific for each therapeutic modality as well as consideration of important differences in activity between solid tumors and heme malignancies.

The therapeutic success of ICI monotherapy spurred interest in combination therapies, which demonstrated significant improvements in clinical outcome. This led to the first approval of combined anti-PD-1 and anti-CTLA-4 therapy in 2015 and anti-PD-1 plus anti-LAG-3 in 2022.[14 15] Different ICI regimens attempt to target the full spectrum of the four MoA strategies inclusive of T cell priming by anti-CTLA4, activation and expansion of T cells by anti-CTLA4, anti-PD-(L)1 and anti-LAG-3, promoting T cell trafficking (eg, anti-CTLA4 enhancing T cell trafficking from lymph nodes into the tumor and anti-PD-(L)1 promoting PD-1+T cell trafficking from the periphery into tumors), and anti-PD1 and anti-LAG3 reversing inhibitory signals present in the TME. Beyond solid tumors, cancer immunotherapies have also been approved for hematologic malignancies including daratumumab and elotuzumab in 2015, as well as seven chimeric antigen receptor (CAR) T cell therapies (tisagenlecleucel and

axicabtagene ciloleucel in 2017, brexucabtagene autoleucel in 2020, idecabtagene vicleucel and lisocabtagene maraleucel in 2021, ciltacabtagene autoleucel in 2022, obecabtagene autoleucel in 2024),[16 17] and 10 bispecific antibodies within both heme and solid tumors beginning with blinatumomab in 2014.[18] Most recently, in 2024, two cell-based therapies—a tumor-infiltrating lymphocyte (TIL) product, lifileucel, and a T cell receptor (TCR)-T cell, afamitresgene autoleucel—were approved for solid tumors in melanoma and synovial sarcoma, respectively. Collectively, this represents substantial clinical progress in the past decade, across both solid tumor and hematologic malignancies, coupled with a deeper understanding into the dominant mechanisms linked to therapeutic benefit.

In contrast to ICI, the cell therapy approved treatments primarily target the first two MoA strategies as they involve infusion of in vitro manipulated autologous or allogeneic T cells into patients. ACT includes several different types of therapies, such as CAR T cells (CAR T), TCR T cell (TCR T) cancer immunotherapy, and TIL. CAR T cells are T cells engineered to express surface fusion proteins (CARs) to target antigens expressed on cancer cells.[19] The process of manufacturing CAR T involves patients' leukapheresis, T-cell transduction (with a vector containing the target-specific CAR sequence through lentiviral, retroviral, or transposon system), and CAR T in vitro expansion.[20] Following lymphodepleting chemotherapy (typically a combination of fludarabine and cyclophosphamide to deplete endogenous immune cells and increase the availability of homeostatic cytokines, such as IL-7, IL-15, and IL-21, to support the survival and expansion of infused cells) CAR T cells are reinfused into patients.[21] Once reinfused, CAR T cells specifically recognize target antigens on the surface of cancer cells through the CAR. The CAR structure includes an antibody-derived single-chain variable fragment (scFv) that binds to the target antigen. This scFv is linked to intracellular signaling domains, typically composed of one or both costimulatory domains (4-1BB or CD28) and a CD3ζ signaling domain. This allows CAR T cells to recognize a broad range of surface targets with a high binding affinity. This interaction triggers T cell activation, proliferation, and the release of cytotoxic molecules, such as perforin and granzymes, leading to the direct killing of tumor cells.[22 23] TCR T are T cells engineered to express a TCR recognizing specific peptides-major histocompatibility complexes (MHC). TCR T manufacturing follows the same steps (leukapheresis, transduction, in vitro expansion) as CAR T manufacturing.[24] Differently from CAR T, the activation of TCR T occurs only when the TCR recognizes peptides non-covalently bound to MHC complexes on the surface of antigen-presenting cells or tumor cells.[25] TCR T, unlike CAR T, can identify both surface and intracellular antigens but are limited by MHC restriction. TILs are T cells collected from tumor biopsies, expanded in vitro, and reinfused into patients. TILs recognize and mediate tumor killing in an MHC-I-restricted manner similar to TCR-T; however, they are not necessarily genetically engineered.[26] A TIL product has been approved by the FDA for metastatic melanoma in patients progressing to targeted therapies (if BRAF mutated) and anti-PD-1 immunotherapy.[27]

While ICI therapies have shown robust and durable responses in a subset of patients with solid tumor malignancies, there has been limited success in hematological malignancies beyond Hodgkin's lymphoma. In contrast, ACT therapies have demonstrated significant efficacy in treating malignancies of the blood, with complete response rates (CRR) ranging from 28% to 86%,[17] depending on the specific cellular therapy and disease. While promising efficacy has been reported in solid neoplasms (overall response rates: 31–39%), complete and durable responses are rare[17 27 28] with ACT therapies. Optimization of these approaches in solid tumors will likely require directing our attention to the latter two MoA strategies, which focus on T cell infiltration (which may not be as critical in heme malignancies that exist in the blood) and reversing immune suppressive mechanisms within the TME. Similar themes apply to TCEs, which are molecules designed to simultaneously engage both neoplastic cells and T cells. All FDA-approved TCEs are bispecific molecules designed to bind simultaneously to specific tumor-associated antigens and the invariant component of the TCR complex. This simultaneous binding brings the effector T cell in proximity to the tumor cell and triggers cytotoxicity.[29 30] Similarly to what has been observed with ACT therapies, TCEs induce complete remission in a significant proportion of patients with hematological malignancies (CRR: 18–69% based on disease and specific TCE) while in solid neoplasia CRRs are significantly lower (0–4.3%).[31–35] Thus, future improvements may materialize when missing components of effective cancer immunotherapy MoA are rationally combined.

WHY DOES IMMUNOTHERAPY FAIL TO WORK?

During the last decade, the use of preclinical models and modern technologies for detailed molecular analysis of specimens obtained from patients treated with cancer immunotherapies has supported the identification of multiple features associated with sensitivity and resistance to treatment. While differences have been recognized across specific tumor types, therapeutic regimens and line(s) of treatment, a few common themes have consistently emerged across studies as strongly associated with reduced clinical benefit to immunotherapy. In general, these can be categorized into four MoR,[36] and further categorized as factors coming from tumor cells (intrinsic) or non-tumor cells (extrinsic) (table 2).

