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Society for Immunotherapy of Cancer (SITC) consensus statement on essential biomarkers for immunotherapy clinical protocols

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ABSTRACT

Immunotherapy of cancer is now an essential pillar of treatment for patients with many individual tumor types. Novel immune targets and technical advances are driving a rapid exploration of new treatment strategies incorporating immune agents in cancer clinical practice. Immunotherapies perturb a complex system of interactions among genomically unstable tumor cells, diverse cells within the tumor microenvironment including the systemic adaptive and innate immune cells. The drive to develop increasingly effective immunotherapy regimens is tempered by the risk of immune-related adverse events. Evidence-based biomarkers that measure the potential for therapeutic response and/or toxicity are critical to guide optimal patient care and contextualize the results of immunotherapy clinical trials. Responding to the lack of guidance on biomarker testing in early-phase immunotherapy clinical trials, we propose a definition and listing of essential biomarkers recommended for inclusion in all such protocols. These recommendations are based on consensus provided by the Society for Immunotherapy of Cancer (SITC) Clinical Immuno-Oncology Network (SCION) faculty with input from the SITC Pathology and Biomarker Committees and the Journal for ImmunoTherapy of Cancer readership. A consensus-based selection of essential biomarkers was conducted using a Delphi survey of SCION faculty. Regular updates to these recommendations are planned. The inaugural list of essential biomarkers includes complete blood count with differential to generate a neutrophil-to-lymphocyte ratio or systemic immune-inflammation index, serum lactate dehydrogenase and albumin, programmed death-ligand 1 immunohistochemistry, microsatellite stability assessment, and tumor mutational burden. Inclusion of these biomarkers across early-phase immunotherapy clinical trials will capture variation among trials, provide deeper insight into the novel and established therapies, and support improved patient selection and stratification for later-phase clinical trials.

INTRODUCTION

The rapid evolution of immunotherapy and its integration as a pillar of cancer treatment has created opportunities and challenges for biomarker identification and selection in the design of clinical trial protocols. The "paradox" that tumors continue to grow despite immune recognition has been known for many years, since the pioneering work of the Hellstrom, identifying cytolytic immune cells within tumors.[1–3] Decades of research have since demonstrated that the twin factors of tumor cell genomic instability and the immense host immune repertoire drive the mutual evolution of the tumor and antitumor immune response over space and time.[4] This adaptive immune response draws on extraordinary diversity generated by combinatorial changes in the T and B cells. We now understand the process of malignant transformation and the tumor's interaction with immune cells over 7–10 years of coevolution involves substantial cancer editing through three defined phases (elimination, equilibrium, and escape) culminating in tumor progression.[5]

According to the National Institute for Health (NIH), biomarkers are characteristics that can be objectively measured and

Table 1 Comparing biomarker classification systems.

	SITC recommendations	NCI recommendations
Clinical trial types emphasized	Early-phase immunotherapy clinical trials	Large (≥100 patients), randomized phase II treatment trials or in any randomized phase III clinical trials
Content	Prioritization framework and recommendations for specific biomarker tests to standardize clinical trial design and data reporting	Prioritization framework to support funding of biomarkers in clinical trials
Biomarker categories	**Essential** A list of biomarkers recommended for inclusion and data reporting for all early-phase immunotherapy clinical trials **Eligibility** A subset of essential biomarkers that are relevant only in a particular trial context (eg, tumor type or treatment-specific biomarkers) **Emergent** Potential future essential biomarkers, pending data, standardization of methodology, and/or feasibility (eg, affordability or reimbursement)	**Integral** ► A class of biomarkers that are central to the design of a specific trial and required for all patients ► Supports a trial hypothesis ► Used in the design and conduct of the trial: for example, for eligibility, randomization, stratification, or treatment assignment **Integrated** ► Included for validation of potential future integral biomarkers ► Includes a hypothesis and preplanned statistical design ► Included as a secondary objective

NCI, National Cancer Institute; SITC, Society for Immunotherapy of Cancer.

used as indicators of normal biological processes, disease processes, or pharmacologic responses to therapy.[6 7] Biomarkers identified within the tumor cells themselves and the associated tumor microenvironment (TME) (eg, fibroblasts, endothelium, and immune cells) provide an invaluable biologic context for the development and evaluation of immunotherapies.[8–12] For those entering the field of immuno-oncology (IO) and preparing novel clinical protocols, guidance is needed to prioritize biomarkers of demonstrated utility. The Society for Immunotherapy of Cancer (SITC) has previously developed an informative checklist to guide the design of high-value Phase III clinical trials.[13] We proposed similarly that IO clinical trial evaluation involves a set of biomarkers to define eligibility and those that are essential, even in the earliest of trials.[14] For clarity, we have removed the previously suggested "level" labels in favor of the intuitive categories themselves of essential, eligibility, and emergent (table 1). We proposed in our previous commentary on the measurement and interpretation of biomarkers for early clinical trials, that essential markers should have: (1) strong evidence of clinical relevance and/or biological relevance, (2) broad support across tumor types and treatment approaches, and (3) high feasibility (ie, standardized testing methods, routine or readily available testing).[14] The eligibility tier captures feasible markers with largely the targets of the immunotherapy in specific contexts. A subset of immunologic therapies thus is targeted, using largely antibodies or cells to enable antitumor efficacy only in a subset of individual tumors usually expressing a cell surface molecule (eg, Her2/neu, EGFR). For this purpose, we have defined these markers as eligibility, given that the expected clinical utility would be more limited, recognizing that they do not meet the other essential criteria of greater pan-tumor use but represent special cases. Candidate markers with limited evidence of clinical utility or lacking a well-described, analytically validated assay are relegated to the emergent tier, with the expectation that they will eventually establish their value and move into the essential or eligibility class, or else drop out of consideration. Furthermore, we provide criteria for the evaluation of promising emergent biomarkers to allow promotion to essential based on sound scientific principles and guided by suitable fiscal support. While the National Cancer Institute (NCI) terminology of integrated, integral, and investigative biomarkers defines ways in which biomarkers may be incorporated into clinical trial protocols, the recommendations for essential, eligible, and emergent biomarkers are based on evidence supporting standardized adoption of specific biomarker tests and data reporting practices across IO clinical trials.[15] We plan to make this a living document and review and revise it every few years or as necessary based on rapid advances in the field.

CONCEPTUALIZING BIOMARKER PRIORITIZATION: ESSENTIAL, ELIGIBILITY, AND EMERGENT BIOMARKERS

Here we examine the critical elements for early-phase clinical trial design (although many of the principles of course extend to late-stage protocols) focusing on essential, eligibility biomarkers, and exploratory or

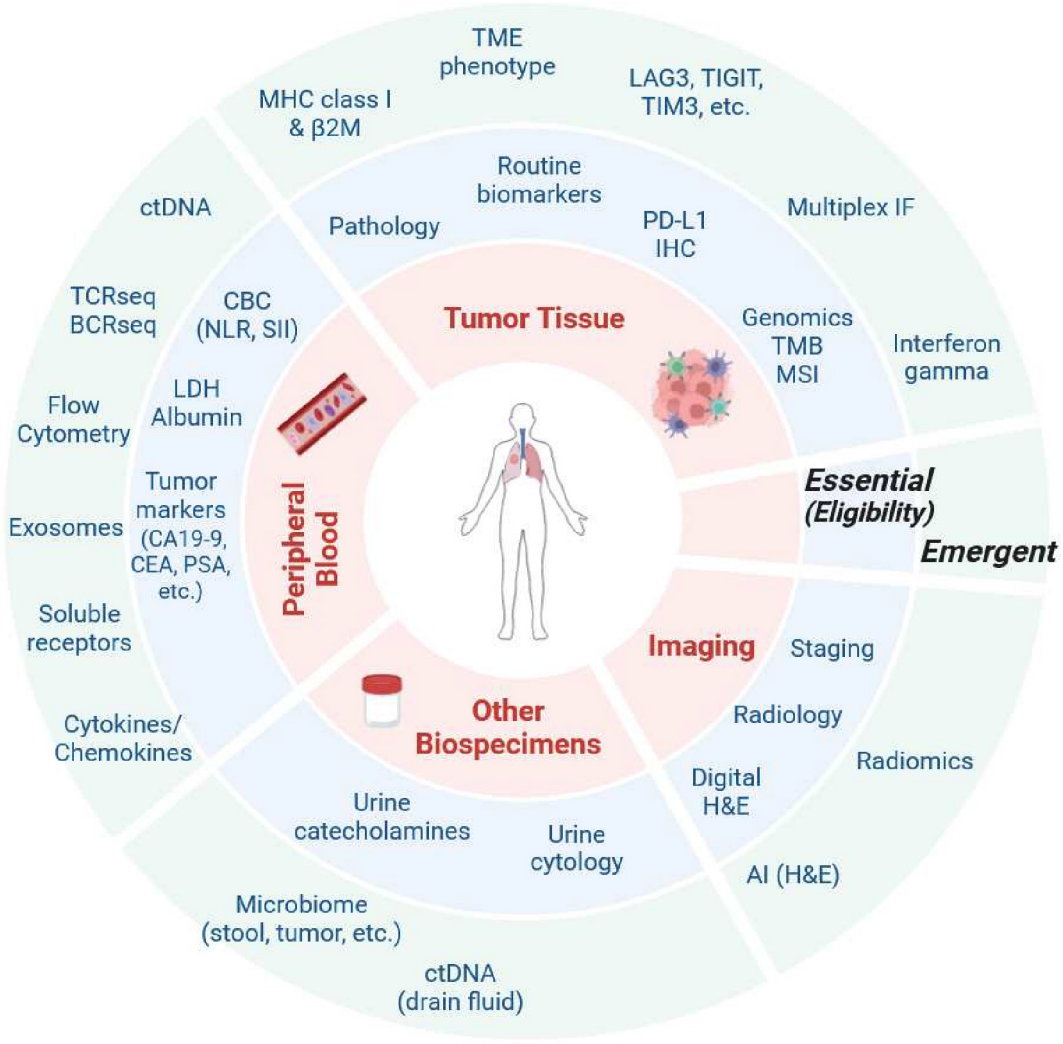

Figure 1 Essential, eligibility, and emergent biomarkers for early-phase immunotherapy clinical trials. Biomarker testing performed on peripheral blood, tumor tissue, and other biospecimens or imaging data (red) provides insight into the interacting systems of cancer cells, the tumor microenvironment, and the patient's immune system that dictate immunotherapy outcomes. Essential biomarkers (blue) were selected based on strong evidence of biological and/or clinical relevance across tumor types as well as high feasibility for testing (see also box 1, table 2). Essential biomarkers also include tumor-specific biomarkers that are used in routine clinical care. A subset of essential biomarkers, eligibility (or integral) biomarkers are incorporated into the design of a clinical trial, that is, required for patient enrollment. Emergent (or exploratory) biomarkers (green) have early evidence of biological and/or clinical relevance but may be limited by a lack of standardization or data across tumor types (see also tables 2 and 3). AI, artificial intelligence; β2M, β2-microglobulin; BCRseq, T cell receptor sequencing; CBC, complete blood count; ctDNA, circulating tumor DNA; IF, immunofluorescence; IHC, immunohistochemistry; LDH, lactate dehydrogenase; MHC, major histocompatibility complex; MSI, microsatellite instability; NLR, neutrophil-to-lymphocyte ratio; PD-L1, programmed death-ligand 1; SII, systemic immune-inflammation index; TCRseq, T cell receptor sequencing; TMB, tumor mutational burden; TME, tumor microenvironment.

emergent biomarkers in modern immunotherapy protocols (figure 1). By essential, we mean those that are clinically relevant in multiple tumor settings, either predictive of response to therapy or prognostic for important endpoint variables such as disease-free survival (DFS), progression-free survival (PFS), and overall survival (OS). Examples of PFS measures that have been validated in many tumor types both for conventional chemotherapeutic approaches as well as now for immunotherapy include neutrophil-to-lymphocyte ratio (NLR) or 'systemic imune-inflammation index' (SII). Measures of the tumor similarly associated with severity include conventional tumor markers (prostate specific antigen (PSA), carcin-0embryonic antigen (CEA), CA19.9, CA125) and serum levels of lactate dehydrogenase (LDH) and albumin. For B cell malignancies, minimal residual disease assessments by flow cytometry or next-generation sequencing have informed survival analyses for antibody-drug conjugates (ADC),[16] bispecific T cell engagers[17] and chimeric antigen receptor (CAR) T cells.[18 19] These measures should be included in every early-stage protocol as they are readily available, cost-effective, and components of current practice at most institutions.

