Immunity
Perspective

Top 10 Challenges in Cancer Immunotherapy

Priti S. Hegde[1] and Daniel S. Chen[2,*]
[1]Genentech Inc., South San Francisco, CA, USA
[2]IGM Biosciences, Mountain View, CA, USA
*Correspondence: dschen@igmbio.com
https://doi.org/10.1016/j.immuni.2019.12.011

Cancer immunotherapy is a validated and critically important approach for treating patients with cancer. Given the vast research and clinical investigation efforts dedicated to advancing both endogenous and synthetic immunotherapy approaches, there is a need to focus on crucial questions and define roadblocks to the basic understanding and clinical progress. Here, we define ten key challenges facing cancer immunotherapy, which range from lack of confidence in translating pre-clinical findings to identifying optimal combinations of immune-based therapies for any given patient. Addressing these challenges will require the combined efforts of basic researchers and clinicians, and the focusing of resources to accelerate understanding of the complex interactions between cancer and the immune system and the development of improved treatment options for patients with cancer.

Introduction

Therapeutic advances in cancer immunotherapy (CIT) have rapidly emerged in the past few years, reflecting the importance of the interaction between the human immune system and cancer. Despite the successful application of CIT across a broad range of human cancers, only a minority of patients with otherwise terminal cancer experience life-altering durable survival from these therapies. These outcomes likely reflect the complex and highly regulated nature of the immune system. Similar to other intricate and well-designed systems, a series of biological steps needs to be sequentially fulfilled prior to successful immunologic elimination of cancer cells. Additionally, the system is coupled with a multitude of fail-safes, negative feedback loops, and checkpoints that enable both precision control and the ability to disengage and shut down an immune response. Furthermore, cancer itself is complex, adaptable, and heterogeneous, and is the product of a myriad of genetic alterations that can disturb the normal function and behavior of cells. However, the genetic alterations that are central to the oncogenic process can also lead to the cancer cell appearing increasingly foreign to the immune system, providing an opportunity for CIT.

Cancers present differently in different patients, and tumors can vary within a given patient based on differences in the clonality of the cancer cells themselves (Parsons, 2018) and/or the surrounding microenvironment. Furthermore, some malignancies arise following chronic inflammatory states, whereas others can subvert and/or co-opt an immune response as part of progression and metastasis. The resulting interplay between the evolving entities of the human immune system and an emergent cancer can lead to several different outcomes: complete immunologic eradication of cancer, a chronic tug-of-war between the two, or uncontrolled cancer growth that has evaded an immune response. Modification of these outcomes toward cancer eradication is the goal of CIT.

The ultimate successful application of CIT must be defined by the ability to achieve durable responses and survival for patients with terminal as well as earlier-stage cancer. CIT can be successful for individual patients; however, the field of CIT research faces many challenges in the pursuit of fulfilling the broader societal goal of "curing cancer." Here we define ten key challenges facing CIT (Table 1). This list is an attempt to focus in on important challenges facing the field and is intended to stimulate collaboration and help galvanize a broader effort. Further, this list is not presented in a prioritized fashion but rather from basic research to clinical development-related opportunities. Given the enormity of the current CIT effort, coordination and collaboration within the larger community are beneficial, if not essential, to the overall success of this endeavor.