Tumor cell intrinsic factors include both those existing within the tumor cell itself or those secreted from tumor cells into the TME (figure 4). Not surprisingly, most of these factors involve alterations in key immunomodulatory pathways, and they can be complex involving multiple cell types and cellular signaling events. Immunologically, tumors can be categorized as having a) limited

Table 2 Mechanisms of resistance to effective antitumor immunity

Mechanism of resistance	Tumor cell intrinsic or extrinsic
1. Failure in T cell activation	Intrinsic and extrinsic
2. Barriers to access TME	Extrinsic
3. Counter inhibitory suppressive activity	Intrinsic and extrinsic
4. Tumor intrinsic resistance to killing	Intrinsic

TME, tumor microenvironment.

(or absent) productive adaptive immune responses in the TME characterized by a lack of costimulation and reduced local proinflammatory cytokines that restrict antitumor effector T-cell responses (eg, "immunologically cold" tumors) (MoR 1); b) low immunogenicity of cancer cells displaying a limited amount of antigenic (neo) epitopes and/or defects in HLA class-I antigen presentation machinery (eg, tumors adapted to resist immune pressure (MoR 1); c) defective TME immune responses characterized by the presence of both proinflammatory and immunosuppressive signals and/or cells associated with T-cell dysfunction or inhibition (eg, chronically inflamed tumors with immunosuppression) (MoR 3); or d) systemic or extratumoral immunomodulatory features affecting both the spontaneous tumor rejection and/or the therapeutically induced immunity (eg, microbiome, vascular or stromal physical barriers, or metabolic features) (MoR 2 and MoR 4). While our understanding in this area continues to expand,[36–39] and as our technological tools for exploration advance, there remains the need to more specifically characterize and ascribe the resistance mechanism relevant to each tumor type and disease setting (eg early stage versus advanced metastatic stage). For example, the immunological MoR described in melanoma and lung cancer appears to be distinct from those that exist in microsatellite stable colorectal cancer (MSS CRC) or pancreatic cancer. Further, the MoRs may even differ within one anatomical space as is the case between MSS CRC and microsatellite instability high or DNA mismatch repair-deficiency (dMMR) CRC.

It is relevant to note that the features currently associated with resistance may not represent the entire spectrum of anticancer immunotherapies, are not expected to be mutually exclusive, and are described as the extreme states of a biological continuum where they can exert different roles or degrees of involvement in a given patient, at a specific time, or with a given medical circumstance. In addition, not all of the reported features associated with resistance have a clear mechanistic basis and some rely largely on correlative science. Despite prominent advances in the understanding of mechanisms mediating immunotherapy resistance, efforts to rapidly translate them into the clinic for use as predictive biomarkers for patient selection and/or to design optimal therapeutic interventions have been limited and there remain many opportunities for advancement (box 3).

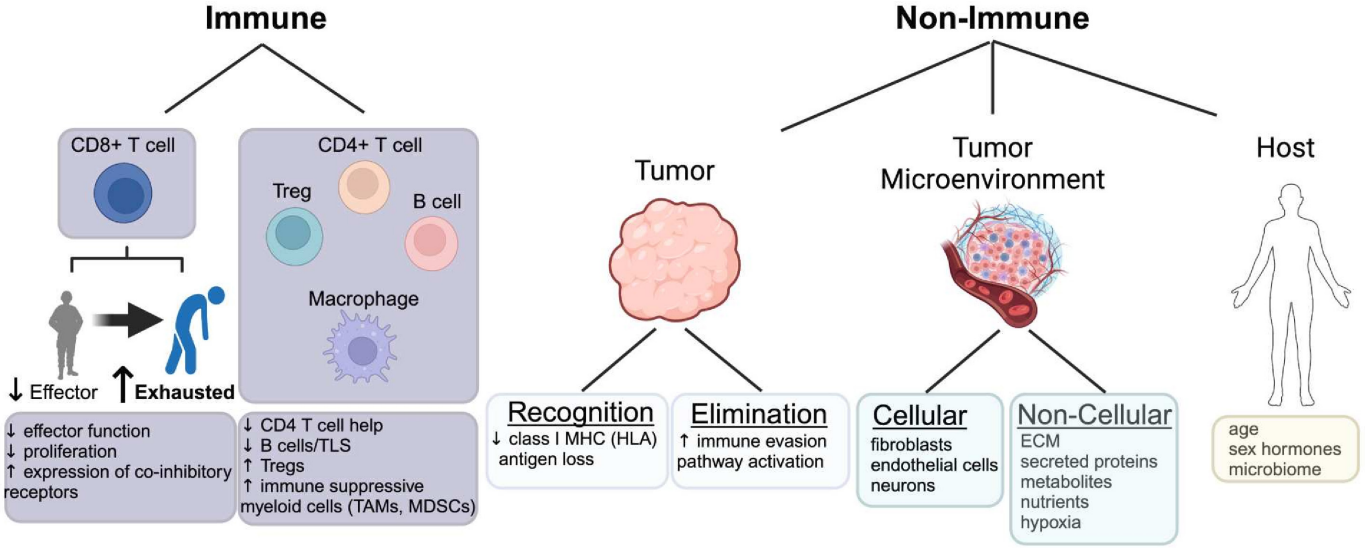

Figure 4 Immune and non-immune variables influencing response and resistance to cancer immunotherapy. *Immune variables*: The quantity and quality (effector versus exhausted) of CD8+T cells and the relative abundance of other immune cell populations that promote or restrict tumor immunity. Exhausted CD8+T cells are hypoproliferative with diminished effector function and high expression of co-inhibitor receptors (eg, PD-1, CTLA-4, LAG-3, TIM-3). *Non-immune variables*: Tumor-specific variables include those that result in impaired recognition by the immune system (eg, decreased expression of class I MHC/HLA, antigen loss) and impaired elimination of cancer cells (eg, activation of tumor intrinsic immune evasion pathways). Cellular and non-cellular factors in the tumor microenvironment can also promote IO resistance. Host-specific factors, including age, biological sex, sex hormones, and microbiome compositions can also influence IO sensitivity. ECM, extracellular matrix; IO, immunotherapy; MDSCs, myeloid-derived suppressor cells; MHC, major histocompatibility complex; TAM, tumor-associated macrophage; TLS, tertiary lymphoid structures; Tregs, T regulatory cells.