For agents requiring the positive expression of individual proteins or markers (eg, mutant KRAS for T cells targeting this molecule, programmed cell death protein-1 (PD-1) or programmed death-ligand 1 (PD-L1) on immune or tumor cells, or overexpressed EGFR for antibodies targeting these molecules), entry criteria defining eligibility for therapy are now well-defined and should be specified in the protocol synopsis. To assess both the nature of the tumor and the host response, other essential and exploratory (emergent) measures should be specified. Emergent tumor biomarkers include circulating tumor (ct)DNA or circulating tumor cells (CTCs) that are not yet fully validated and incur additional costs. Similarly, promising emergent markers of the immune response include diversity and clonality of T and B cells, expression of major histocompatibility complex (MHC) Class I and the associated β2-microglobulin (β2M), and the presence of tertiary lymphoid structures (TLS) within the tumor. Grading of immune infiltrate[20] within the tumor (infiltrated), only within the stroma (excluded), and without substantial infiltrate (immune desserts)[21] is an area being carefully considered by the pathology committee and leadership of SITC. Many of these emergent biomarkers will be examined as resources, validated methodology in Clinical Laboratory Improvement Amendments (CLIA)-approved laboratories, and scientific merit allow for consideration as essential biomarkers.

Predictive versus prognostic biomarkers

All biomarkers provide information about the state of a biological process at a discrete time point (eg, a patient's tumor, immune system, metabolic health). Prior to initiation of therapy, baseline biomarkers serve as a vehicle to define, in part, the status of the tumor and the host response. Longitudinal tracking of biomarkers can be valuable for detecting alterations in tumor biology and/or treatment response. A distinction is often made between prognostic biomarkers versus predictive biomarkers.

Prognostic biomarkers relate tumor and host variables to clinical outcomes independently of treatment and can be identified using various statistical and machine-learning tools.[22] Tumor-related factors include variables that reflect its biology and pathology, such as tumor size, lymph node involvement, metastasis, and molecular markers such as PTEN gene overexpression and the presence of the androgen receptor variant AR-V7. Standardized reporting of prognostic tumor-related histologic features is included in all diagnostic pathology reports (eg, as per College of American Pathologists, CAP guidelines). Host-related factors pertain to patient characteristics that impact survival outcomes, including Eastern Cooperative Oncology Group performance status and comorbidities. These biomarkers can be used to: (1) stratify randomization by disease risk, reducing heterogeneity within subgroups and maximizing it across subgroups; (2) identify potential treatment targets; and (3) direct therapy to specific patient subgroups. For example, in a recent trial with 1,311 patients with advanced prostate cancer, randomization was stratified according to the predicted 24-month survival probability, determined by a prognostic model of OS.[23 24] This approach was used to balance critical baseline factors between the two treatment arms.

Predictive biomarkers are linked to treatment responses and help identify patients whose tumors are more likely to benefit from or be resistant to specific therapies. Predictive biomarkers are often directly related to the mechanism of action of a particular cancer therapy (eg, molecular targeted therapies or PD-1/PD-L1 blockade). Predictive biomarkers are typically discovered through retrospective analysis of large, randomized phase II and phase III trials. When the outcome is a time-to-event endpoint that is subject to censoring, the Cox proportional hazards (PH) model is employed. To ascertain whether a biomarker is predictive of the treatment being studied, a statistical test of interaction between the biomarker and the treatment is performed.[25] Significant resources are dedicated to discovering and validating predictive biomarkers, which are essential for the effective delivery of personalized medicine. For instance, patients with breast cancer with estrogen and progesterone receptors are treated with endocrine therapy as they are expected to respond well to it, while HER2-positive patients benefit from trastuzumab treatment. Similarly, in patients with advanced prostate cancer, several drugs targeting the androgen receptor axis are approved for treating patients with advanced disease. Developing genomic signatures provides a valuable opportunity to identify patients who are most likely to benefit from specific therapeutic options. Genomic classifiers have been widely applied in phase III clinical trials. For instance, Oncotype DX has been used to select patients for the TAILORx trial.[26] Similarly, the Decipher signature, a 22-gene genomic classifier designed to predict metastasis in patients with prostate cancer following radical prostatectomy has been employed as a stratification variable for randomization in a phase III trial.[27]

It is important to note that some biomarkers can be prognostic, predictive, or both. For example, the presence of estrogen and progesterone receptors serves as both prognostic and predictive factors for the efficacy of hormonal therapy. In the context of a rapidly expanding arsenal of immunotherapeutic strategies, the overlap between prognostic and predictive biomarkers is significant.[28] For example, many studies have identified the expression of PD-L1 as a prognostic feature in retrospective cohorts of patients with cancer who did not receive anti-PD-1 therapy.[29–32] Conversely, in most instances the presence of tumor-infiltrating neutrophils is a well-established biomarker of poor prognosis that will likely be a useful predictive biomarker for emerging neutrophil targeting therapies.[33] Still, some recent studies suggest that neutrophils may be important immune effectors in the setting of effective T cells within the tumor.[34] Moreover, a single therapy may have multiple mechanisms of action, for example, tumor cell cytotoxicity and immune activation effects of traditional chemotherapy.[35] Combination

therapies seem to have synergistic efficacy, such as PD-1 blockade plus chemotherapy. Unfortunately, changes in clinical practice are often outpacing the research needed to understand the underlying mechanisms driving treatment response.

Given the ambiguity, overlap, and fluidity of biomarker classification into prognostic and predictive subsets, we have opted to de-emphasize the distinction between prognostic and predictive biomarkers in favor of focusing on the biological rationale, available data, and practical feasibility when evaluating candidate biomarkers for early-stage immunotherapy clinical trials.

Role for defining stratification in future trials

Implementing a requirement for essential biomarkers also allows some insights into variable responses and outcomes that may be useful in stratifying patient randomization and post hoc analysis based on informative biomarkers. For example, the NLR has been demonstrated to be prognostic in many protocols.[36] Clinical outcome models will likely be heavily employed for both patient selection and stratifying randomization in future trials. It is expected that artificial intelligence (AI) and machine learning will likely expedite the development and validation of these models from both clinical trials and real-world data. We also anticipate that serial measurements will be used in these models. The notion of enriching early-phase clinical protocols with patients more likely to respond based on these markers is undergoing active consideration in SITC.

PROCESS DESCRIPTION-COMMENTARY, CALL FOR SUGGESTIONS; SCION AND SITC COMMITTEE REVIEW

Five years ago, SITC leadership approved the development of a hands-on intensive SITC Clinical Immuno-Oncology Network (SCION) workshop to promote and extend the best science in meaningful early-phase clinical protocols. Now with a few years of experience with this successful program, we realize that informative biomarkers could be more easily considered as essential, eligible and emergent. An engaged SCION faculty group working closely with the established SITC Biomarker and Pathology committees constructed a commentary, requesting nomination and consideration of candidates for essential biomarkers.[14] Furthermore, this current essential biomarkers manuscript has been reviewed and commented on by the faculty of SCION (including oncologists, immunologists, and biostatisticians as well as the lead patient advocate) who have reviewed and approved it prior to publication based on their experience and thus are part of this consensus document.

To develop consensus concerning the inclusion of biomarkers across the "Essential" and "Emergent" sections, participants took part in an anonymized Delphi survey effort. All authors were asked to provide agreement or disagreement for each individual biomarker within both sections. Biomarkers that met a 75% threshold of agreement were included within each respective list. The minimum level of consensus reached for both sections was 80%. Additionally, survey participants were offered the opportunity to provide comments on each respective biomarker, all of which were considered during the drafting of each section here.

Box 1 Goals and criteria for essential biomarkers

Goals

⇒ Accelerated identification of active agents.
⇒ Early elimination of toxic and non-effective treatments.
⇒ Development of means to assess the patient's tumor for likelihood of response.
⇒ Allow comparison between studies performed early in an agent's evaluation, allowing assessment of tumor virulence and immune competence in individual patients.

Criteria

⇒ Strong evidence of clinical and/or biological relevance.
⇒ Broadly relevant to the patient's tumor response and outcomes across tumor types and treatment approaches.
⇒ High feasibility (routinely collected as part of routine clinical care or readily available).

CRITERIA FOR ESSENTIAL BIOMARKERS

We are hopeful that the goals and criteria for essential biomarkers (box 1) that we have detailed meet the approbation of colleagues across the regulatory and the NCI Clinical Trial Network cooperative groups as we integrate immune biomarkers into the more conventional, well-established measures of tumor biology and required elements in clinical protocols.

We can readily agree on a few principles: essential markers should have clinical trial-proven relevance in multiple tumor types and stages of the disease, and consistent evidence of broader utility from preclinical models and preliminary human studies. There must be assays available with clear protocols and demonstrated analytic validity when applied in independent labs (eg, CLIA certification), and correlation between competing assays, but are necessarily case dependent and even then, difficult to agree on. We suggest that the markers included in the essential tier here (table 2) were selected because we already routinely measure them in immunotherapy studies. We readily invest scarce research resources to measure PD-L1 because it is proven in many diseases to yield specific, actionable information about our patients, and because we want to know what role it plays, if any, in other contexts. Its development as an assay was hard-won and required agreement between pathologists and oncologists in its validation.

For all immunotherapy clinical trials, high-value biomarkers are associated with patient outcomes across multiple tumor types. This rationale is based on the idea that immunoregulatory mechanisms are

Table 2 Essential biomarkers for early-stage immunotherapy clinical trials

Essential biomarker(s)	Details	Role	References
Patient-level (systemic immunity and tumor burden)			
Baseline peripheral blood biomarkers	► Lactate dehydrogenase ► Albumin ► CBC with differential (eg, neutrophil–lymphocyte ratio) ► Circulating tumor marker levels (eg, CA19-9, CEA, CA125, PSA)	Tumor Nutrition/host Host Host	95 281–284
Clinical stage	Per AJCC guidelines/NCCN guidelines https://www.nccn.org/guidelines/category_1	Tumor	
Radiographic imaging	Pretreatment and on-treatment (eg, CT and/or PET scans)	Tumor	76
Cancer cells			
Tumor grade and stage	► Histologic subtype ► Pathologic stage and features (eg, per AJCC/CAP guidelines)	Tumor Tumor	95
Routine biomarkers	Disease site-specific (eg, hormone receptors, PSA, CA19-9, CA125, Ki67, viral status)	Tumor	122 285–287
Genomic sequencing (if routine clinically)	► Driver mutations ► MSI ► Tumor mutational burden	 Tumor Tumor Tumor	285–287
Tumor microenvironment			
PD-L1 IHC	Tumor proportion score, combined positive score	Tumor	288
Digital pathology	H&E pretreatment tumor (TLS, TIL, etc)	Host	184

AJCC, American Joint Commission on Cancer; CAP, College of American Pathologists; CBC, complete blood count; CEA, carcinoembryonic antigen; IHC, immunohistochemistry; MSI, microsatellite instability; NCCN, National Comprehensive Cancer Network; PD-L1, programmed death-ligand 1; PET, positron emission tomography; PSA, prostate specific antigen; TIL, tumor-infiltrating lymphocytes; TLS, tertiary lymphoid structures.

often agnostic to tumor types, such as the association of PD-L1 expression or microsatellite instability (MSI)-high with response to immune checkpoint blockade (ICB). Variation in the biology and immune context by tumor site of origin is well documented[37] and we acknowledge that an immune phenotype (eg, immune desert) may predominate among tumors from a particular site. However, particularly in early-phase clinical trials, clinically actionable biological insights can be gained by capturing the heterogeneity within a tumor type. A notable example is the recognition of a single patient with anti-PD-1 responsive colorectal cancer (CRC) leading to the identification of mismatch repair deficiency as a tumor-agnostic biomarker for anti-PD-1 therapy.[38]

Recognizing practical constraints and the burden on patients associated with clinical trials, the essential biomarkers are currently limited to reimbursable tests performed in routine clinical practice. These essential biomarkers should also be included in all early-phase clinical trials, as they are readily available at most hospitals. This is contrasted with the many exciting emergent biomarkers discussed below, which can be associated with specialized collection strategies and measurements, some at substantial cost, precluding their application beyond research institutions and academic medical centers. This is an exciting area and emergent biomarkers will be added to future updates as essential when the consensus from our consultant groups concurs that they have met the essential biomarker criteria.

While the recommendations for essential biomarkers are based on data supporting biological relevance and associations with patient outcomes across tumor types, we acknowledge that there will be specific contexts in which a single essential biomarker may not predict treatment outcomes. Importantly, emerging data suggest analysis of multiple biomarkers improves outcome predictions,[37 39–41] likely as a result of capturing relevant interacting tumor, immune, and patient-related factors. Our strong recommendation that the essential biomarkers be included in all early-phase immunotherapy clinical trials is intended to provide clear guidance and standardization for the field. While this is not a substitute for regulatory guidance, this expert consensus is intended to synthesize the best available evidence to align relevant stakeholders from new clinical trialists to regulatory bodies.