Challenge: Development of Pre-clinical Models That Translate to Human Immunity

Cancer drug discovery relies on preclinical models to prioritize drug targets and study mechanisms of action, delivery approaches, treatment dose and schedule, and safety management. The use of these models has led to many key discoveries in cancer treatment and immunotherapy, including the anti-tumor effects of CTLA-4 and programmed death-ligand 1/programmed death-1 (PD-L1/PD-1) blockade (Dong et al., 2017; Freeman et al., 2000; Leach et al., 1996). However, human cancer immune biology is not always well reflected in the models that are typically used (Ebert et al., 2016; Shrimali et al., 2017; Spranger et al., 2014). Major differences include composition of immune cells in the microenvironment, tumor antigens, and complexity of immune cell suppression that results from chronic immune recognition and exposure (Dranoff, 2011; Gould et al., 2015). For example, the murine colorectal cancer (CRC) MC38 model was derived from a mouse exposed to carcinogen and is commonly used to study cancer immunology preclinically. While MC38 tumors have a high tumor mutational load, which reflects the hypermutated or microsatellite instable (MSI) form of CRC, the nature of these mutations differs from those typically seen in human cancers (Homet Moreno et al., 2016; Efremova et al., 2018). Additionally, another CRC model CT26 shares features with non-hypermutated or microsatellite stable (MSS) aggressive, undifferentiated, and refractory human CRC (Castle et al., 2014) but also has a re-expressed endogenous retrovirus immunodominant antigen that promotes immunogenicity (Huang et al., 1996).

Table 1. Ten Key Challenges for Cancer Immunotherapy
1. Development of pre-clinical models that translate to human immunity
2. Determining the dominant drivers of cancer immunity
3. Understanding organ-specific tumor immune contexture
4. Understanding the molecular and cellular drivers of primary versus secondary immune escape
5. Elucidating the benefit of endogenous versus synthetic immunity
6. Effective and efficient assessment of cancer immunotherapy combinations in early-phase clinical studies
7. Full characterization of the impact of steroids and immune suppression on cancer immunotherapy and autoimmune toxicities
8. Maximizing personalized approaches through composite biomarkers
9. Developing improved regulatory endpoints for cancer immunotherapy
10. Optimizing long-term survival with multi-agent cancer immunotherapy combination regimens

An issue with commonly used pre-clinical models is that these rely on implantation of cancer cell lines. Tumors that grow subsequent to implantation often do not recapitulate the tumor immune contextual characteristics that influence an immune response in human cancers (Fridman et al., 2012). In addition, whereas the most commonly used preclinical models can perform as subcutaneous implants, they do not reflect the complex tissue- and/or organ-specific aspects of tumor growth and development that also influence immune response to cancer upon intervention. Related to this last point, in humans, cancer is most often believed to evolve over years, during which the relationship and interaction with the human immune response is shaped. In this context, genetically engineered mouse models come closest to representing human disease. These models have traditionally been developed by knocking out tumor suppressor genes or induction of somatic mutations using the Cre-LoxP system in an organ-specific manner resulting in *de novo* tumors (Kersten et al., 2017). As this method of tumor induction does not mimic the sequential accumulation of mutations that occur through the course of human cancer evolution, these models represent immunologically cold and genetically stable cancers that typically do not respond well to CIT. Therefore, thus far, the translation of preclinical findings to human cancer has been limiting. This reality has led to the need to address the majority of scientific questions through experiments conducted during clinical trials. Although this approach has led to important insights due to the broad efficacy of many of these agents, in particular, checkpoint inhibitors (CPIs) and chimeric antigen receptor (CAR) T cell therapy, it has also resulted in an enormous number of clinical trials, especially those aimed at defining the effectiveness of combination therapies. Further, the complicated interactions that exist with the immune system, particularly as it relates to priming and activation, cytotoxic activity and regulation, and formation of a memory response, highlight the need to understand therapeutic sequencing and schedule in addition to dose selection. Such therapeutic optimization is nearly impossible in clinical trials, due to the staggering combinatorial possibilities to be tested. As such, this paradigm cannot continue, and new techniques and technologies that better reflect the biology of human cancer immunology are needed (Zloza et al., 2017).