Box 3 Overcoming challenges for translating mechanisms of resistance research to clinical impact

Efforts to rapidly translate mechanisms mediating immunotherapy resistance into the clinic for use as predictive biomarkers for patient selection and/or to design optimal therapeutic interventions can be improved by:

⇒ Better characterizing the multidimensional nature of such mechanisms.
⇒ Approaches that help determine their relative contribution and independence/redundancy.
⇒ Better defining heterogeneity across patients.
⇒ Development of improved methods to reliably study cell–cell interactions and functional immune parameters in clinical-grade specimens.
⇒ Use of animal models able to accurately recapitulate the complex nature of immunotherapy-resistant human malignancies.
⇒ Modeling the dynamic nature of both cancer-cell and immune-cell adaptations (including leveraging computational modeling).
⇒ Inclusion of prespecified biomarker analysis and biomarker data generation in pivotal Phase 3 clinical trials (ie, to allow differentiation of MoR against comparator antiPD-(L)1 therapy).
⇒ Defining mechanisms of immunotherapy resistance that may be distinct between clinical resistance categories.

As the clinical and molecular characteristics of resistance are defined, there is an opportunity to better link specific MoR with clinical presentation. For example, if a given mechanism or MoR to ICI is more likely associated with primary or secondary resistance, then strategies to identify patients to enroll in trials that target these specific mechanisms will be more streamlined and drug development more efficient. Additionally, if the mechanisms that drive primary resistance are present in pretreatment samples, they could be identified prior to therapy, and thus could be targeted with frontline immunotherapy approaches to overcome this de novo resistance rather than in the second or third line when treatment typically is less effective. It is also worth noting that treatment-naïve patients with tumors showing absence of naturally occurring local adaptive immunity (eg, PD-L1 expression and/or interferon (IFN)-γ responses or TILs and a limited cancer cell antigenic profile/repertoire (eg, somatic mutations and/or tumor neoantigens) showing progression after ICIs may not conceptually qualify as having therapeutic resistance due to the lack of mechanistic basis to expect treatment activity. These cases could instead be classified as being naturally insensitive to ICIs due to their native molecular composition and could display distinct dominant mechanisms of immune evasion. The study of these cases, independent from those with primary resistance, harboring biological features expected to mediate sensitivity to ICIs could provide novel biological insights with clinical potential.

It is worth noting that MoRs to therapies beyond ICI may be distinct. Though ACT therapies and TCE have shown promising results, only a fraction of the patients respond long-term. Tumor intrinsic resistance factors include antigen loss (due to the selective pressure of the ACT therapies, TCE or cell-intrinsic genetic and/or epigenetic mechanism that decreases antigen expression),[40–43] antigen masking,[44] reduction of MHC,[45] expression of inhibitory molecules (eg, PD-L1[46]), or intrinsic apoptosis resistance.[47] TME extrinsic-related mechanisms include the presence of immunosuppressive stromal cells and inhibitory signaling between tumor, myeloid cells, and T cells[47] as described for ICI.[48 49] On the other hand, T cell functionality plays a critical role in shaping ACT therapies or TCE functionality. Indeed, terminal differentiation, exhaustion, or regulatory T cell (T_{reg}) phenotype have a significant impact on driving resistance to ACT or TCE therapies[50–54] and barriers to infiltrating the TME remain a resistance mechanism to these therapeutic approaches. Given that progress on methods to overcome barriers to accessing the TME and intrinsic resistance to killing could be applied to both ICI and ACT therapies, these may represent areas of focus that should be prioritized collectively.

OPPORTUNITIES TO APPLY MOA AND MOR INSIGHT TO OPTIMIZE NOVEL COMBINATION APPROACHES

As the field drives towards identifying regimens with potential for transformational benefit to patients, a key opportunity also exists for leveraging novel combination approaches primarily intended to either complement an active MoA (figure 3) or, alternatively, to synergize by overcoming a MoR (figure 4) identified as dominant and specific to the treatment and setting. The combination of anti-CTLA-4 plus anti-PD-(L)1 is an example of an existing approach that primarily leverages complementary MoAs. Anti-CTLA-4 and anti-PD-(L)1 ICIs use distinct mechanisms within different anatomical regions to unleash potent antitumor effector T cells. Anti-CTLA-4 enables B7-1/B7-2 costimulatory signals to re-engage with the CD28 coreceptor on activated T cells, typically within secondary lymphoid organs. Following anti-CTLA-4-mediated priming in the lymph nodes, effector T cells traffic to the tumor whereupon they can eliminate cancer cells. In contrast, anti-PD-1 abrogates PD-L1 and/or PD-L2-mediated suppression in peripheral tissues and thereby can facilitate the reinvigoration of effector T cells within the tumor.[15 55] Recent data has indicated that the efficacy of anti-PD-1 also requires signaling through CD28, suggesting some shared MoA between anti-CTLA-4 and anti-PD-1 therapy though anatomic locations of this signaling likely still differ.[56 57] In addition to boosting effector T cell responses, anti-CTLA-4 and anti-PD-(L)1 can disrupt the suppressive function of T_{regs},[58] although the extent to which ICI modulation of T_{regs} contributes to tumor regression is not fully understood. Combined anti-CTLA-4 and anti-PD-1 therapy augments antitumor immunity through both overlapping and unique MoA as compared with the single agents. Specifically, anti-CTLA-4 and anti-PD-1 exhibit complementary effects including more robust T cell expansion and differentiation leading

to improved tumor regression and survival. However, combination treatment also elicits stronger Th1-like $CD4^+$ T cell expansion and expansion of a less exhausted $CD8^+$ T cell population as compared with the monotherapies,[59] perhaps reflecting the unique roles of CTLA-4 and PD-1 signaling in regulating $CD4^+$ versus $CD8^+$ T cell development and function.[60]