ESSENTIAL BIOMARKER DATA COLLECTION RECOMMENDATIONS

Patient data and peripheral blood biomarkers

Patient demographics including performance status and immune function are necessary as per conventional protocol entry criteria. These would include broad well-validated screening assays such as complete blood count and differential (also allowing calculation of the NLR), LDH, albumin levels, and a comprehensive metabolic panel. Their further explication is beyond the scope of this article and there are multiple well-considered and detailed rationales published for their inclusion in both immune-based and conventional therapies.[42–44]

Neutrophil-to-lymphocyte ratio, systemic immune-inflammation index

NLR has emerged as an important and we believe essential biomarker that predicts better response to ICB.[45–48] This has been confirmed in patients with many tumor types and not only checkpoint therapies but also tumor-infiltrating lymphocytes (TIL) adoptive transfer, interleukin-2 (IL-2) therapy, and even several chemotherapeutic agents (that likely mediate some of their efficacy through immune mechanisms). These include those patients with melanoma,[45 49] non-small-cell lung carcinoma (NSCLC),[47 50 51] small cell lung carcinoma,[52] gastric cancer (GC),[53] renal cancer,[54] and pancreatic cancer.[55] Those individuals receiving TIL therapy for melanoma responded better to therapy when they had a low NLR.[56] Similar observations have been made in response to chemotherapy and radiation therapy for patients with a variety of neoplasms including NSCLC, renal cancer, and ovarian cancer. We believe that the neutrophil increases that are observed reflect increased circulating mediators promoting myelopoiesis as well as a reflection of tumor necrosis with recruitment of nominally immunosuppressive neutrophils.[57–59] Similarly, the so-called systemic immune-inflammation index (SII) is a biomarker of inflammation and immune competence. The SII represents both the peripheral blood platelet count and the NLR and is a biomarker of inflammation and immune competence. In some contexts, the SII is more reflective of patient outcome than NLR alone, such as in the case of patients with pancreatic cancer receiving exogenous granulocyte colony-stimulating factor who experience poorer responses on average when receiving conventional neoadjuvant chemotherapy.[57 59] This could be formally tested in randomized, prospective trials in this or other neoplasms.

Albumin ± total protein

Albumin and total protein levels are essential biomarkers in clinical trials for cancer, providing valuable insights into the patient's nutritional status, liver function, and overall systemic health.[60] Albumin, the most abundant plasma protein, plays a crucial role in maintaining oncotic pressure and serving as a carrier for various endogenous and exogenous substances. Hypoalbuminemia is associated with poor prognosis in patients with cancer, reflecting malnutrition, systemic inflammation, and advanced disease stages.[61–63] Total protein, which includes albumin and globulin fractions, gives a broader overview of the protein status in the body. Together, these biomarkers can help predict treatment outcomes, guide nutritional and therapeutic interventions, and monitor disease progression.[64 65]

In clinical trials, baseline levels of albumin and total protein can stratify patients, identify those at higher risk of adverse outcomes, and tailor treatment plans accordingly. Moreover, albumin and total protein levels can serve as surrogate markers for other underlying conditions, such as liver dysfunction or chronic inflammation, which may impact the patient's response to treatment. By integrating albumin and/or total protein measurements into clinical trial protocols, researchers can enhance patient stratification, optimize therapeutic strategies, and ultimately improve the overall management and outcomes for patients with cancer.

Lactate dehydrogenase

Data across tumor types and global patient populations consistently confirm an association between elevated serum LDH and poor survival outcomes[23 66–69] including in those treated with ICB therapy.[11] A recent review of 22,882 patients found elevated LDH levels (median serum LDH of 245 units per liter (U/L) in these studies with adult LDH levels ranging from 140 to 280 (U/L)) that were associated with an HR for OS of 1.7 (95% CI 1.62 to 1.79; p<0.00001).[69] The effect was most prominent in patients with renal cell, melanoma, gastric, prostate, nasopharyngeal and lung cancers.

Potential biological impacts of elevated LDH include metabolic alterations associated with tumor necrosis, lactic acidosis within the tumor bed, and other measures of debris released from the tumor including HMGB1 and ATP.[70 71] LDH is a tumor marker associated with so-called Warburg metabolism with aerobic glycolysis.[71] Multiple studies suggest LDH levels may correlate with other prognostic biomarkers, including NLR and C-reactive protein (CRP).[72–75] An intriguing study of 418 patients with solid tumors treated with ICB with PD-1/PD-L1 found a correlation between post-treatment LDH and more severe immune-related adverse events (irAEs).[71] Thus, we recommend including a baseline and post-treatment LDH level in all patients with advanced disease on immunotherapy trials as essential. Although a majority (>75% of those polled) considered it essential it was deemed "less" essential than N/L ratio and MSI status.

Radiology/radiomics

Conventional assessment of tumor therapies has traditionally relied on radiographic measures of tumor response, such as the RECIST (Response evaluation criteria in solid tumors) criteria and is deemed essential. Initially established in 2000, RECIST has undergone multiple revisions, culminating in RECIST V.1.1 and

iRECIST, the latter specifically tailored for evaluating immunotherapy (although iRECIST is not approved or used by regulatory agencies for registration currently).[76] These standardized criteria are essential in clinical drug development but exhibit certain limitations. They are a one-dimensional measurement of the sum of the longest diameter of up to five lesions, defined as "target lesions", selected by a radiologist according to lesion size and characteristics, and measurement repeatability, thus subjected to intrareader and inter-reader variability. Furthermore, as the tumor size of the largest lesions is not the only driver for treatment success, (part of) the relevant tumor burden might not be represented by RECIST. Therefore, various groups are investigating other treatment evaluation approaches based on radiological images, such as volumetric measurements,[77] the inclusion of total tumor volume,[78] modeling of tumor growth and decay kinetics,[79] and the inclusion of other characteristics such as tumor density.[80] Despite their potential, these methods have not yet become standard practice and lack a comprehensive view of the tumor characteristics.

In the last decade a new comprehensive and non-invasive approach to tumor characteristics as visualized at imaging has been proposed and widely studied. This approach, called radiomics, can add important information, not only for the prediction of treatment response, but also for diagnosing tumor biology non-invasively.[81] Radiomics quantifies various tumor features, such as size, shape, texture, and intensity, and enables the building of predictive models combining radiomic features into tumor signatures. It has been demonstrated that different types of biologic profiles, such as PD-(L)1 status, EGFR, and ALK mutations, can be detected or quantified solely from radiological images.[82] Treatment outcomes have been predicted based on baseline images alone or by combining baseline and early follow-up images, according to a methodology known as delta-radiomics.[83 84] Radiomics can be applied to all diagnostic images, including CT, MRI, ultrasound, and PET (positron emission tomography). PET has become over the past two decades a crucial tool in IO as it provides metabolic and functional information on tumors, fundamental to identify active cancer regions, assess metabolic activity, and monitor treatment response.[85] Most recently, new PET tracers such as those targeting prostate specific membrane antigen (PSMA) emerged as powerful tools to be used both for the detection and delivery of drugs (known as theranostic agents), making PET imaging not only suitable for whole body disease evaluation, and treatment monitoring, but also to drive personalized treatment planning.[86] The application of radiomics to PET imaging along with CT and MRI can exploit the unveiled characteristics of tumor heterogeneity in both underlying biology and treatment response. Some radiomic measures may be used or developed to assess changes in immune infiltrate and the presence of TLS.

Pathology staging

Multiple CAP-suggested measures of tumor biology following resection have been previously identified as essential and include grade, nodal involvement, resection margins and other well-defined characteristics associated with prognosis. These include neural invasion (NI) and lymphovascular (lymphatic) invasion. NI, the invasion of cancer cells into the nerves, influences the pathological characteristics of malignant tumors. In a meta-analysis on the influence of NI on survival and tumor recurrence in pancreatic ductal adenocarcinoma, NI was significantly associated with decreased OS, DFS and PFS. As a strong independent prognostic factor for decreased OS and early tumor recurrence in pancreatic ductal adenocarcinoma (PDAC), it was suggested that patients may benefit from adaptation of their adjuvant therapy regimens according to their severity of NI.[87] Similarly, with peri-NI (PNI), the invasion of cancer cells into tissues surrounding the nerves, PNI was reported as a predictive and prognostic indicator for head and neck cancer, oral squamous cell carcinoma, and GCs.[88–90] Although patients had significantly poorer DFS and OS, the presence of PNI was also an indication for adjuvant therapy, where patients benefited from additional targeted radiation therapy.[88 89]

Lymphatic invasion (LI) is the invasion of cancer cells to regional lymph nodes and distant sites. In the reporting of LI, many studies have reported LI and vascular invasion as a single variable of lymphovascular invasion (LVI). For instance, LVI has an independent negative prognostic impact on early-stage breast cancer OS, DFS and MFS,[91] LVI-positive patients with CRC have up to a 55% decrease in OS and significantly reduced DFS,[92] and from 16 observational studies, LVI was associated with nodal metastases in CRC.[93] However, given the difference in LI and vascular invasion in esophageal squamous cell carcinoma prognosis,[94] LI should be reported separately from vascular invasion according to the eighth American Joint Commission on Cancer (AJCC) Cancer Staging Manual.[95] As a single variable, in a retrospective study of 396 patients with thoracic esophageal squamous cell carcinoma, LI is an independent predictive factor for DFS, and patients with LI were more likely to suffer nodal metastasis.[96]

Nodal and metastatic assessment is essential as early detection of nodal involvement or metastases allows for appropriate treatment strategies. In early GC, the presence of lymph node metastasis is a significant prognostic factor that influences the choice of treatment and prognosis; the 5-year survival rate decreases with increasing numbers of positive nodes.[97] Metastasis to tumor-draining lymph nodes by breast cancer cells is also correlated with poor prognosis and is associated with local immunosuppression with accumulation of CD80-expressing regulatory T cells.[98] Apart from nodal metastasis, distant metastasis is another main cause of treatment failure in patients with cancer. In locally advanced rectal cancer, stratification of patients at high risk for distant metastasis identified patients who can benefit from adjuvant chemotherapy.[99]

Programmed death-ligand 1

PD-L1 is a well-established biomarker to guide patient selection for ICB treatment and can be quantifiable using immunohistochemistry (IHC) assays in terms of Tumor Proportion Score (TPS) which assesses the percentage of PD-L1 positive tumor cells or Combined Positive Score (CPS) which assesses the ratio of PD-L1 positive tumor and immune cells to tumor cells. Several studies have shown that higher PD-L1 expression was associated with better ICB response and prognostic outcomes. For instance, a recent study found that CPS≥5 was the only predictor significantly associated with survival in multivariable analyses, including tumor mutational burden (TMB), MSI, or Epstein-Barr virus (EBV) in GC. The overall response rate (ORR) in patients with GC increases with increasing CPS.[100] Similarly, the KEYNOTE-059 trial also reported higher ORR in the PD-L1 CPS≥1 group than in the CPS<1 group in pembrolizumab-treated advanced patients with GC.[101 102] In advanced triple-negative breast cancer (TNBC), the combination of atezolizumab with nab-paclitaxel resulted in a clinically meaningful improvement in the OS of patients with positive PD-L1 expression.[103]

In contrast, other clinical trials involving various cancer types have also concluded that the benefit of ICB was irrespective of PD-L1 expression. From the KEYNOTE-024 trial, pembrolizumab-treated patients with NSCLC had better OS and response rates than chemotherapy-treated patients, irrespective of PD-L1 expression (TPS≥50%, ≥20%, ≥1%).[103 104] Similarly, CheckMate 141 reported that nivolumab significantly improved OS in head and neck squamous cell carcinoma (HNSCC) irrespective of PD-L1 expression[105] and KEYNOTE-045 reported that the benefit of pembrolizumab in patients with advanced urothelial cancer appeared to be independent of PD-L1 expression on tumor and infiltrating immune cells.[106] On that note, a longitudinal analysis comparing matched early-stage (preoperative and surgical samples) and relapsed NSCLC revealed dynamic changes in PD-L1 expression during disease, not significantly affected by oncological treatment.[107]

Presently, various PD-L1 IHC assays (Dako 22C3, Dako 28–8, Ventana SP142, and Ventana SP263) have been approved as companion diagnostics for treatment response prediction in cancer types such as NSCLC, GC, TNBC, and HNSCC.[108] With an increasing number of approved ICBs, each paired with a different PD-L1 antibody clone, there remains a need for harmonization. Using TNBC as a paradigm, although an overall good concordance was observed between Ventana SP142, Dako 22C3 and Dako 28–8, the Ventana SP263 was not interchangeable with the former three assays.[109] Furthermore, simultaneous quantification of three individual PD-L1 antibodies using multiplex IHC showed moderate-to-strong correlations in PD-L1 positivity on TNBC tissues (Dako 22C3, Ventana SP142, and Ventana SP263),[110] but not on GC tissues (Ventana SP142, Dako 22C3 and Dako 28–8).[110] Other challenges with regard to conventional IHC assays remain; different protocols, interobserver variability, and different sample types (tumor microarray (TMA), whole tissue sections, tissue biopsies, resected tissues) are some examples. Specifically, tumor heterogeneity,[107] tissue-specific considerations,[111] drug-specific performance,[112 113] and detection of PD-L1 splice variants.[114] Assay standardization and uniform data reporting practices would facilitate a biomarker database (see Data reporting recommendations). While the field continues to work through these challenges, we recommend reporting continuous PD-L1 data (eg, percentage of tumor and immune cell PD-L1 expression in 5–10% increments and/or tumor type-specific scoring method) along with details of the staining assay.