Preclinical models are being further refined, including humanized mouse models, genetically re-engineered mouse models to improve on the immunogenicity of tumors, organoids, and mammospheres derived from human tumor stem cell precursors, and *ex vivo* technology (Zitvogel et al., 2016). *Ex vivo* human tumor explant models are especially enticing for CIT, as they allow tumor-immune cell interactions to be observed using a combination of immunostaining and real-time imaging (Salmon et al., 2012). Additional challenges come from the complexity of the immune system itself, which relies on numerous different cell types within the tumor microenvironment (TME) to function and therefore cannot be easily replicated within a single cell type. Some of these complexities are reflected in the immune infiltrate, as human tumors can be arranged on the tumor immunity continuum and categorized as having inflamed, immune desert, or immune-excluded phenotypes based on the spatial localization of immune cells with respect to the tumor and stromal compartments (Hegde et al., 2016). Inflamed tumors are associated with close proximity of immune cells with tumor cells, immune-excluded tumors associated with immune cells embedded in the surrounding tumor stroma away from tumor cells, and immune deserts associated with tumors devoid of tumor-infiltrating lymphocytes (TILs). When tumors derived from the murine CT26 syngeneic tumor model were examined, they were found to have immune infiltrates characterized by the presence of cytotoxic immune cells (Lechner et al., 2013), while the infiltrates from MC38- or EMT-6-derived tumors were found to be largely composed of immunosuppressive cells, including myeloid-derived suppressor cells (MDSCs), with T cells being excluded (Mariathasan et al., 2018; Mosely et al., 2017). In addition to the tumor immunity continuum, other ways to differentiate and categorize tumors by immunogenicity have been suggested, including the six immune subtypes classification proposed by Thorsson et al. (wound healing, interferon [IFN] γ dominant, inflammatory, lymphocyte depleted, immunologically quiet, and transforming growth factor (TGF)β dominant) (Thorsson et al., 2018). Regardless of the type of categorization used, care is needed when selecting preclinical models to test hypotheses to ensure that key features of the human cancer are retained.

Reverse translation, which is taking scientific insights derived from clinical studies and applying them back to pre-clinical models, has helped further characterize some of the specific deficiencies that exist. This drive toward developing more translatable models, if successful, could improve the field's understanding of the biology underlying new therapeutic agents and their targets. Application of such models might then assist in potentially helping avoid Phase III clinical trial failures, such as those involving combination studies of drugs that target the indoleamine 2,3-dioxygenase enzyme or MEK pathway and PD-L1/PD-1 inhibitors (Barlesi et al., 2018; Eng et al., 2019; Long et al., 2019; Rizvi et al., 2018).

Challenge: Determining the Dominant Drivers of Cancer Immunity

Tumors are defined as inflamed based on high expression of PD-L1 by tumor cells or tumor-infiltrating immune cells and/or high