In contrast, the doublet ICI therapy of anti-LAG-3 plus anti-PD-1 (nivolumab and relatlimab), approved for use in advanced melanoma, is a combination approach intended to synergize with anti-PD-1 through a variety of mechanisms. Endogenous LAG-3 cooperates with PD-1 to modulate $CD8^+$ T cell exhaustion, in part through the transcription factor TOX, which regulates the generation and maintenance of exhausted T cells. While PD-1 and LAG-3 are both involved in suppressing effector T cell responses, they differ in their functional impact as PD-1 primarily regulates T cell proliferation, while LAG-3 is more important for modulating T cell effector function.[61–64] Several preclinical studies have worked to determine whether combined LAG-3 plus PD-1 blockade would overcome LAG-3-mediated resistance to anti-PD-1 monotherapy. These data revealed synergistic effects of dual therapy, primarily through enhancing T cell effector function, which was confirmed in biospecimens from patients treated with anti-LAG-3 plus anti-PD-1.[61 62 64 65] Mechanistically, there is evidence from analysis of clinical samples that the combination of nivolumab and relatlimab boosts TCR signaling in $CD8^+$ T cells, augmenting $CD8^+$ T cell differentiation and boosting effector function. Additionally, this combination also enhanced functional signatures of clonally expanded $CD8^+$ T cells compared with monotherapy.[66] Regarding mechanistic aspects for specifically overcoming resistance to anti-PD-1, recent work revealed that increased frequencies of $LAG\text{-}3^+$ T cells in the peripheral blood,[67] termed immunotype-1, were associated with reduced response to anti-PD-1 monotherapy in patients with metastatic melanoma or urothelial cancer. A recent report linked T_{reg} reprogramming to the antitumor activity of anti-PD-1 plus anti-LAG-3 specifically in anti-PD-1 resistance settings.[68] While LAG-3 is also expressed on natural killer cells and other cell subsets, the impact of dual LAG-3 and PD-1 blockade on these cell populations, and how that might affect clinical efficacy across diverse disease settings, remains under investigation. The next wave of rational combinations should leverage the learnings from these clinically successful combination approaches.

The development of resistance through various T cell intrinsic and/or extrinsic mechanisms remains a significant problem limiting the efficacy of doublet ICI regimens. Some of the same MoRs may exist for both, including the upregulation of additional inhibitory receptors, such as TIM-3 and/or VISTA.[66 69–72] Identification of additional MoRs to doublet ICI will require further elucidation from ongoing studies and samples, and the emerging mechanistic insights will need to be proactively leveraged to rationally design novel combination approaches. Ideally, this approach would be prioritized over combining existing therapies simply because they are available for clinical testing. In addition, more granular details around key differences in the target T cell populations that mediate optimal clinical activity (box 4), between available immunotherapy combination regimens, need further refinement to identify biomarkers that could predict benefit for each.

Box 4 Mechanisms of action lingering questions around T-cell states

- ⇒ $CD8^+$ T cell states associated with clinical benefit to immunotherapy are defined by increased expression of genes associated with stem or memory-like T cells, with relatively lower expression of genes and gene programs involved in effector T cell function and are relatively diverse with low T cell receptor clonality.[91 92] *Is benefit similarly associated with CD4 stem-like or progenitor exhausted T cells? Or an alternate CD4 phenotype? Is this association true for doublet immune checkpoint inhibitor (ICI) with anti-CTLA-4 + anti-PD-1 and anti-LAG-3 + anti-PD-1?*
- ⇒ The role and fraction of tumor-antigen specific T cells within tumors relative to bystander cells is unknown and most tumor-reactive and clonally expanded $CD8^+$ T cells show terminal differentiation programs, express high levels of exhaustion/dysfunction markers and are hypoproliferative with diminished effector function. *Is there a certain threshold or effective ratio of bystander or progenitor cells to more terminally differentiated cells that is indicative of tumor control?*
- ⇒ Multiple studies have demonstrated that T cell exhaustion or dysfunction is associated with irreversible epigenetic changes.[93 94] *Do clonally expanded, terminally exhausted $CD8^+$ T cells directly contribute to effective antitumor immune responses to mono ICI or doublet ICI?*

DEVELOPING MORE IMPACTFUL BIOMARKERS

A major challenge in the field is identifying predictive biomarkers to guide patient selection and/or optimize therapeutic combinations to maximize the benefit of cancer immunotherapy. Further, despite more than a decade of mechanistic research since the approval of the first T cell checkpoint inhibitor, there remains a lack of biomarkers linked to the therapeutic MoA strategies (figure 3) that can reliably predict which patients will respond to a given immunotherapy approach and guide treatment strategies. For example, assessment of PD-L1 expression by immunohistochemistry (IHC) is an FDA-approved biomarker assay used to select those patients most likely to respond to anti-PD-(L)1 in certain cancer types (linked to MoA 4; ablate immunosuppressive pathways). It has limited utility as a biomarker, however, partly due to the variability of PD-L1 expression observed in tumor specimens (formalin-fixed paraffin-embedded (FFPE) tissue) from pretreatment biopsies, differences in expression between primary and metastatic disease sites, and the wide variety of IHC assays used clinically.[36] Additionally, PD-L1 expression has demonstrated limited correlation with sensitivity to anti-PD-(L)1 in some

diseases.[36] As a single biomarker, and as the treatment landscape evolves, it may be increasingly difficult to differentiate who will benefit from novel combinations that are layered on top of anti-PD-(L)1. A variety of other biomarkers, linked to therapeutic MoA 1 (tumor antigenicity), have been evaluated including tumor mutational burden (TMB), dMMR, and assessment of various tumor antigenicity measures (eg, hypermutated phenotype, neoantigen burden). As well as a variety of immune parameters (eg, IFN-γ signatures, TILs) expected to be associated with MoA 2 and 3 (priming, expansion, trafficking). However, only TMB and dMMR status have received regulatory approval, and similar to PD-L1, their utility as single biomarkers capable of differentiating the benefit of novel combinations over approved immunotherapies may be difficult.

The identification of biomarkers that can accurately identify which patients will benefit from which available immunotherapy regimen will likely require novel approaches beyond the tumor cell-centric assays that are currently validated (eg, monoplex PD-L1 tumor proportion score (TPS) IHC assay or dMMR genomic assay). Cancer immunotherapy efficacy is centered on manipulation of key immune cell types, distinct for each approach. Thus, the next wave of biomarker progress will likely need to measure aspects of the immune system linked to drug MoA. Additional opportunities may lie in leveraging combinations of biomarkers that integrate both tumor target cell features as well as immune cell features. This is of particular importance to help identify patients who will benefit from one regimen over another now that so many cancer immunotherapies are used within the same indication (eg, ipilimumab monotherapy versus nivolumab and ipilimumab versus nivolumab and relatlimab, where PD-L1 has proven to be a poor biomarker for patient selection in melanoma). Novel biomarker assays assessing immune parameters, such as IFN-γ signatures or TILs have not been analytically validated, nor have cut-offs been defined across diverse tumor types. Given that future predictive biomarkers for immunotherapies may target immune effectors themselves, improvements may also be gained by assessing targets across a range of expression instead of binary cut-offs (eg, positive or negative) that were effective for tumor-targeted therapies (eg, BRAFmut or BRAF WT). Such approaches that depend on ranges of biomarker expression, instead of a single cut-point, may also require the implementation of digital pathology approaches to enable consistency in scoring across the field. This will require assay development from diagnostic companies in collaboration with the company's developing therapies for deployment in large Phase 3 clinical trials. Identifying and validating novel predictive biomarkers of sensitivity and resistance remains a critical need, along with developing model systems that can effectively replicate the human TME to understand MoA associated with immunotherapy response which could help identify biomarkers linked to underlying mechanistic biology.