Tumor mutational burden

TMB is defined by the total number of mutations present in a specimen and is deemed essential. A recent pan-cancer analysis comprising 20 solid cancer types revealed that TMB had a significant impact on OS in 14/20 cancers. Although high TMB was associated with worse OS in patients with adrenocortical carcinoma (ACC), cholangiocarcinoma (CHOL), colon adenocarcinoma (COAD), esophageal carcinoma (ESCA), kidney renal clear cell carcinoma (KIRC), liver hepatocellular carcinoma (LIHC), mesothelioma (MESO) and pancreatic adenocarcinoma (PAAD), it predicted better OS in patients with bladder cancer (BLCA), cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), kidney renal papillary cell carcinoma (KIRP), ovarian serous cystadenocarcinoma (OV), stomach adenocarcinoma (STAD) and uterine Corpus Endometrial Carcinoma (UCEC).[115] Another study with a focus on how TMB may predict ICB response similarly reported an association between high TMB and improved prognosis in ICB-treated patients.[116] However, it is important to note that “high TMB” is subjective as the TMB cut-off value varied markedly between cancer types, ranging from the top 10–50%.

From the pan-cancer analysis, TMB had no prognostic association with lung adenocarcinoma (LUAD) and lung squamous carcinoma (LUSC). However, a meta-analysis reported that patients with high TMB showed significant clinical benefits from ICB compared with those with low TMB.[117] This inconsistency in reporting sheds light on potential variations in mutation, pathological stages, and assay variability. This is another notable challenge of TMB since there is currently no consensus as to which mutations should be included in the calculation. While some authors report all mutations, other authors report only certain types of mutations, such as non-synonymous,[116] missense,[118] and miscoding[119] mutations. Despite these limitations, clinical trials have assessed the role of TMB as a predictive marker for ICB response. In CheckMate 227 and CheckMate 026 for example, PFS was significantly longer among ICB-treated patients with NSCLC with high TMB, regardless of PD-L1 expression.[118 120] Similarly, a separate NSCLC study also concluded that high

TMB improved the likelihood of benefit to ICB, without correlation with PD-L1 expression.[121] With other cancer types including but not limited to melanoma, head and neck cancer, and renal cell carcinoma (RCC), higher TMB was also able to predict favorable outcomes for ICB.[122] Given its promise to serve as a predictive biomarker for a response, many studies have aimed to standardize TMB assessment in clinical samples to guide immunotherapy treatment decision-making.[123–125]

Plasma TMB has been proposed as an alternative to tissue TMB due to potential sampling bias because of tissue heterogeneity. However, plasma TMB rarely matches those in the tissue, possibly due to low levels of ctDNA.[119] An in-depth review comparing tissue TMB and blood TMB likewise suggested that these two are not equivalent.[126 127] High tissue TMB had a strong correlation with prognostic outcomes of NSCLC. In contrast, ICB-treated patients with NSCLC with high blood TMB demonstrated superior PFS and higher ORR although no assessment on OS was made.[128] In a follow-up study by the same authors, blood TMB was found to be not associated with favorable OS after immunotherapy, regardless of cut-off value.[129] As with tissue TMB, plasma TMB faces challenges involving sample heterogeneity and assay variability as well.

RATIONALE AND CONSIDERATIONS FOR ELIGIBILITY BIOMARKERS

Eligibility biomarkers are a subset of essential biomarkers that are relevant in a particular trial context (eg, tumor type or treatment-specific biomarkers). Perhaps the clearest examples of this class are those markers used to guide treatment decisions for targeted therapies (eg, DNA repair defects as indicators for PARP inhibitors). In this context, eligibility biomarkers serve as gatekeepers, determining if a patient's tumor possesses the necessary characteristics for a given treatment, enhancing trial efficacy, minimizing unnecessary exposure, and facilitating personalized medicine. We would also include disease site-specific markers such as fibrin for monitoring BLCA in this category.

Rationale for incorporating eligibility biomarkers

1. Enhancement of trial efficacy: by enriching the study population with patients who have a higher likelihood of responding to the therapy, eligibility biomarkers can enhance the efficacy of the trial. This enrichment can potentially shorten the time required to observe clinical benefits and reduce the sample size needed to achieve statistical significance. For example, PD-L1 expression is commonly used as an eligibility biomarker in trials for ICB, ensuring that only patients whose tumors express PD-L1 are included.[130]
2. Minimization of unnecessary exposure: these biomarkers help minimize patient exposure to treatments unlikely to be effective, thereby reducing the incidence of unnecessary side effects and improving overall patient safety. For instance, the presence of specific genetic mutations, such as EGFR mutations in NSCLC, can help determine eligibility for targeted therapies, sparing patients without these mutations from ineffective treatments.[131]
3. Facilitation of personalized medicine: eligibility biomarkers align with the trend toward precision medicine by enabling the development of more targeted treatment regimens. They allow for interventions to be tailored to the unique molecular and immunological profiles of individual patients. For example, BRCA1/2 mutations are used to select patients for PARP inhibitor treatments in ovarian and breast cancer, targeting the therapeutic approach.[132]

Considerations for implementing eligibility biomarkers

1. Clinical and biological relevance: the chosen biomarkers should have strong clinical and biological relevance, supported by robust evidence demonstrating their predictive value for treatment response across multiple tumor types and therapeutic contexts. The NIH defines predictive biomarkers as those indicating the likely benefit from treatment compared with the patient's condition at baseline, helping to classify patients with tumors that respond versus non-responders. An example of this is HER2 overexpression in breast cancer, which predicts response to HER2-targeted therapies.[133]
2. Feasibility and standardization: it should be feasible to measure the biomarker using standardized, reproducible assays that are readily available in clinical settings. This ensures that the tests can be consistently performed across individual laboratories. For example, measuring TMB requires standardized next-generation sequencing techniques that are widely accessible.[134]
3. Regulatory and cost considerations: the biomarkers should comply with regulatory requirements and be cost-effective to implement. This ensures their widespread adoption in routine clinical practice and prevents cost from becoming a barrier. For instance, the use of blood-based biomarkers such as ctDNA for detecting EGFR mutations in lung cancer is both cost-effective and minimally invasive, facilitating easier implementation.[135]
4. Ethical considerations: the use of eligibility biomarkers must be ethically justified, ensuring that they do not unjustly exclude patients from potentially beneficial treatments. It is crucial to balance precision in patient selection with equity and fairness in clinical trial design. For example, ensuring that biomarker tests are accessible to diverse patient populations can prevent disparities in trial participation.[136]

In summary, eligibility biomarkers are important tools that optimize clinical trial design and advance personalized immunotherapy. By selecting patients most likely to benefit from treatment, these biomarkers enhance trial efficiency and safety, ultimately aiming to improve patient outcomes and treatment efficacy. The NIH definitions

Table 3 Emergent biomarkers for early-stage immunotherapy clinical trials

Emergent biomarker(s)	Details	References
Patient-level (systemic immunity and tumor burden)		
ctDNA	Specifically correlates with early tumor recurrence; high priority as assessed	141 142
Peripheral blood cell phenotyping	Specifies only profound derangements and not yet suitable for widespread application	145
T and B cell repertoire analyses	Defines immune competence in the periphery (diversity) and immune response in tumor (clonality)	206
Microbiome	In stool/tumor predicting outcome	289
AI-based radiomic biomarkers	Can correlate with immune infiltrate	290
Cytokines and chemokines	Assess storm in acute immune response	291 292
Exosomes	Serum tumor markers (exosomes)	293 294
Cancer cells		
MHC class I, β2-microglobulin	Well-accepted importance, needs championing CLIA-supported study; high priority as assessed	152 153
Tumor microenvironment		
TME phenotype	nflamed, cold, desert	39
Tumor infiltrating immune cells	cellular quantification/phenotyping	184
Immune checkpoint molecule expression on T cells	LAG3, TIM3, TIGIT, etc.	295
Interferon gamma signature	Transcriptomic profiling of tumor	296
AI-based H&E biomarkers	Readily accessible and cost-effective	297
On-treatment biopsy	Treatment effect, Successful delivery of immune cells adoptively transferred for cell therapy trials within the immune context.	298 299

AI, artificial intelligence; CLIA, Clinical Laboratory Improvement Amendments; ctDNA, circulating tumor DNA; MHC, major histocompatibility complex; TME, tumor microenvironment.

provide a framework for understanding the role and implementation of these biomarkers, highlighting the importance of rigorous validation and standardization.[7]

CRITERIA AND RATIONALE FOR EMERGENT BIOMARKER EVALUATION

Emerging biomarkers, with promising early evidence of association with patient well-being, disease progression, or clinical benefit in multiple trials still require independent prospective validation (table 3). They often encompass those detected by still-evolving methodologies and/or those where additional funding considerations may be needed. Examples include T cell receptor (TCR) repertoire analyses from blood or tumor,[137] serum or plasma soluble analytes,[138] tissue transcriptomic signatures including at the single-cell level or spatially resolved,[139] autoantibody profiling,[140] serum exosomes,[141 142] ctDNA (see below), stool and/or tumor[143] microbiome,[144] radiomics organ imaging,[145] MHC Class I/β2M expression in the tumor (see below), and even potential cross-modality combinations of these different methods using machine learning. Individually, these biomarkers offer some of the most relevant approaches to discovering pathways of importance in tumor immunotherapy. Their rationale is typically justified following a demonstration of utility using a hypothesis-driven study design, even if some of these biomarkers originally emerged from the generation of biased, descriptive data sets. Of note, the high-dimensional, complex nature of these assays makes them less amenable to simplification into a panel-based test, since their value is often derived from composite signatures. Due to their broadly encompassing nature, emergent biomarkers could also reflect signals that could be surrogates of other underlying processes (inflammation, tumor gene alterations, MHC Class I loss or β2M loss, etc). Therefore, though very exciting, emergent biomarkers should fulfill certain requirements if they are to be used broadly for patient inclusion or for early prediction of responsiveness or adverse events.

To evaluate them for clinical utility, cost-effectiveness, and availability, the following checklist should be considered (table 4). If three or more categories are marked as

Table 4 Checklist for evaluation of emergent biomarkers

Checkbox questions	Examples, additional information
Are these emerging biomarkers showing similar outcomes across more than one study with sufficient power?	Examples include infiltration of tumors with TCF7 stem-cell-like T cells[300] as defined by scRNAseq, bulk or panel-based transcriptomics, or multiplex immunostaining. They also include clonality/diversity/overlap of TCR[301]
Is the method to detect the biomarker reproducible and applicable broadly with achievable harmonization of results?	Are sensitivity/specificity/reproducibility metrics compatible with multicenter implementation? Are there reference materials to use for standardization?
Does the proposed biomarker platform offer a significant advantage over existing essential markers?	For example, could radiomic imaging metrics represent a non-invasive approach to quantify tumor heterogeneity and immune infiltration[302]? Is a multiplex immunostaining method able to encompass existing tests such as PD-L1 expression and HLA loss?
Is the methodology used to detect the biomarker signature approaching maturity and could it be simplified to a minimal set of actionable variables?	For example, spatial transcriptomics has only recently achieved single-cell resolution with unbiased RNA detection (Visium HD, Stereo-seq), despite still relatively low sensitivity. From multiplex soluble analytes, could a minimal number of variables recapitulate the AUC achieved with the full panel?
Is the material required to assess the biomarker simple to obtain?	scRNAseq is still largely dependent on fresh tissues, stool requires at-home kits to be distributed. Are there reference materials to use for standardization?
Is the cost compatible with a phase III biomarker-driven study? Can the test be eventually reimbursed by insurance carriers in its current state?	If no, would alternative methods be possible to simplify the approach?

AUC, area under the curve; HLA, human leukocyte antigen; PD-L1, programmed death-ligand 1; scRNAseq, single cell RNA sequencing; TCR, T cell receptor.

no, the biomarker is likely to require additional considerations before being included in the design of a prospective study.