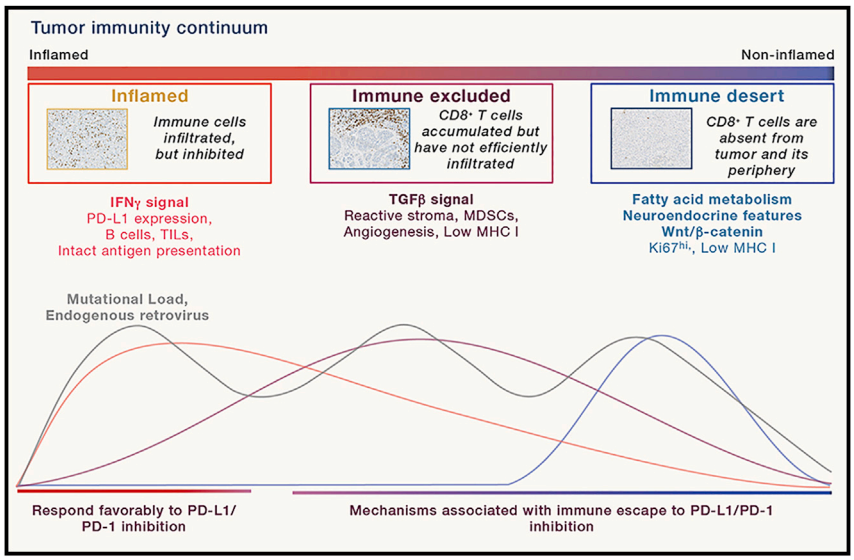

Figure 1. The Tumor Immunity Continuum

A gradient of three immunophenotypes are observed based on spatial distribution of CD8+ T cells in the tumor microenvironment. Mapping of these immunophenotypes with gene expression and/or immunohistochemistry (IHC) reveals molecular pathways associated with the phenotypes. Inflamed tumors are associated primarily with IFNγ signaling and related features including high tumor PD-L1, TILs, B cells, and intact antigen presentation (i.e., intact HLA and expression of MHC class I on the surface of tumor cells; red tracing). Immune-excluded tumors are defined by the presence of TGFβ signaling, reactive stromal biology, presence of myeloid-derived suppressor cells (MDSCs), and tumor angiogenesis (peri-tumoral variant histology shown; purple tracing). Immune desert tumors are devoid of T cell infiltration and primarily present with neuroendocrine features or highly proliferative tumor cells with increased fatty acid metabolism (blue tracing). Wnt/β-catenin signaling has also been shown to be associated with these tumors. Both immune-excluded and immune desert tumors are associated with low MHC class I expression, potentially due to reduced influence of IFNγ signaling in the tumor milieu. Neoantigenic signals provided by tumor mutation load or endogenous retroviruses show a wide distribution of prevalence across the three phenotypes. Histologic images and staining for CD8 T cells representing cancer immune phenotypes are provided by Hartmut Koeppen MD PhD (personal communication). Adapted from Hegde et al., 2016.

prevalence of TILs (Galon and Bruni 2019; Herbst et al., 2014; Ayers et al., 2017; Ayers et al., 2019). These inflamed tumors are associated with response to immune CPI, particularly PD-L1- and PD-1-directed antibodies. Histopathologically, the T cells are interspersed in the intra-epithelial spaces, in close proximity to and in contact with tumor cells (Kowanetz et al., 2018). A wealth of data now exists on biomarkers of inflamed tumors beyond tumor PD-L1, including IFNγ signatures, B cells, and genomic instability as defined by MSI or high tumor mutational burden (TMB) (Figure 1). While IFNγ signatures represent tumors with high PD-L1 expression and mark similar patient populations, the recent association of B cells with efficacy to CPIs underscores the importance of B cells in supporting T cell activation, survival, and anti-tumor immunity. Tumors containing an abundance of plasmablast-like B cells are associated with exquisite survival benefit to CPI therapies compared to chemotherapy (Griss et al., 2019).

Genomic instability as described by MSI or TMB status provides one mechanism for creating unique antigenicity for a cancer cell, providing the host immune system something specific and foreign (non-self) to "latch onto" a tumor-associated antigen bound to the major histocompatibility complex (MHC). In general, mutations that occur early in oncogenesis and are shared across cancer cells (clonal mutations) may generate a more effective anti-cancer T cell response versus later branch mutations limited to a subpopulation of the cancer cells (subclonal mutations) (McGranahan et al., 2016). This immune recognition signal of cancer antigens, particularly neo-antigens, is critical for initiation of the cancer immunity cycle (step 1) (Chen and Mellman, 2013). Consequently, tumors with very high TMB, of which MSI represents the highest fraction of the TMB continuum, respond favorably to CPIs based on early data in metastatic bladder cancer, non-small cell lung cancer (NSCLC), colon cancer, and multiple other cancer types describing the same (Rizvi et al., 2015; Rosenberg et al., 2016; Cristescu et al., 2018; Le et al., 2015; Samstein et al., 2019). However, given that inflamed tumors undergo immune editing by promoting loss of heterozygosity (LOH) in the human leukocyte antigen (HLA) locus (McGranahan et al., 2017; Rosenthal et al., 2019), the question remains whether high TMB tumors from highly inflamed cancer like NSCLC ultimately derive enhanced survival benefit from CPI therapies. Recent results from randomized Phase III trials in front-line NSCLC including Checkmate-227 and MYSTIC suggest high TMB may not be effective at predicting for survival benefit from a combination of PD-1 and CTLA-4 inhibitors (Peters et al., 2019a; Peters et al., 2019b). It remains to be seen if HLA -LOH confounds the readout of these complex biomarkers and should be taken into consideration when defining an immunogenic tumor driven by step 1 of the cancer immunity cycle. Loss of antigen presentation on a malignant cell would clearly impact the ability of T cells to recognize neoantigens generated by mutations. It is also important to note that it is not yet clear what defines the impact of relative T cell responses against different antigens or epitopes. Presumably, the T cell immune response is directed against a variety of epitopes, and the relative importance of a response against a specific epitope is stochastic, resulting from temporal competition of the responding T cells to priming, activation, stimulation, and survival. This appears to be true even after bystander or "tourist" T cells in the TME that do not recognize cancer epitopes are removed from consideration. This is consistent with analyses of T cell responses detectable in blood and tumors. However, temporal variability in response complicates such studies in humans. In most cancer patients, multiple immunogenic neo-antigens are present, and may elicit a T cell response. The breadth of these T cell responses likely contributes to the durability and depth of CIT immune responses. However, the importance of a given T cell response against an individual neo-antigen is more difficult to assess, given intratumoral heterogeneity, relating to the stochastic nature of T cell stimulation and inhibition encountered within the TME. (Linnemann et al., 2014; Scheper et al., 2019; Rizvi et al., 2015; Jamal-Hanjani et al., 2017; McGranahan and