UNDERSTANDING, PREDICTING, MANAGING, AND PREVENTING TOXICITY

The relationship between cancer immunotherapy sensitivity and toxicity is complex. While many studies have identified a modest correlation between toxicity and benefit from ICI, many patients develop toxicity without clinical benefit, while others experience clinical benefit without relevant toxicity. This suggests that the mechanisms driving response and toxicity do not fully overlap. The precise factors predisposing individuals to irAEs—including underlying vulnerabilities to autoimmune diseases, specific molecular pathways, and immune cells or mechanisms—remain incompletely understood.

Up to ~70% of patients treated with ICIs develop irAEs as a complication of treatment. irAEs can arise any time on treatment or after treatment discontinuation and can affect any organ system. The relationship between ICI responses and toxicity is not easy to analyze, thus, the precise molecular pathways and immune subsets underlying irAEs are not fully understood. At the cellular level, clonally expanded and highly activated $CD8^+$ T cells have been identified in single-cell analyses of irAE target tissues, along with a population of inflammatory $IL\text{-}1B^+$ $TNF\text{-}a^+$ myeloid cells that associate with more severe irAEs.[73] Th17 cells, and expression of the Th17-related cytokine IL-6, are increased in patients who develop irAEs, and in preclinical models IL-6 blockade has a dual function of both enhancing tumor rejection while simultaneously ameliorating autoimmunity.[74] Collectively, these data provide initial support that the toxicity and antitumor immunity can potentially be partially decoupled. The development of novel and more effective strategies for specifically treating, intercepting, and preventing irAEs is a major area of unmet need.

When combination cytotoxic chemotherapy was developed, it became clear that there were dose-limiting side effects that prevented optimal dosing and tumor control. With the development of immunotherapy, there are similar issues that need to be sorted out and thus an opportunity exists to rethink the delivery of immunotherapy to make it safer and possibly more effective. One challenge not faced with combination chemotherapy is that prevention of toxicities such as nausea and vomiting with antiemetics, profound myelosuppression and sepsis with granulocyte-stimulating and broad-spectrum antibiotics, and hemorrhagic cystitis with mesna would not limit the antitumor activity of the chemotherapy. However, generalized immune suppression may blunt the antitumor response of immunotherapy. Overall, the improved clinical efficacy of doublet ICI compared with monotherapy reveals that doublet therapy alleviates unique mechanisms of T cell inhibition driven by each inhibitory receptor, leading to enhanced antitumor efficacy along with the potential for immune-related toxicity. As the collective understanding of what drives toxicity as well as antitumor response improves, it is hoped that strategies will be developed to uncouple drivers of toxicity and response. In addition to improving the management of toxicity without mitigated

therapeutic benefit, there may be a possibility of building regimens to prevent, or at least reduce the risk of, toxicity and improve efficacy by allowing longer treatment with combination immunotherapy regimens. Further, there is an opportunity to generate biomarker strategies to identify which patients would be best candidates for lower-intensity or higher-intensity therapy (eg, single-agent anti-PD-1 versus doublet ICI blocking CTLA-4, PD-1, and LAG-3), as well as which patients are at greatest risk for toxicity to better understand the risks and benefits of therapy at the individual patient level. Finally, targeted drug delivery will allow for a wider therapeutic window as tumor-specific delivery of cytokines, small molecules, and checkpoints will enable intensification of therapy in the TME while limiting the effects of therapy on the rest of the body.

ACT and TCE immunotherapies are associated with several significant toxicities such as cytokine release syndrome (CRS), immune effector cell-associated neurotoxicity syndrome (ICANS), hemophagocytic lymphohistiocytosis (HLH), cytopenias, B-cell aplasia, and infections. CRS and ICANS are immune syndromes characterized by increased levels of serum cytokines and inflammatory molecules following CAR T, TCR T, and TCE but not commonly after TIL.[75] Immune effector cell-associated HLH-like syndrome is a rare but potentially life-threatening hyperinflammatory syndrome caused by concurrent CAR T cell and macrophage activation, which triggers a self-sustaining inflammatory loop. Cytopenias, including neutropenia, thrombocytopenia, and anemia, are prevalent in both ACT T and TCE treatments. In ACT treatment, the lymphodepletion regimen significantly impacts the onset, severity, and duration of both CRS, ICANS, and hematologic toxicities.[76 77] Finally, the risk of developing secondary neoplasia, in particular T-cell lymphoma, has been studied by multiple groups following the FDA's warning about the risk for secondary T-cell malignancies post-CAR T.[78] Both single-center reports and large registry or meta-analysis studies have indicated that the risk of secondary neoplasia after CAR T is approximately 3–6.5%.[79–83] Additionally, the occurrence of T cell lymphoma is rare, with only 22 reported cases out of more than 27,000 CAR T infusions, and even fewer cases involving transformation of CAR positive T cells.[84] It remains unclear whether transduction of autologous or allogeneic T cells with a viral vector expressing a CAR construct plays a causative role in these rare cases. Overall, although follow-up remains relatively short, the risk of secondary neoplasia and T cell lymphoma is not increased relative to similar populations of patients with hematologic malignancies who have been treated with chemoimmunotherapy (5–20%, based on specific treatment and length of follow-up)[85 86] but did not receive CAR T.[81] Looking forward, a more personalized and mechanistically grounded approach is essential to fully realize the potential of ACT and TCE therapies while minimizing patient harm. The integration of high-throughput tools, such as single-cell and spatial transcriptomics, proteomics, and metabolomics, is beginning to unravel the complex immune and tumor-intrinsic mechanisms that drive both therapeutic efficacy and immune-related toxicities. These platforms are enabling the discovery of novel mediators that can guide early risk stratification, inform pre-emptive interventions, and support the rational design of next-generation therapies. An urgent and underexplored area lies in the optimization of lymphodepleting chemotherapy, which plays a critical role in shaping the immune milieu contributing to CAR T expansion and toxicities. Comparative, prospective trials are needed to define the optimal lymphodepletion strategies that balance clinical efficacy with minimization of hematologic and immune adverse events. In parallel, the field must address emerging concerns about long-term safety, particularly the rare risk of secondary T cell malignancies, through long-term monitoring and mechanistic studies that are needed to clarify the underlying mechanism. Additionally, rational engineering of next-generation ACT products and combinatorial strategies with small molecules or immunotherapies should aim to decouple efficacy from toxicity. Finally, to ensure broader, more scalable access, it will be critical to implement remote and decentralized monitoring platforms and develop safer products that reduce the need for patient monitoring. Altogether, a future in which ACT and TCE therapies are safer, more effective, and accessible across diverse clinical settings will require coordinated innovation across biological insight, clinical trial design, and therapeutic engineering.