CONSIDERATIONS FOR SPECIFIC EMERGENT BIOMARKERS

Microbiome

Recent preclinical and observational studies across cancer types have identified the gut microbiome as a prognostic and predictive biomarker for immunotherapy with ICBs.[146] Factors such as higher alpha and beta diversity reflecting the overall health of the gut microbiome and the presence of specific commensal genera, including *Eubacterium*, *Faecalibacterium*, and *Ruminococcus*, have been linked to better outcomes in patients with cancer.[147] Notably, the use of antibiotics prior to ICB treatment is predictive of poor outcomes in various cancer types, including melanoma, NSCLC, RCC, and BLCA. Moreover, generating avatar mouse models with patients' stool microbiomes can predict response to immunotherapy in patients treated with immunotherapy alone or combined with fecal microbiota transplantation.[148] Given the effect of the gut microbiome on systemic and local immunity, widespread use of antibiotics in patients with cancer, and other cancer-related factors that affect the microbiome, such as chronic inflammation, developing microbiome-based biomarkers is imperative (and an emergent biomarker) for the development of immunotherapeutics.

Given the possibility of assessing the gut microbiome profile and function from stool samples and the possibility of measuring bacterial-made metabolites in stool and blood, developing microbiome-based biomarkers has a high potential for serial sampling and analysis of the patient's microbiome on treatment and over time which is not readily achievable with tissue-based biomarkers such as pathology IHC and PD-L1 status. Efforts are already underway to develop microbiome biomarkers across cancers. For example, clustered species-interacting groups derived from metagenomics analysis of 245 patients with NSCLC correlated with OS. A person-based calculation of a topological score combined with species *Akkermansia* levels was able to predict OS in patients with NSCLC and genitourinary (GU) cancer who received ICBs.[149] Longitudinal metagenomics profiling of the gut microbiome of 175 patients with advanced melanoma revealed distinct patterns in species-level genome bins in patients achieving PFS of 1 month or longer (PFS≥12) versus patients with PFS shorter than 12 months (PFS<12).[150] Future microbiome-based biomarker development should also focus on profiling microbial metabolites in stool and blood as well as bacterial probe set-based quantitative PCR scoring approaches with a quick and actionable turnaround time that overcomes the longer time required for metagenomics analysis. These emergent biomarkers will require standardization, development of positive and negative controls, and assessment

across individual tumor types before they can be considered essential.

MHC Class I and β2M

The MHC-I complex comprises a heavy chain (human leukocyte antigen (HLA)-I) and an invariant light chain (β2M) to maintain its structural stability and position on the cell surface. MHC-I downregulation has emerged as a common mechanism of immune evasion in tumors.[151] This phenomenon has a negative impact on immunotherapy as studies have revealed that patients with reduced HLA-I expression tend to have worse prognosis and poorer response to ICB.[152 153] This was described as a mechanism of intrinsic and acquired resistance to immunotherapy.

Recent studies highlight the critical role of the classical HLA-I loci, namely HLA-A, HLA-B, and HLA-C, in immune surveillance and antigen presentation to $CD8^+$ T cells. HLA-I downregulation was similarly observed to be a hallmark of ICB resistance following RNA sequencing and flow cytometry analysis on pretreatment and on-treatment melanoma biopsies.[154] Additionally, the HLA-I genotype has been implicated in influencing ICB response. From relapsed Merkel cell carcinoma tumor, *HLA-B* was significantly downregulated but not *HLA-A*, suggesting that transcriptional suppression of specific HLA-I locus may underlie resistance to immunotherapy.[155] More specifically, patients with melanoma with the HLA-B44 supertype had extended survival, whereas the HLA-B62 supertype (including HLA-B*15:01) or somatic loss of heterozygosity at HLA-I was associated with poor outcome.[156] While maximal heterozygosity was associated with improved OS after ICB, HLA-I homozygosity in at least one locus, especially HLA-B, was associated with reduced OS. In ICB-treated patients with NSCLC, HLA-I loss of heterozygosity was a significant negative predictor of OS. Survival prediction improved when combined with TMB, suggesting that the combination may better identify patients likely to benefit from ICBs.[157] Similarly, in tumors with HLA-Class II molecule expression, loss of expression has been reported (eg, in lymphomas, CRC and melanoma).[158]

β2M encodes the β-chain of MHC I molecules, and is crucial for the formation of peptide-MHC-I complexes. The loss of β2M decreases immune cell recognition of cancer cells, consequently leading to acquired resistance to ICB therapy.[159] Pan-cancer analysis revealed that *DUX4* re-expression on cancer suppresses both HLA-ABC and β2M expression and promotes ICB resistance; increased transcriptional activity was associated with failure to respond to ICB and decreased OS following ICB.[160] In a cohort of patients with melanoma treated with ICB, patients with a complete response had higher pretreatment β2M levels than those with a partial response or progressive disease. This showcases the potential of β2M in predicting ICB response.[161] Interestingly, β2M expression was downregulated in LUAD and tumors with high β2M expression contained more $CD8^+$ T cells than those with moderate or low β2M expression. This suggests that high β2M expression might be a prerequisite for optimal T-cell responses.[161] However, among these tumors, only 40% of tissues had positive PD-L1 staining (≥1%), hinting that the remaining patients may not benefit from ICB treatment due to impaired $CD8^+$ T cell recruitment and cytotoxicity. Assessing PD-L1 with β2M expression may provide a more comprehensive approach to patient selection. We consider measures of MHC I and β2M on tumors as highly prioritized emergent biomarkers, awaiting standardized measures performed in a CLIA-certified laboratory. ctDNA

ctDNA is becoming commonly incorporated into trial designs. These nucleic acid fragments are typically around 166 bp in length and are released from tumor cells through various processes including apoptosis and necroptosis.[162] Numerous assays have been developed to capture and quantify the presence of ctDNA in blood and other biofluids, resulting in a non-invasive diagnostic test with multiple applications. One application is the ability to obtain a tumor mutational profile through ctDNA analysis. As compared with a standard tumor needle biopsy or excisional biopsy, a ctDNA assay is non-invasive and can provide faster results in a clinical setting so that patients can be allocated to appropriate treatment arms more quickly.[163] Additionally, ctDNA provides valuable prognostic information; across tumor types and stages, ctDNA positivity at the time of diagnosis has been shown to correlate both with PFS and OS.[164–166] Therefore, randomization of patients in a clinical trial with stratification by ctDNA status is crucial to achieve balance in assortment and unbiased results. Furthermore, ctDNA positivity can also identify patients with the highest risk of recurrence, and thereby select patients that may benefit most from trial enrollment and treatment escalation in the setting of neoadjuvant and adjuvant studies. ctDNA can predict and predate radiographic evidence of recurrence and progression.[167 168] As such, ctDNA has been used as a trial endpoint to provide an earlier read-out of treatment efficacy.

Certain limitations of ctDNA still exist. Importantly, while improved assays are continuously being developed, the current sensitivity and specificity of ctDNA for early-stage cancer are relatively low. The sensitivity for detection is typically less than 50% for stage I cancer.[169] This significantly limits the applicability of ctDNA across tumor stages. The significant variability in ctDNA assays can also pose problems with reproducibility. ctDNA quantification can be done through sequencing, methylation patterns, and fragmentomics, so it is important to recognize that one ctDNA assay may not be equivalent to others.[170 171] We therefore recommend that ctDNA, although highly prized by the consensus group, be considered an emergent biomarker in early-phase trials, and that further development and validation

are needed to improve relevance and feasibility across tumor stages.

Tumor-infiltrating immune cell profiling

The role of TIL as a prognostic biomarker has been extensively studied across various cancer types, with notable observations in TNBC, where enrichment in TIL was observed in patients with favorable outcomes.[172 173] Alongside the traditional AJCC/ Union for International Cancer Control (UICC) tumor, node, metastases classification system, an immune-based scoring system known as Immunoscore was developed to quantify $CD3^+$ and $CD8^+$ T cells in the tumor core and invasive margin of CRC.[174] The prognostic impact of Immunoscore was then showcased in patients with CRC, providing a reliable estimate of recurrence risk.[175] The prognostic and predictive value of Immunoscore was recently confirmed in a large study of patients with stage III CRC receiving adjuvant chemotherapy.[176] Despite growing interest in the potential of TIL as a predictive biomarker, their clinical utility in predicting immunotherapy response remains limited. The term "TIL" encompasses multiple immune subsets, complicating efforts to elucidate their specific role in tumor immunity and immunotherapy response. This prompted many studies to focus on single immune subsets, such as CD8+ alone or in combination with Treg assessment, and their implication on treatment outcomes.

In NSCLC and advanced urothelial carcinoma tissues, ICB-treated patients with high CD103 expression, a marker suggestive of CD8+ tissue-resident T cells, had increased OS.[177] Additionally, increased plasma cell signatures associated with the presence of TLS and/or lymphoid aggregates were predictive of OS in ICB-treated patients with NSCLC but not chemotherapy-treated patients, independent of $CD8^+$ T cell signals.[178] In other solid tumors, the presence of mature TLS was also associated with improved ORR, PFS and OS in ICB-treated patients, independently of $CD8^+$ T cell density and PD-L1 expression status.[179] Other immune cell types were also evaluated; high pretreatment NLR is significantly associated with poorer OS and PFS, and lower probability of ICB response across multiple cancer types[46 180] and high infiltration of intratumoral $CCR5^+CD66b^+$ neutrophils was associated with superior therapeutic response to pembrolizumab in muscle-invasive bladder cancer.[181] In general, the assessment of predictive value in these studies was retrospective and based on the observation of improved OS in ICB-treated patients. Thus, it is important to note that these immune biomarker analyses are exploratory to identify TIL-associated biomarkers with the potential to stratify patients for response. Thus, we currently consider CD8+T cell infiltrate and associated PD-1 expression as emergent biomarkers but acknowledge that they are likely to be found validated and added to essential biomarkers once qualified assays have been developed in CLIA-certified laboratories. Assessment of overall density across the tumor or micro-niches within it is within the capabilities of modern digitized pathology coupled with AI (see below).

Immunophenotyping

Distinctive patterns of immune infiltration, known as immunophenotypes, have been recognized based on the presence or absence of immune cells in the TME and their spatial distribution. At a high level, these can be categorized into three phenotypes: immune-desert, immune-inflamed, and immune-excluded. The immune-desert phenotype is characterized by a lack of lymphocytes within the TME, implying a lack of pre-existing antitumor immunity.[182] In contrast, the immune-inflamed phenotype is marked by the presence of lymphocytes near tumor cells, reflecting an active immune response. The immune-excluded phenotype is defined by the confinement of immune cells to the stromal compartment, without infiltration into the epithelial tumor cell regions, which limits immune cell access to the tumor.[21]

Although the biology underlying these patterns is complex and may involve immunologic, metabolic, and tumor-intrinsic pathways, multiple studies have identified prognostic and predictive associations. The majority demonstrate an association between the density of immune infiltration and better outcomes, and several studies have shown a lack of response to immunotherapies in non-inflamed phenotypes.[183] While it is anticipated that immune phenotypes will have value as both predictive and prognostic biomarkers, the strength of these associations will need to be validated across tumor types and treatment regimens. AI-based approaches have shown potential to evaluate immune phenotypes from diagnostic H&E-stained slides using morphology-based cell and tissue classification, opening opportunities to integrate this assessment into routine diagnostic workflows.[184–186]

Despite the relative ease of identifying desert and inflamed phenotypes, challenges exist in the classification and quantification of these phenotypes, particularly for the immune-excluded group. Most studies rely on IHC, typically focusing on CD8+T-lymphocytes, and face challenges related to tumor heterogeneity and the reproducibility of quantification methodologies. Gene expression signatures represent an alternative methodology, with genes associated with immune response, angiogenesis, and stromal activation typically being part of the classifier.[187] Lastly, the recent development of novel technologies enabling the spatial visualization of RNA expression in a morphological context promises not only to enhance the classification of immune phenotypes but also to provide mechanistic biological insights into the pathways sustaining a particular tumors' immune phenotype.[188]

Multiplexing

Immune cell phenotyping through multiplex IHC/immunofluorescence (mIHC/IF) enables the staining and simultaneous viewing of up to 300 protein biomarkers on a single tissue section.[189] This technique reduces the need

for multiple serial sections and minimizes potential alignment issues that can arise from using multiple serial tissue slides.[189 190] In addition to retaining biomarker spatial distribution, the fluorescent intensity is also proportional to the biomarker's abundance. This allows for computational quantification of complex and intricate biomarker combinations, with minimal human biases.[191]

Several mIHC/IF platforms, including but not limited to PhenoCycler and COMET, have emerged.[189] PhenoCycler offers ultra-high-plex imaging for in situ, cellular and subcellular analysis. Using a 56-marker PhenoCycler panel, characterization of the composition, spatial organization, and functional immune status of the TME of cutaneous T cell lymphoma is possible.[192] Similarly, in advanced patients with CRC, PhenoCycler analysis revealed that local enrichment of PD-1$^+$ CD4$^+$ T cells correlated with survival in high-risk patients.[193] COMET, an automated sequential staining and imaging platform,[194] enabled detailed TME analysis to characterize immune cell infiltration levels, along with tumor-intrinsic features and stromal compartments of different cores of a lung cancer TMA.[195] The intratumoral immune and neural phenotypes among patients with cutaneous squamous cell carcinoma who underwent anti-PD-1 therapy demonstrated that inflammation with tumor-associated nerves promotes resistance to anti-PD-1 therapy in patients with cancer.[196]

Despite its promise in robust cell phenotyping, the adoption of mIHC into the clinic has been limited by financial constraints, lack of technical standardization, and regulatory hurdles.[190] However, evidence-based mIHC/IF platforms are now anticipated to provide valuable diagnostic and prognostic insights in clinical settings to predict immunotherapy responses across various cancer types. This potential is further bolstered by the requirement for only small numbers of tissue sections, advances in automation, AI, and increased affordability.[39 197 198]

Flow cytometry

Flow cytometry profiling is usually applied to peripheral blood rather than tumor-infiltrating cells, since the latter are best studied spatially, via multiplexed imaging techniques. Most profiling of peripheral blood is also done without regard to tumor-specific cells. This is because (1) tumor-specific T cells tend to be very rare in the blood, and (2) identifying them (especially in any comprehensive way) is difficult if not impossible, without knowing all the specific antigens that may drive an immune response in a particular patient's tumor. As such, we consider here the utility of non-antigen-specific profiling of peripheral blood by multiparameter flow or mass cytometry.