Swanton, 2015; Joshi et al., 2019). This contrasts from anti-viral T cell immunity, where a dominant clonal T cell response against a specific viral epitope most commonly emerges until that viral epitope is lost, after which a new dominant clonal T cell response against a different viral epitope may emerge.

While high TMB represents step 1, PD-L1 expression or IFNγ signatures most commonly represent step 7 of the cancer immunity cycle, the presence of pre-existing immune response. This signal suggests that the TME is permissive of existing functional T cell responses. PD-L1 expression is also associated with response to CPIs and has thus far been the more reproducible biomarker of survival benefit compared to TMB with Phase III trials in NSCLC including Keynote-024, Keynote-042, IMpower110, and Checkmate-227 (Hellmann et al., 2018; Herbst et al., 2019; Peters et al., 2019b; Spigel et al., 2019). TMB and PD-L1 are independent markers and do not necessarily coincide in patient tumors (Cristescu et al., 2018; Gandara et al., 2018). Remarkably, metastatic bladder cancer patients with high TMB but low pre-existing immunity (immune deserts) derive similar benefit from CPIs as patients with high tumor PD-L1 expression (Mariathasan et al., 2018; Rosenberg et al., 2016). It can be hypothesized that these tumors do not undergo immune editing via HLA loss given the lack of immune pressure for survival. Thus, high TMB in non-inflamed immune desert tumors may indeed translate to increased presentation of antigens, with alternate mechanisms that prevent immune cells from infiltrating the TME. The enriched response rates observed to pembrolizumab monotherapy in TMB high (28.3% objective response rate [ORR]) versus TMB low (6.5% ORR) tumors in the pan tumor KEYNOTE-158 trial (Marabelle et al., 2019) investigating multiple typically non-inflamed cancers including anal, biliary, cervical, endometrial, salivary, thyroid cancers, mesothelioma, and SCLC further lends credence to this hypothesis. This does also suggest an additional role for CPIs in priming new T cell responses in the periphery, which in turn results in durable anti-tumor immune responses. The fraction of patients whose tumors present with both high TMB and pre-existing immune response signals represents the population that most often derives truly transformative benefits from CPIs (Balar et al., 2017; Herbst et al., 2019).