Lastly, there remains a true unmet need and critical opportunity to develop better models of immunotherapy efficacy, resistance, and toxicity. In particular, the TME is composed of tumor cells as well as various *cellular* (eg, fibroblasts, endothelial cells, neurons) and *non-cellular* factors (eg, extracellular matrix, secreted proteins, hormones, hypoxia, nutrients, metabolites) (figure 4). As such, the evaluation of microenvironment-specific features associated with immunotherapy resistance requires model systems capable of manipulating, modifying, and measuring one or more of these cellular and/or non-cellular factors. In addition to these microenvironment-specific factors, host-specific factors, such as sex hormones,[87] age,[88] and the gut microbiome[89 90] are increasingly appreciated as key determinants of tumor immunity that can impact overall organismal immune health and/or influence tumor-specific immune function. There are several approaches that can be undertaken including the development of patient-derived model systems, such as patient-derived xenografts, organoids or ex vivo studies using patient-derived tumor fragments. However, they have limitations due to their ex vivo character and lack of cross-talk between circulating immune cells and other factors relevant for systemic immunity, including immune cell priming and dynamics that take place in sites distal to the tumor including lymph nodes. Recent efforts have mostly focused on optimizing the technical setup and suitability of the respective models for immunotherapy (IO) research. In the future, the combination

of ex vivo models with high-dimensional single-cell and spatial-omics approaches may offer unique insights into the dynamic cellular interactions underlying therapy response, resistance, and even toxicity. As large cohorts are necessary to confirm the predictive value of ex vivo observations, prospective integration of patient-derived models in clinical trials will be critical in the coming years. While all models—at least to some extent—recapitulate immunotherapy-induced immune activation, it is important to be aware of key differences and current limitations, making it critical to select the right model for the right question (table 3). For example, co-culture models provide less complexity as compared with more "noisy" models such as patient-derived organotypic tumor spheroids, air-liquid interface patient-derived organoids or explants. The latter are, however, better suited for combination with spatial technologies that will become critical to address the emerging role of immune organization. One essential requirement for all models is access to high-quality tissue samples that will be important to

Table 3 Preclinical models

Mouse models	Uses	Challenges
Immunocompetent syngeneic models	▶ Frequently used to investigate ICI responses. ▶ Have been critical to discover the role of immune checkpoints such as PD-1 and CTLA-4.	▶ Lack of natural tumor growth and critical differences in immune components can make the translation of findings to patients challenging.[95] ▶ Many immunotherapies showed encouraging responses in mice but failed during clinical development.
Genetically engineered mouse models (GEMMs)	▶ Allow for more natural tumor development. ▶ Essential for understanding the impact of genetic alterations on the TME.	▶ Due to low mutation rates and neoantigen burden, responses to ICI have been modest.[96] ▶ Current efforts aim to facilitate the expression of de novo epitopes in these models (eg, NINJA mice[97]).
Humanized mouse models	▶ Mimic some aspects of the human immune system (immunodeficient mice reconstituted with human PBMC or hematopoietic stem cells). ▶ Allow to test human therapeutics or antibodies to human gene products.	▶ Develop prominent graft-versus-host disease a few weeks following human PBMC engraftment. ▶ Only partially functional immune systems after hematopoietic stem cell engraftment.[98]
Patient-derived xenograft (PDX) models	▶ Human tumor fragments are grown in immunodeficient or humanized mice. ▶ Predictive potential for targeted therapy responses.[99]	▶ Lack of tumor-stroma interactions in these models limits their current use for immunotherapy given that reduced T cell infiltration and inhibitory signals from tumor vasculature and stromal cells may be a common mechanism of resistance.[100]
Human modeling systems	**Uses**	**Challenges**
Classical patient-derived organoids (PDOs)	▶ Consist exclusively of tumor epithelium. ▶ Encouraging results in predicting sensitivity to chemo or targeted therapies.[101–107]	▶ Not suitable for immunotherapy research due to lack of immune compartment.
Reconstituted PDO models	▶ Organoids that contain one or more TME components. ▶ Allow preservation of tumor and TME components for weeks to months. ▶ Ex vivo system formats and culture setups:[108 109] – PDO co-cultures with individual or multiple TME components.[110–112] – Micro-organosphere (MOS) cultures established using droplet emulsion microfluidics.[113] ▶ Offer the possibility for genetic modifications to investigate the contribution of individual cell types or pathways.	▶ Enzymatic tissue dissociation into single cell tumor suspensions for PDO/MOS generation may alter epitope expression. ▶ Lack of original tumor architecture. ▶ Require the addition of growth factors, small molecule inhibitors and/or cytokines that by themselves may influence responsiveness to immunotherapies.
"en bloc" PDO or patient-derived explant (PDE) models	▶ Retain the native composition and structure of the TME. ▶ Formats and culture setups: – Patient-derived organotypic tumor spheroids (PDOTS, 40–100 µm): enzymatically digested tumor pieces in a microfluidic device.[114] – Air-liquid interface (ALI) PDOs: minced tumor samples embedded in a matrix and exposed to air on one side for better oxygenation.[115] – Patient-derived tumor fragments (PDTFs): ~1 mm 2-sized PDEs embedded in matrix preserving architecture and intratumor heterogeneity.[116] – Organotypic tumor slices: ~250 µm thick, microtome-generated tumor sections cultured either floating in medium or embedded in matrix.[117–120]	▶ Models with enzymatic tissue dissociation may display altered epitope expression and lack of architecture. ▶ Models with larger explant sizes often display substantial heterogeneity between individual explants. ▶ Preservation of the entire TME makes it challenging to link treatment effects to specific cell–cell interactions. ▶ PDE models are short-term cultures, in which tissues usually can be kept alive for a few days maximally. ▶ Genetic modifications to investigate the contribution of individual cell types or pathways have so far not been possible due to the presence of multiple cell types and retention of three-dimensional structure.