There is a long history of using flow cytometry to profile immune cell phenotypes in the context of cancer clinical trials. Blood-based cellular biomarkers are appealing in that blood is more readily and universally accessible than tumor tissue, and it tends to be a more uniform sample type compared with biopsy material. In addition, recent technical developments, including mass cytometry[199] and spectral flow cytometry,[200] have allowed the use of higher parameter panels (40–50+markers), permitting the analysis of more cell lineages and more detailed phenotypic cell subsets than ever.

Despite these advances, the correlations of cell subset frequencies with clinical outcomes have not been strong or consistent across trials. For example, some studies have found an association of baseline levels of central memory[201] or effector memory[202] CD8 T cells or those producing IL-2[201] with positive immunotherapy outcomes in melanoma. In NSCLC, a lower proportion of central memory CD4 T cells and higher double-negative T cells have been associated with longer PFS.[203] Circulating NK cells and PD-1+CD8 T cells have also been associated with increased PFS in NSCLC.[204] On the other hand, measurement of CD19+cells by flow cytometry to document B cell aplasia as an "on-target, off-tumor" effect of CD19 CAR T cells has emerged as a surrogate biomarker of CAR T cell persistence and activity in children and young adults with B cell leukemia.[18 205] Loss of B cell aplasia is highly associated with relapse.[19]

A further difficulty of biomarker identification with flow cytometry is that findings are likely to vary based on the type of cancer and the precise immunotherapy regimen. Furthermore, in many studies, the frequencies of the implicated cell types tend to overlap to a large degree between tumor responders and non-responders. As such, high-parameter flow cytometry has not been proven to be much more predictive of immunotherapy outcome than much simpler hematologic measures such as NLRs.[206] Nevertheless, we consider the goal of developing new systemic biomarkers to be worth pursuing, especially in the context of novel immunotherapies that may rely on particular cell subsets for their efficacy. Therefore, immune cell profiling should be considered an emergent assay, suitable for biomarker discovery and validation in a large variety of clinical trial settings, but not yet productive of a clear biomarker with consistent evidence in the literature.

SUMMARY OF OTHER EMERGENT BIOMARKER LEVELS AND PLANS FOR FUTURE EFFORTS

We must thus consider when an emergent marker is ready for routine use in clinical trials generally, or in an eligibility-specific context. There are numerous well-considered examples of evaluating biomarkers for targeted therapy, including the recent proceedings of the ASCO/College of American Pathologists Immune Checkpoint Inhibitor Predictive Biomarker Summit[207] which reviewed the evidence for markers of checkpoint blockade among others, outlined current limitations, and proposed next steps for overcoming those issues. Similar, and broader efforts to establish coherent criteria for predictive biomarkers include the European Society for Medical Oncology's Scale of Clinical Actionability for Molecular Targets[208] and Memorial Sloan Kettering (MSK)'s Precision Oncology Knowledge Base[209] each of

which provides a detailed framework for evaluating scientific support for molecular markers of targeted therapy.

However, these efforts and many others chiefly address the question of when a marker is ready for routine clinical use, emphasizing the importance of thorough analytical evaluation and the highest level of clinical validity as established by large-scale clinical trials. Our question of when a marker is justified for routine use in the research setting of early-stage clinical trials is new. We cannot require rigorous, context-specific evidence from randomized clinical trials before applying markers in early-stage studies, and it will sometimes be impractical if not frankly infeasible to insist that assays be fully standardized and harmonized across studies with complete analytic validation and locked-down decision rules. Conversely, outside of hematology, we place the entire class of ctDNA-based markers in the emerging tier. Despite great promise and sometimes very strong supporting data, there is little agreement on which genomic or epigenomic features to measure, or how to interpret the results, among other issues. No single approach has broad evidence of utility, or widespread community support. This suggests that a market-value-based model could be effective for evaluating markers. Such markers must meet basic evidentiary criteria to be considered. Prioritization for inclusion in the essential list should be determined by a review of the literature, registered clinical trials and surveys of the research community.

NEOADJUVANT OR PERIOPERATIVE WINDOW OF OPPORTUNITY TRIALS

The neoadjuvant or perioperative setting presents the ideal opportunity when designing trials to evaluate correlative predictive and prognostic blood and tissue biomarkers, to study the TME, and to assess the immunologic mechanisms of immunotherapeutics. Comparisons of baseline pretreatment blood, body fluids, stool, imaging, and tissue biopsy samples with post-treatment samples and surgical specimens in clinical trial designs allow the closest correlation and prediction of pathologic and radiologic response, and prognostic factors for recurrence and survival outcomes. Not only have neoadjuvant and perioperative approaches with immunotherapy enabled mechanistic determination of immunological action and assessment of correlative biomarkers, but the earlier use of immunotherapy in these settings has led to improvements in response and survival.

Adjuvant approaches with immunotherapy involving single or dual ICB have been successful and proven in improving DFS or OS using largely biomarker unselected trial approaches for high-risk melanoma, high-risk clear cell RCC, resected stage II or III esophageal and gastro-esophageal junction cancer and high-risk muscle-invasive urothelial carcinoma,[210] with only PD-L1 positive expression required for adjuvant ICB therapy in resected NSCLC.[211] However, despite successful approval of adjuvant ICB for these indications, there is limited deselection of individuals who may not benefit and/or suffer unnecessary toxicities in this setting. Neoadjuvant trials are much more effective at assessing and validating the selection factors for tumor response and survival than advanced metastatic trial designs. Furthermore, neoadjuvant therapy may yield superior immunological results than treatment in the adjuvant or metastatic setting.[210] Preclinical mouse models show that immunotherapy given prior to surgery results in stronger immune responses when the initial tumor and/or lymph nodes are still in place and results in improved survival when compared with immunotherapy given as adjuvants.[212] Moreover, immunotherapy given in earlier settings has a higher chance of efficacy than in the advanced setting where complex heterogenetic immunologic resistance mechanisms play a more prominent role.[213] Remarkable major pathological response rates (MPR) of 59% with 8.0% partial response rates were observed with neoadjuvant ipilimumab and nivolumab in a phase III trial of stage III melanoma,[214] and MPR rates of 95% with 68% pathological complete responses (pCR) in a phase II trial of locally advanced non-metastatic mismatch repair-deficient CRC.[215] Promising results in RCC[216 217] as well are changing the treatment landscape for these immunogenic cancers in favor of earlier ICB therapy and neoadjuvant approaches for all solid malignancies.

The risk of neoadjuvant strategies in patients with earlier stages of the disease is that tumors that do not respond to neoadjuvant therapy may progress, becoming unresectable or metastatic before surgery. Therefore, predictive, and prognostic biomarkers should be used to anticipate the response to therapy and create personalized clinical trial designs with selected therapy. For example, a neoadjuvant trial determined that patients with melanoma with tumors bearing a low INF-γ signature were less likely to achieve a pathologic response and have a higher likelihood of disease recurrence in response to single-agent immunotherapy, whereas the converse was observed in patients with high INF-γ scores.[218] Moreover, in the advanced, metastatic setting, high levels of TMB can potentially predict the high efficacy of ICB treatment.[219] Therefore, INF-γ, TMB and other potential predictive markers can be used in precision biomarker-driven treatment trial designs in the neoadjuvant setting to intensify treatment or evoke combinational approaches for potential non-responders and minimize treatment-related toxicity with de-intensified treatment or single-agent therapy for potential responders. Currently the Food and Drug Administration (FDA) is considering requiring four-arm clinical trials focusing on OS and PFS in patients on such neoadjuvant/perioperative treatment trials.

Neoadjuvant and perioperative approaches involving chemotherapy and immunotherapy were first established as the standard of care in localized TNBC and resectable NSCLC. The phase III KEYNOTE-522 trial demonstrated that perioperative pembrolizumab with chemotherapy preoperatively followed by surgery and adjuvant pembrolizumab for stage II or III TNBC compared with placebo

increased the pathologic response rate and improved event-free survival (EFS) meeting both these co-primary endpoints leading to FDA regulatory approval as standard of care.[220] Exploratory biomarkers demonstrated that pre-treatment tumor PDL1 expression (CPS) and stromal tumor TILs correlated with pCR to pembrolizumab and chemotherapy.[221] For resectable stage Ib (≥4 cm), stages II and III NSCLC with no known EGFR or ALK genetic driver mutations, nivolumab added to neoadjuvant platinum-based doublet chemotherapy improved the pCR rates without decreasing the percentage who underwent definitive surgery or increasing the incidence of grade ≥3 adverse events in the Checkmate 816 study.[222] While the prespecified interim analysis for the secondary endpoint of OS did not cross the threshold for statistical significance at the time of the study report, the primary endpoints of median EFS and pCRs were improved with nivolumab, leading to FDA approval.[223] Exploratory analyses determined that EFS was longer in those who achieved a pCR than those who did not, suggesting that pCR could potentially be a surrogate for survival. ctDNA clearance appeared to correlate to the longer EFS in the nivolumab and chemotherapy arm.[222] More recently, perioperative pembrolizumab (pembrolizumab plus platinum-based chemotherapy preoperatively followed by surgical resection and adjuvant pembrolizumab) was demonstrated to improve the pCR rate and the dual endpoints of EFS and OS when compared with placebo for stage II to IIIB NSCLC in the phase III Keynote-671 trial leading to FDA approval on October 16, 2023.[224]

Pretreatment and on-treatment biomarkers assessed in phase III neoadjuvant trials such as PD-L1 expression, tumor mutation burden, presence of TIL, ctDNA and gut microbiome and others are undergoing exploration and validation as to how well they prognosticate and correlate to outcomes such as pCR, EFS and OS. Neoadjuvant or perioperative approaches of combination ICB and chemotherapy combinations appear promising in ongoing phase III clinical trials in many solid tumors including gastrointestinal malignancies, HNSCC, breast cancer, NSCLC, and muscle-invasive urothelial cancer and others.[210] Importantly, these biomarkers evaluated during neoadjuvant therapy will set the stage for the selection of effective subsequent therapy or additional adjuvant therapy for early-stage disease or can be used to predict response and guide therapy in the recurrent and/or metastatic setting. For example, the gut microbiota profile has been shown to predict the pathological response to nivolumab plus ipilimumab in melanoma in the neoadjuvant setting.[146] Subsequent examination of gut microbiota changes was then found to correlate with response to PD-1 checkpoint blockade for potential use as a predictive biomarker for melanoma and a variety of epithelial cancers in the advanced metastatic setting.[225 226]

Pathologic response as an endpoint for neoadjuvant trials

In clinical trials in the neoadjuvant/periadjuvant setting, pathologic response (ie, how much residual viable tumor (RVT) is left at the time of definitive resection) is frequently a primary or secondary endpoint. %RVT has been shown to associate with EFS in several large clinical trials in multiple tumor types, including melanoma,[227] NSCLC,[228] and cutaneous squamous cell carcinoma,[229] suggesting the role of pathologic response as a surrogate for patient survival. The association of pathologic response with EFS frequently outperforms other proposed surrogate markers of survival, including radiographic response and ctDNA.[228] The relationship between pathologic response and OS has yet to be determined as OS data from many of these trials has still not matured.[230]

Scoring systems for pathologic response that are inclusive of histologic features seen in response to immunotherapy have been developed[231] and have been applied across tumor types.[232] Efforts are underway to harmonize pathologic response assessment strategies, and this pan-tumor approach to scoring pathologic response, akin to RECIST in the advanced disease setting, has been shown to be highly reproducible among pathologists.[232]

Various categories of pathologic response have been proposed, primarily pCR (defined as 0% RVT) and major pathologic response (MPR, ≤10% RVT), originally based on data supporting their association with patient survival following treatment with neoadjuvant chemotherapy.[233] More recently, additional empiric thresholds such as pathologic partial response (pPR, <50% RVT) have been suggested.[234] However, assessment of the full spectrum of %RVT beyond categories such as pCR, MPR, and pPR, associates with EFS,[228] suggesting that there may be more nuanced thresholds of %RVT that are clinically meaningful.