ICI, immune checkpoint inhibitor; PBMC, peripheral blood mononuclear cell; TME, tumor microenvironment.

consider in future diagnostic or therapeutic procedures. Successful model improvements would include systems that can address the lack of a systemic immune compartment or lymph nodes that critically contribute to antitumor immunity. In this regard, more complex culture setups are currently being explored, including bioreactors or tumor-on-a-chip technologies in which an artificial "blood flow" containing peripheral blood immune cells or multiple connected ex vivo compartments can be modeled. Finally, increased engagement with those in the autoimmunity field to gain insight into autoinflammatory states akin to irAEs offers an opportunity to identify targets that could uncouple antitumor immunity from irAEs and test promising compounds.

PATIENT PERSPECTIVE

Patients with cancer and their caregivers are greatly encouraged by the incredible advances in, and the growing list of viable options for, cancer treatments today. The expanding use and approval, and documented efficacy, of immunotherapy across multiple cancer types and in earlier stages is driving much of this encouragement and hope. In this evolving treatment environment, a greater number of caregivers are seeing loved ones respond more favorably and sustainably to these new cancer treatments than ever before. This truly is great news!

In the midst of this progress, however, lies the reality that a significant number of patients with cancer will not respond to these therapies, or will respond initially but not sustainably, and/or will experience difficult quality of life ramifications from their treatment. Unfortunately, there is not an assessment tool, resource, marker, test for each unique patient that can predict, with reasonable certainty, whether the patient will have a favorable response to treatment and/or will experience debilitating adverse effects. As a result, patients do not know if the treatment that is being recommended to them will work specifically for their unique disease situation, nor if they will suffer harm from the treatment along the way. There is great support from the patient community to develop new approaches to overcome immunotherapy resistance and to mitigate the adverse effects of treatment, as well as develop better tools to predict patient outcomes.

The patient community fully supports an aggressive pursuit by the field towards gaining a much "better understanding of the mechanisms of response, resistance, and toxicity," an understanding that will lead to the delivery of highly efficacious, and less harmful, patient-specific treatment pathways. Towards that end, the patient community fully supports the identification and prioritization of, and the actionable pursuit of solutions to, the "challenges" and "opportunities" listed above.

To maximize the impact of these initiatives, it will be crucial to connect into the lattice work of patient advocacy. This is important for several reasons. First and foremost, the patient advocacy community serves as the field's conscience, demanding that the patient remain at the center of the research. Receiving input from this community will help ensure that the field is continuously pursuing the kinds of patient-focused research that will help meet their most critical care-related needs to ultimately deliver to them clinical care that is both highly efficacious and as harmless as possible.

Second, any efforts to uncover the mechanisms of response and resistance will require obtaining high-quality research-only biopsies from patients who are currently dealing with "active" disease. As these biopsies can come at a risk to these individual patients without directly benefiting them in their specific cancer situation, the field will need the informed understanding and support of patients and their advocates to make these efforts successful.

Third, the patient advocacy community has continuously and successfully worked with the major funders of cancer research, namely the National Cancer Institute (NCI) and Department of Defense (DoD), to secure financial support for dedicated preclinical and clinical research of immunotherapy (ie, response, resistance, toxicity). Additionally, many cancer-specific philanthropic organizations exist because of patient advocacy and fund-raising efforts, and these organizations serve an important role in funding often complementary research to that supported by the NCI and DoD, as well as providing dedicated support to trainees and junior faculty.

For patients with the most advanced cancer presented with the option of immunotherapy, their #1 concern is not, *"What is the possible harm (irAE) that I might suffer at some unknown point in the future from this treatment?"* but rather *"Will this treatment work for me right now*?". This is not necessarily the case for patients with earlier stage cancer who are being presented with immunotherapy as a possible option for them. For a number of these earlier stage patients, surveillance is an option, and immunotherapy might be perceived as more of a "preemptive" approach than a "necessary" pathway, at least for their immediate future. For these patients, the potential for significant irAEs can be a much greater, and more important, consideration in their decision-making process. Their risk:benefit analysis may be more heavily weighted towards potential harm than towards possible efficacy.

Immunotherapy drugs are very powerful, and potentially very effective, treatment tools to help patients in their battle against cancer. Unfortunately, these drugs can also be highly toxic to patients, potentially causing a number of adverse events that can negatively impact a patient's quality of life. These adverse events can be temporary in nature and impact, or chronic. During a patient's treatment journey, it is possible to experience side effects at different times (during or post-treatment), with varying severity levels, potentially necessitating different treatment approaches, each of which may cause their own toxicity necessitating other interventions. All of these can have an incredibly negative impact on that patient's quality of life during treatment and potentially after treatment. Patients will greatly benefit, both in their

decision-making processes and in their quality of life, from having more fact-based prognostic information related to what the potential side effect risk is for them specifically.

CONCLUSIONS ON THE PATH AHEAD

Ultimately, it will take integration of multiomic clinical datasets and ex vivo model data, with refined therapy-specific clinical response and resistance definitions, to define biomarkers capable of predicting patient benefit (including reduced toxicity) and to fully optimize cancer immunotherapies moving forward. Global collaboration across academia, industry, and diagnostic partners will be key to progress, as well as consistent incorporation of the patient perspective and voice along the way.

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Acknowledgements The authors wish to thank SITC staff including Mary Dean for strategic guidance, Christina Mooney for project management and Kaitlin Johnson, PhD for editorial assistance. Additionally, the authors thank SITC for supporting the manuscript development.