Thus, given the robust association of %RVT with EFS, we recommend that neoadjuvant trials not only report pathologic response categories, but also %RVT at 10% increments to allow for the identification of clinically meaningful thresholds of response as trial data continues to accrue.

DATA REPORTING RECOMMENDATIONS

There is a well-established body of consensus recommendations for reporting study results, such as the [235]Standards for Reporting of Diagnostic Accuracy (STARD) to the Consolidated Standards of Reporting Trials project[236] to standardize reports on the conduct of clinical studies. We would refer the interested reader to the comprehensive recommendations of the REMARK initiative (Reporting recommendations for tumor MARKer Prognostic Studies), which outlined principles for testing new biomarkers and reporting performance to facilitate evaluation and compare results across studies[237] and has been extensively expanded and updated since establishment.[238]

A call to establish a common database for biomarkers. Harmonization of patient factors, immune factors and tumor factors to allow comparison across trials is desirable to develop many aspects during early clinical development, and we here echo previous calls to establish a

common database for trial biomarkers.[239] Although no common database for meta-analysis currently exists, informed scientists and clinicians could develop these with data from individual pharmaceutical firms and academic labs, applying the principles developed here. For example, requirements for a final report on completed clinical trials within 365 days[240] could use a common reporting mechanism to capture these "essential" biomarkers, especially if patient-level data could be included. Having comparators across individual smaller biotechnology firms could be an important means for validation and informed judgments by regulatory groups, scientists and investors, placing such evaluation on the critical path, even for early drug development. Furthermore, if validated as being informative, such measures could be useful with a potential role for defining stratification in future trials.

Having a suitable common database could provide multiple benefits, especially for early trials with limited patient numbers. It could be contributed to and accessed when comparing outcomes with a novel agent to prior treatment protocols. How to develop, fund, and maintain such a common database could be a goal for individual tumor types and taken up by governmental and philanthropic agencies. An example of a significant governmental effort is the European Health Data Space (EHDS), which aims to centralize health data across the European Union. This initiative facilitates access to and reuse of health data for research and public health purposes, enhancing evidence-based decision-making and patient care while ensuring strict data security and privacy standards.[241] Efforts like these also reflect similar wishes within patient communities that want data shared and used effectively so that new biomarkers can drive more relevant medical approaches and combinations of treatment. Existing database efforts are described further in the appendices.

PATIENT-REPORTED OUTCOMES AND INSTRUCTIONS FOR PATIENTS

Several issues need to be considered in the context of informing patients of the value of biomarkers and their interpretation. The individual types of biomarkers need much better explanations to help people understand their context, from both essential as well as emergent biomarkers. Health literacy principles are critical for providers and patients and their families to fully understand the value of the information and how their healthcare providers will use it. Strategic plans for SITC and other immunotherapy groups need to consider how advances will be communicated.[242]

Invasive biopsies for serial measurements in clinical trials should be minimized by including them with other procedures needed for patient care when possible. It is also important to note that patients consider bone marrow biopsies invasive, even if they do not fit the traditional definition. The clearer we can make the myriad of markers that are often discussed with patients, the better. Context is needed to help them understand what type of biomarker we want to test, why, and how. There are many other patient issues. Some top ones regarding biomarkers and testing include access, cost, timing, potential risk of insurance ineligibility, and toxicity from testing. This will be especially important as "emergent" biomarkers are developed and vetted.

Immunotherapy biomarkers predictive for toxicity and irAEs were not sought out early and research continues to try to catch up to define their incidence. A comprehensive approach to toxicity, including more innovative approaches, allows clues that were overlooked before such as plasma metabolomics in patients with lung cancer treated with PD-1/PD-L1 blockade. Predictive markers hold the most promise for patients, and yet have been the most elusive to date. Additional biomarkers create opportunities for patients since some trials have shown tumor response even though "negative" PD-1 and PD-L1 values were expressed, initially suggesting that they would not respond to therapy.

Biomarker tests are not reimbursed by all health systems so costs associated with testing and resulting actions should be planned in each clinical trial as well as clear information that explains what is in the report, and how that information is used to determine treatment, prognosis, and how it will be communicated to the patient and their family. Early-phase clinical trials are designed to measure toxicity as well as identify early signs of toxicity. Quality of life measures may be premature but could help provide patient-reported measures that are sometimes difficult to gage by healthcare practitioners. Other clinical trial advances, such as incorporating electronic health records when real-world data are useful, may also help more quickly identify viable immunotherapy biomarkers. Patient-reported outcomes (PROs) are not yet ready for definition as "essential" and have here provided a URL for a validated tool that is available online for the capture of this data: https://www.promishealth.org.

CONCLUSION

Over the past dozen years, the field of cancer immunotherapy has made significant progress in altering the cancer treatment landscape with the advent of checkpoint inhibition and cellular therapies. The challenge facing the field is to ensure that this momentum continues and that such novel agents and treatment strategies continue to develop. It has become apparent, given current research, that personalized, biomarker-driven strategies may enhance patient outcomes by better matching novel agents to populations that will derive benefits. Our efforts within this consensus effort seek to further advance biomarker-based strategies by advocating for a minimum, yet evidence-based list of essential biomarkers that should be measured to ensure patient benefit, serve to allow comparison between trials, as well as fuel future IO drug development. Here we suggest as essential biomarkers,

NLR, LDH serum levels, albumin levels, PD-L1 expression on the tumor, and measurement of TMB and microsatellite stability (MSI high or low) as a start. Emergent biomarkers of great interest include ctDNA, MHC Class I expression on the tumor (as well as β2M) and CD8 and PD1 expression (as density measures) within the tumor. Other measures, including repertoire analysis in the periphery and tumor, need greater study but appear very promising.

One challenge that must be recognized is the balance between biomarker data collection and patient considerations within clinical trials. A pragmatic trial design is critical to ensure patient participation and comfort. In addition, regulators are currently evaluating the pragmatic trial design and characterizing minimal data sets required for registrational studies. As the field advances, additional biomarkers must meet a critical, evidence-based setpoint before being mandated within all IO clinical trials.

Data sharing and collaboration will be required to further advance biomarker characterization and standardization. This will require broad stakeholder collaboration and the potential development of open repositories. This has become especially important given the advent and availability of omics data sets and the accelerated analyses of large-scale studies. SITC, as an entity with strong collaborative processes and convening power, will continue to address data sharing in this setting.

SITC and SCION recognize that the IO biomarker landscape will rapidly evolve as studies continue to mature. As such, our authors, largely SITC volunteers, will provide regular updates to this effort, towards the goal of providing an approachable, yet important resource, for future IO clinical trialists. SITC will also set foundational education efforts around the incorporation of these recommendations with broad, field-wide dissemination as the goal.

APPENDICES

Primers and analysis strategies for biostatisticians

Defining outcomes. A well-designed trial inherently demands a meaningful and reliable outcome. Explicitly defining the primary endpoint at the study's outset is crucial to mitigate potential investigator selection bias. OS is typically the standard endpoint in phase III immunotherapy cancer trials. Often considered the most objective measure, it is frequently referred to as the "hardest" endpoint in cancer trials. Numerous immunotherapy randomized trials have been designed with OS as the primary endpoint and have demonstrated benefits to patients. Other commonly used endpoints include PFS, DFS, and recurrence-free survival. The main limitation of employing any of these endpoints is that the effect of immunotherapy treatment may affect one endpoint (death) differently than the other (such as progression or recurrence). Consequently, several studies consider dual primary endpoints.

Justifying marker inclusion. The concept of an essential biomarker presupposes that some are so well-founded and broadly applicable that they can be justifiably incorporated into a new study without making detailed, context-specific assessments of utility, but does not absolve us of the broader obligation to respect the sacrifices that patients make to participate in trials and to use scarce research resources wisely. We expect that this guidance is most applicable when deciding to include emerging biomarkers in a study, and perhaps in the converse situation where it makes contextual sense to exclude an essential marker.

Table 4 provides a checklist for using an emerging marker in a clinical trial. The items listed there, for example, is it financially and procedurally feasible to collect and evaluate the required data, have accuracy and precision been demonstrated in previous studies, are there cheaper, more reliable, or better understood alternatives available? To couch the issue in the simplest mathematical terms, the checklist guides the user to consider whether the cost of obtaining the marker is affordable in the context of the study, and whether benefits exceed cost. This is easier said than calculated as many of the factors that would inform the decision are qualitative rather than quantitative, and the checklist does not provide guidance on integrating a marker into the study design and analysis plan.

Sample size. There are several approaches for calculating sample size requirements, depending on the questions to be answered. The reader may be most familiar with classical power analysis,[243] applied when performing a hypothesis test, to ensure that the experiment has adequate power to discriminate clinically significant alternatives from the null hypothesis of no treatment benefit. This approach is commonly used in Phase II and III trials where demonstrating efficacy is the primary objective and will be applicable when making decisions about markers as well, when the hypothesis testing framework is relevant, for example, when the objective is to establish whether an emergent biomarker is correlated with response to the therapy under consideration.

A second approach, applicable when the goal is to estimate a rate or other quantity, characterizes sample size requirements in terms of the precision of the estimate, usually expressed as the width of a 95% CI. This has broad applications in early-phase clinical trials where objectives might include (1) accurate and precise characterization of pharmacokinetic factors, (2) identifying clinical and molecular variables associated with increased toxicity, or (3) estimating the sensitivity and specificity of a putative marker of response. Other approaches include Bayesian and information-theoretic options. A general summary of methods applicable to clinical trials can be found in the appendices to the DELTA2 guidance.[244] We cannot establish rules here for minimum power, precision and the like, the landscape of early trial biomarkers, and the objectives they serve are too diverse to support such an endeavor. These matters should be determined in

context, and under close consultation with study statisticians. The appendix on statistical considerations includes further guidance on these matters, including prototype analysis plans for the essential markers, and more specific advice on how to evaluate sample size requirements for the various types of biomarkers represented there.

Choosing marker thresholds. To apply biomarkers derived from trials in a clinical setting, it is essential to establish biologically meaningful clinical cutoffs for each biomarker. In the case of a continuous biomarker, cutpoint selection is often used to dichotomize or otherwise stratify patients into groups based on association with a given clinical endpoint. The most widely accepted method for selecting an optimal cutpoint is the Youden index,[245–247] which maximizes the correct classification rate and therefore minimizes the misclassification rate. Importantly, the cut point for clinical benefit based on a given biomarker may differ across different cancer types and should be selected in a data-driven manner whenever data is available. For data reporting, a threshold should be identified and clearly stated and the underlying data should be available to verify that the cutpoint selected is indeed optimal.

Randomization and stratification factors. Given patient heterogeneity and the targeted nature of most IO therapies, it is anticipated that patients with positive biomarkers may have different responses than those with negative biomarkers. Therefore, the biomarker of interest could be incorporated as a stratification factor in the randomization process. The primary objective is to achieve a balance between the two treatment arms concerning the biomarker (or any other baseline characteristics of the patients that might impact the outcome) to reduce bias. For instance, several trials have considered PD-L1 status as a stratification factor. The key concern is to ensure the availability of biomarker status on every patient prior to randomization. It is assumed that the biomarker is binary (positive or negative, although some do not have clear cutoff points) and underwent analytical validation, and its cutoff has been established and validated before being incorporated into the study.

While trials involving immunotherapies have contributed to enhanced patient care and prolonged survival benefits, they have also presented challenges. Several phase III trials have revealed instances where treatment effects were not consistently maintained or where the PH assumption was not satisfied. Notably, data from several clinical trials have demonstrated various violations of the PH assumption. Some studies have shown delayed separation or crossing hazards, or even a cured proportion in the Kaplan-Meier (KM) curves for the primary endpoint.[248] This suggests that the risk of progression or death between novel immune-oncology drugs and control was not uniform over time. These findings significantly impact the design and the interpretation of treatment benefits for patients. Conventional metrics such as the HR and estimated median time-to-event endpoints may not fully capture treatment effects. It may be valuable to consider alternative measures such as the weighted HR, piecewise HR, restricted mean survival time (RMST), absolute difference in survival, and milestone estimates (landmark survival rates at specific time points).