Contributors RJS and CG-B served as co-chairs of the SITC Manuscript Development Group and provided guidance and oversight of the manuscript development. As such, they are listed as first and last authors. ARC, RLF, RWJ, HMK, MK, EJL, LP, WLR, MR, KAS, DST, JKT, and MY participated equally in the manuscript development process as well as provided critical review and conceptual feedback on manuscript drafts.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests RJS—Researcher: Merck. Consultant/Advisor/Speaker: BMS, Merck, Novartis, Pfizer, Marengo. ARC—Consultant/ Advisor/ Speaker: AboundBio. RLF—Researcher: AstraZeneca/Medimmune, Bristol Myers Squibb, Merck, Novasenta, Tesaro. Consultant/Advisor/Speaker: Adagene Incorporated; Consulting, Aduro Biotech; Consulting, AstraZeneca/MedImmune; Clinical Trial, Research Funding, Bicara Therapeutics; Consultant, Bristol Myers Squibb; Advisory Board, Clinical Trial, Research Funding, Brooklyn Immunotherapeutics LLC; Consultant, Catenion; Consultant, Coherus BioSciences; Advisory Board, Eisai Europe Limited; Advisory Board, EMD Serono; Consultant, Everest Clinical Research, Corporation; Consultant, F Hoffmann-La Roche; Consultant, Federation Bio; Consultant, Genocea Biosciences; Consultant, Genmab; Advisory Board, Hookipa Biotech GmbH; Advisory Board, Instil Bio; Advisory Board, Kowa Research Institute; Consultant, Lifescience Dynamics Limited; Advisory Board, MacroGenics; Advisory Board, MeiraGTx, LLC; Advisory Board, Merck; Advisory Board, Clinical Trial, Merus NV; Advisory Board Mirati Therapeutics; Consultant, Mirror Biologics; Data Safety Monitoring Board, Nanobiotix; Consultant, Novartis Pharmaceutical Corporation; Consulting, Novasenta; Consulting, Stock, Research Funding, Numab Therapeutics AG; Advisory Board, OncoCyte Corporation; Advisory Board, Pfizer; Advisory Board, PPD Development, LP; Consultant, Rakuten Medical; Advisory Board, Sanofi; Consultant, Seagen; Advisory Board, SIRPant Immunotherapeutics; Advisory Board, Tesaro; Research Funding, Vir Biotechnology; Advisory Board, Zymeworks; Consultant, Adagene Incorporated, Aduro Biotech, Bicara Therapeutics, Bristol Myers Squibb, Brooklyn Immunotherapeutics, LLC, Cantenion, Coherus BioSciences, Eisai Europe Limited, EMD Serono, Everest Clinical Research Corporation, F Hoffmann-La Roche, Genmab, Hookipa Biotech GmbH, Instil Bio, Kowa Research Institute, Lifescience Dynamics Limited, MacroGenics, MeiraGTx LLC, Merck, Merus NV, Mirati Therapeutics, Nanobiotix; Consultant, Novartis Pharmaceutical Corporation, Novasenta, Numab Therapeutics AG, OncoCyte Corporation, Pfizer, PPD Development LP, Rakuten Medical, Sanofi, Seagean, SIRPant Immunotherapeautics, Vir Biotechnology, Zymeworks, Adaptimmune—Advisory Board, CureVac—Advisory Board, Cytoagents—Advisory Board, Regeneron—Advisory Board, Royalty and Patent Beneficiary: potential for royalties in the future from Molecuvax; Other: Novasenta—Stock Holder, Mirror Biologics; Data Safety Monitoring Board, stock holder. RWJ—Consultant/ Advisor/ Speaker: Xsphera Biosciences (SAB member, ownership interest); Incyte (invited speaker), G1 Therapeutics (advisory board), Bioxcel Therapeutics (invited speaker). Royalty and Patent Beneficiary: RWJ has ownership interest in US patents US20200399573A9 and US20210363595A1. HMK—Consultant/ Advisor/ Speaker: Iovance, Merck, Chemocentryx, Bristol Myers Squibb, Signatero, Gigagen, Pliant Therapeutics, Esai, Invox, Wherewolf, Teva. MK—Consultant/ Advisor/ Speaker (compensated to the institute): Alderaan Biotechnology, AZ/Daiichi, Domain Therapeutics, BMS, Gilead, Medscape, MSD, Roche. Research funding to my institute from AZ/Daiichi, BMS, Roche. Non-financial research support by Natera. EJL—Consultant/Advisor: Agenus, Bristol Myers Squibb, CareDx, Eisai, Genentech, HUYA Bioscience International, Immunocore, Instil Bio, Lyvgen, Merck, Merck KGaA, Natera, Nektar, Novartis, OncoSec, Pfizer, Rain Therapeutics, Regeneron, Replimune, Sanofi-Aventis, Sun Pharma, Syneos Health. Institutional Research Funding: Bristol Myers Squibb, Haystack Oncology, Merck, Regeneron, Sanofi Stock: Iovance (less than US$10K). LP—Nothing to disclose. WLR—Researcher: Bristol Myers Squibb, Inhibrx, Veana Therapeutics, Shimadzu, Galecto, and CanWell Pharma. Consultant/Advisor/Speaker: Medicenna, Veana Therapeutics, Vesselon. Royalty and Patent Beneficiary: Galectin Therapeutics (belapectin; licensing fees). MR—Employee: University of Pennsylvania. Owner: viTToria biotherapeutics. Researcher: AbClon, Beckman Coulter, viTToria biotherapeutics, ONI, Orflo, Curiox. Consultant/ Advisor/ Speaker: AbClon, Sana Therapeutics, BMS, GSK, viTToria biotherapeutics, GLG. Guidepoint Royalty and Patent Beneficiary: CART technologies, University of Pennsylvania, partly licensed to Novartis, Tmunity, and viTToria biotherapeutics. Other: viTToria biotherapeutics; Scientific Founder. KAS—Consulting Fees: Shattuck Labs, EMD Serono, Clinica Alemana de Santiago, Genmab, Takeda, Merck Sharpe & Dohme, Bristol Myers Squibb, AstraZeneca, Agenus, Repertoire Therapeutics, OnCusp, Parthenon Therapeutics and Ariagen. Fees for Non-CE Services: Bristol Myers Squibb; Fluidigm Corporation; Genmab A/S; Merck & Co; and Takeda Pharmaceutical Company. Contracted Research: Navigate Biopharma, Tesaro/GSK, Moderna, Pierre Fabre, Takeda, Surface Oncology, Merck Sharpe & Dohme, Bristol Myers Squibb, AstraZeneca, Ribon Therapeutics, Akoya Biosciences, Boehringer Ingelheim and Eli Lilly. DST—Researcher: Bristol Myers Squibb, Asher Biotherapeutics. Consultant/ Advisor/ Speaker: iTEOS Therapeutics. JKT—Nothing to disclose. MY—Owner: Adventris. Researcher: Bristol-Myers Squibb, Incyte. Consultant/Advisor/Speaker: Genentech, Exelixis, Eisai, AstraZeneca, Replimune, Hepion, Lantheus. CG-B—Owner: Beyond the Codon, LLC. Publicly Traded Stocks: Bristol Myers Squibb. Employee: Bristol Myers Squibb.

Patient consent for publication Not applicable.

Ethics approval Not applicable.

Provenance and peer review Not commissioned; externally peer reviewed.

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