When designing a clinical trial to assess treatment efficacy, the conventional approach is to use the log-rank test and assume a constant hazard between the treatment and standard care over time; that is the PH assumption is met. Typically, it is assumed that the survival distribution follows an exponential or Weibull distribution for two or more treatment arms. The log-rank test is the most powerful test when the PH assumption is met. However, in several scenarios, it is expected that the hazard varies over time, resulting in reduced power of the trial if the standard log-rank test is used.

There are several approaches that have been used to design IO studies when non-PH is observed. These methods can be categorized into three groups: rank-based, KM-based, and maximum combination tests (based on combining multiple weighted log-rank tests). These include but are not limited to the weighted log rank, weighted KM, maximum combination tests or the RMST. Each of these tests has its advantages or limitations. Thus, it is essential to consider which survival or PFS differences hold the most significance during the design phase (late vs early). One needs to consider the expected type of non-PH if any information on the treatment mechanism is available at the planning stage. Regardless of the chosen test, it is advisable to perform extensive sensitivity analyses to accommodate non-PH during the planning stage of confirmatory trials.

AI and machine learning for marker development. AI, in the form of machine learning and deep learning, also provides means to further define and identify potentially predictive biomarkers. Given that early-stage clinical trials often have small sample sizes, it may not be feasible or possible to apply AI algorithms to a trial's data. To leverage data from multiple clinical trials in an AI algorithm, it is imperative that data collection and reporting, including biomarkers and demographic data, are harmonized and standardized with metadata so that a complete and accurate combined training set can effectively and reliably find true patterns in the data. Bias in data sets also makes it imperative that diverse trial participants are included. AI also provides the benefit of being able to integrate multiple forms of high-dimensional clinical and biologic (multiomic) data using multimodal models and/or feature reduction followed by supervised learning.[249] Studies have shown that individual biomarkers may not be predictive and interactions or associations of combined detection may identify patients more likely to benefit, such as the combination of HLA-I loss of heterozygosity and TMB (referenced in the section on TME biomarkers).[249 250] Clinical trial data collection design should consider the advantages of expanding the types of data collected for patients, which is often costly and time-consuming, as an increased breadth of data will improve the likelihood of finding interactions or biomarkers,

leading to enhanced prediction of irAEs or therapeutic efficacy. Given that AI is well positioned to handle high-dimensional data, reporting all possible prognostic and predictive markers is key to identifying these interactions and combinations. Furthermore, as data augmentation and synthetic data generation powered by generative AI (eg, generative adversarial networks, biologic and clinical large language models) improve, clinical trial simulation, especially for combinations of known immunotherapies and other treatments, could help to identify and expand patient populations who could potentially benefit.

Approaches to biomarker databases in early clinical trials

There are several platforms where data from the NCI and industry-sponsored clinical trials are housed. These include, but are not limited to, the following: NCI,[251] database of Genotypes and Phenotypes,[252] Yale open data access,[253] Vivli,[254] clinical study data request [255] and Project Data Sphere.[256] These platforms capture various elements of data collected from clinical trials, including prognostic factors, stratification factors, endpoints, and safety information. They provide access to both aggregate and patient-level data from clinical trials.

Opportunities for innovation. The development of robust biomarker databases presents a transformative opportunity in precision medicine, particularly for clinical trials in cancer. A novel approach to these databases would involve integrating multiomics data, including genomics, proteomics, transcriptomics, and metabolomics, to provide a comprehensive landscape of disease biology. The feasibility of such databases is enhanced by advances in high-throughput sequencing technologies and machine learning algorithms, which can handle and analyze vast amounts of complex data efficiently. Patient advocates encourage and push for this concept, knowing that the acceleration of biomarker development and the ability to study multiple interactions can create synergistic effects, advancing medical solutions for patients more quickly.

One key application is the creation of dynamic, real-time databases that update continuously with new clinical trial data and patient outcomes, enabling adaptive learning and refinement of biomarker utility. This is particularly critical in early clinical trials, where patient numbers are limited, and robust data integration can significantly enhance trial design and outcome prediction. The Clinical Proteomic Tumor Analysis Consortium exemplifies this by providing proteomic data linked to clinical outcomes.[257] Additionally, incorporating PROs and longitudinal health records, as seen in the EHDS, can provide a holistic view of biomarker performance in diverse populations, which is invaluable for early-phase trials aiming to understand variability in treatment response.[258] Rare as well as pediatric cancers are another area that benefits from biomarker databases.

Such databases could facilitate targeted treatment strategies by identifying predictive and prognostic biomarkers, optimizing patient stratification in clinical trials, and uncovering novel therapeutic targets. This improves trial design by allowing for better patient stratification and enhances real-time data integration to refine biomarker utility continuously. Accelerated drug development is another benefit, as these databases speed up the discovery, development, and qualification of new biomarkers. The NIH Biomarkers Consortium plays a crucial role in this space by fostering public-private partnerships to support the discovery, development, and qualification of biomarkers, thereby accelerating their integration into clinical trials.[259]

Moreover, these databases support the pooling and comparison of data from multiple trials, facilitating powerful meta-analyses and broader insights into treatment effects. They also provide an opportunity to uncover new therapeutic targets and pathways by integrating multiomics data, leading to innovative treatment strategies. Collaboration between academic institutions, healthcare providers, industry, regulatory bodies, and patient communities is essential to ensure data standardization, security, and privacy.

Overall, the establishment of comprehensive and adaptive biomarker databases holds the promise of revolutionizing the design and execution of early clinical trials, improving the efficiency of drug development, limiting and avoiding toxicity, and ultimately enhancing patient outcomes. If possible, given the rapid development in the field, we would also recommend banking peripheral blood mononuclear cells and serum for subsequent analysis at baseline and following completion of therapy.

Special considerations for biomarkers in pediatrics

In pediatrics, biomarkers have emerged in the setting of using immunotherapy for hematologic malignancies and solid tumors. Immunotherapies such as ADC (eg, inotuzumab ozogamicin), bispecific T cell engagers (eg, blinatumomab) and CAR T cells (eg, tisagenlecleucel) all have leveraged measurable residual disease (MRD) measurements by flow cytometry or next-generation sequencing as a means of predicting success or treatment failure in children with B cell acute lymphoblastic leukemia (ALL), with MRD negativity being associated with prolonged survival. For example, children with B cell ALL who are found to be MRD positive by next-generation sequencing 28 days (about 4weeks) after receiving tisagenlecleucel have a higher likelihood of relapse irrespective of the loss of B cell aplasia.[19] Interestingly, B cell aplasia is still used as a surrogate measurement of CAR T persistence, and early loss of B cell aplasia is a biomarker of tisagenlecleucel treatment failure and predicts relapse in children.[19] This has not been observed in adults with non-Hodgkin's lymphoma treated with the same product,[260] but it is unclear if this is related to the patient population or underlying disease. In terms of other biomarkers for hematologic malignancies, PD-L1 expression predicts responses of children with lymphoma to nivolumab[261] and pembrolizumab.[262]

Unlike most adult solid tumors, however, pediatric solid tumors do not generally respond to ICB therapy.[261–264] Two patients with sarcoma did partially respond to a combination of ipilimumab and nivolumab despite having minimal (4%) to no expression of PD-L1.[265] The usage of PD-L1 expression as a biomarker, outside of lymphoma, has not yet been established. In contrast, as in adults, the presence of high MSI, germline mismatch repair deficiency or high TMB serves as a predictive biomarker for response to anti-PD1 therapy in pediatric solid tumors.[266 267] For neuroblastoma, certain KIR-KIR ligand genotypes can serve as biomarkers that predict better responses to anti-GD2 therapy with dinutuximab without[268] or with chemotherapy.[269]

Despite the presence of a few biomarkers that can be leveraged for immunotherapy usage in pediatric cancers, there is still a high need to develop other assays as companion diagnostics that can predict response to treatment or disease burden. Potential biomarkers to predict disease burden could include CTCs, ctDNA or quantitative reverse transcription-polymerase chain reaction (qRT-PCR) of fusion genes unique to pediatric tumors (eg, PAX3-FOXO1 fusion) akin to how BCR-ABL1 is used to track the response of chronic myelogenous leukemia to tyrosine kinase inhibitors. Also, biomarkers may be developed to predict response to monoclonal antibodies or CAR T cells such as examining circulating and tumor GD2 expression, or immune cell subsets by single cell RNA seqeuencing (scRNA-seq) or flow cytometry that correlate with outcome. Overall, there is a high need to support correlative biology studies in early-phase clinical trials involving pediatric tumors so that biomarkers can be co-developed with experimental immunotherapies.

Special considerations for biomarkers in hematologic malignancies clinical trials

In the context of hematologic cancers, biomarkers provide distinct advantages compared with those used in solid tumors, due to the systemic nature and easy accessibility of these malignancies, which allow for easier longitudinal monitoring. Unlike solid tumors, which often require invasive tissue biopsies and exhibit significant heterogeneity within the tumor itself and between the primary tumor and metastatic sites, hematologic cancers can be assessed through less invasive blood samples and bone marrow biopsies, typically showing less cellular heterogeneity. Minimal or MRD, which can be assessed by multiparameter flow cytometry, quantitative RT-PCR or next-generation sequencing of clonal sequences through molecular aberrations such as FLT3, NPM1, and IDH mutations, as well as TCR and immunoglobulin heavy chain (IgH) clonality, plays a pivotal role in hematologic malignancies. MRD provides well-established prognostic value that directly influences treatment decisions by informing prognostication, guiding treatment modifications, and predicting relapse.[270] Liquid biopsies, detecting cancer information through cell-free DNA, offer an accurate reflection of the total tumor burden and are particularly invaluable for real-time genomic profiling and monitoring disease dynamics in hematologic cancers, particularly lymphoma.[271] This method is inherently more applicable to hematologic malignancies due to their circulating nature and provides a dynamic tool for assessing disease status over time. In the context of CAR T-cell and other cellular therapies clinical trials, biomarkers such as cytokine profiles and immune cell profiles, along with inflammatory markers like CRP and ferritin, are critically important, as they help monitor the efficacy and toxicity of the therapies, providing essential insights into the patient's immune response and potential adverse effects like cytokine release syndrome.[272 273] Immunophenotypic markers such as CD19, CD20, CD30, and BCMA are crucial for diagnosis, monitoring therapeutic responses, and directly serving as targets for immunotherapies9.[274] Moreover, tumor burden markers such as LDH and β2M, similar to solid tumors, provide valuable insights into overall tumor load and response to treatment. Clonal evolution in hematologic cancers, tracked straightforwardly through blood samples, provides critical insights into treatment resistance, enhancing the understanding of disease progression and therapy efficacy. This uniformity and the ability to quantitatively monitor biomarkers underscore the unique role of biomarkers in clinical trials for hematologic malignancies, offering a sharp contrast to the challenges posed by tumor heterogeneity and sampling limitations frequently encountered in solid tumor biomarker research.

Notably, TMB and PD-L1, which are widely considered important in solid tumors, serve as crucial biomarkers guiding the use of immunotherapies due to high mutation rates and significant PD-L1 expression that correlate with treatment response. In contrast, their utility in hematologic malignancies is limited; TMB is generally lower and less predictive, and PD-L1's role is more nuanced and specific to lymphoma subtypes.[275]

Special considerations for biomarkers in CNS and meningeal tumors

Brain and central nervous system (CNS) tumors have remained a challenge for immunotherapy. CNS tumors are often considered immunologically inert or "cold tumors" with a relatively low TMB and in a region with a unique lymphatics system.[276 277] Nevertheless, recent studies have highlighted encouraging findings in using immunotherapies to treat CNS tumors, such as CAR-T cell therapy, TIL therapy, and therapeutic/neoadjuvant ICB.[278 279] In clinical trial development and biomarker considerations it is important to note multiple key features that may be unique to these tumors. For example, brain-resident microglia resembling tumor-associated macrophages

have been shown to contribute to the immunosuppressive TME, in addition to the common myeloid-derived suppressor cells, and macrophages in a spectrum of polarization states. While markers to directly distinguish microglia from infiltrating myeloid cells have been identified, such as CX3CR1 and P2RY12, they remain an area of active investigation.[280] Another aspect of biomarker considerations for brain tumors is the lack of frequently available tissue for assessment. Investigators may therefore consider supplementing tumor tissue analysis by examining biomarkers using cell-free tumor DNA, cytokines, and T-cell clone tracking in blood and CSF as potential surrogates of what may be occurring within tumors. Along the same line, advanced imaging techniques such as radiomics or immunoPET imaging of CD8 or other targets may have utility for these tumor types. Furthermore, it is important to note other elements specific to the brain such as the blood-brain barrier which often necessitates the need for specific immunotherapy delivery methods such as delivery directly into the tumor resection cavity or injection into the brain ventricles via Ommaya reservoir. These alternative methods of therapeutic delivery should be considered when designing biomarker studies (for example, sampling of tumor site fluid).

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