A number of cancers are characterized by the presence of inflammation without high TMB. These include renal cell carcinoma (RCC), hepatocellular carcinoma (HCC), triple-negative breast cancer, gastric cancer, and, to an extent, head and neck cancers. Conversely, diseases such as small cell lung cancer (SCLC) represent tumors characterized by high TMB in the absence of other markers associated with inflamed tumors. It is now well understood that genomic instability is not the only source of cancer antigens in tumors. Diseases like clear cell RCC, a subset of prostate cancers, and even ovarian cancers have a high prevalence of human endogenous retroviruses (hERVs). hERVs integrated into the human genome are largely silenced in normal cells but can become dysregulated and re-expressed in cancer; accordingly, hERVs may serve as the tumor antigen signal (Cherkasova et al., 2013; Smith et al., 2018). Indeed, RCC tumors that express high amounts of hERV also express IFNγ signatures, suggesting that these tumors are indeed inflamed (Panda et al., 2018). Moreover, T cell responses to hERV antigens are observed in RCC tumors (Smith et al., 2018) and in peripheral blood from patients with RCC regressing following allogeneic hematopoietic stem cell transplants (Takahashi et al., 2008), all of which suggest that hERVs may indeed provide the antigenic signal in these cancers. In addition to hERVs, viruses such as Epstein-Barr, hepatitis B, hepatitis C, human papillomavirus, and Merkel cell polyomavirus may also provide a source for strongly immunogenic antigens in cancers such as head and neck squamous cell carcinoma, HCC, cervical cancer, Merkel cell carcinoma, and some subsets of gastric cancer (Simoni et al., 2018). While viral status has not been clearly associated with outcomes to CPIs in some of these cancers, the evidence for the role of viral genomes integrating into host genomes leading to genomic instability is well established (Flippot et al., 2016). Thus, the cancer indications associated with inflamed tumors should now broadly encompass not just those that express PD-L1 and have high TMB but also cancers expressing hERV and other viral genomes. While monotherapy efficacy in these tumor types has been observed, combinations with standard-of-care agents have yielded the greatest improvement in overall survival (OS) in cancers such as RCC (e.g., combinations with vascular endothelial growth factor [VEGF] pathway inhibition) (Atkins et al., 2018).

Immune-excluded tumors refer to tumors with an immune suppressed TME represented by T cells clearly embedded in the tumor stromal microenvironment with high TGFβ signaling, myeloid inflammation, and angiogenesis (Galon and Bruni, 2019; Hegde et al., 2016; Hugo et al., 2016; Mariathasan et al., 2018). Immune-excluded tumors appear to be distinct from densely desmoplastic tumors as observed in some cases of melanoma and NSCLC. Desmoplastic tumors exhibit very high expression of PD-L1 on tumor cells (often 80%–100% of tumor cell stain positive for PD-L1) and have been associated with high response rates to CPIs (Eroglu et al., 2018; Kowanetz et al., 2018). While these tumors are densely populated with CD8 T cells in the invasive margin, they histologically appear similar to immune-excluded tumors. Immune-excluded tumors conversely tend to respond poorly to CPIs (Hugo et al., 2016; Mariathasan et al., 2018). Reverse translation studies have demonstrated a major role for the TGFβ signaling pathway in promoting the excluded phenotype, the suppression of which results in phenotype switch to inflamed tumors that respond favorably to the combination of aPDL1+aTGFβ in preclinical EMT6 breast cancer models (Mariathasan et al., 2018), providing one possible combination to address this biology. Trials testing these combinations are currently ongoing (NCT02734160, NCT04064190, NCT02947165). Myeloid inflammation associated with high expression of cytokines such as IL-8 and IL-6 in the TME is also prevalent in both inflamed and immune-excluded tumors (McDermott et al., 2018). It remains to be seen if combinations of CPIs with inhibitors of cytokines such as aIL-8 or aIL6r will result in improved outcomes. Clinical trials with these combinations are currently ongoing (NCT03400332, NCT03866239). Myeloid biology is poorly understood in human cancers and differs significantly between murine models and human disease. Trials with anti-colony stimulating factor 1 receptor (CSF1R), an agent that depletes tumor resident macrophages (Pradel et al., 2016), have largely been unsuccessful. While CSF1R is expressed on macrophages, the plasticity and definition of antigen-presenting macrophages versus immunosuppressive myeloid cells in human tumors are poorly understood. It is

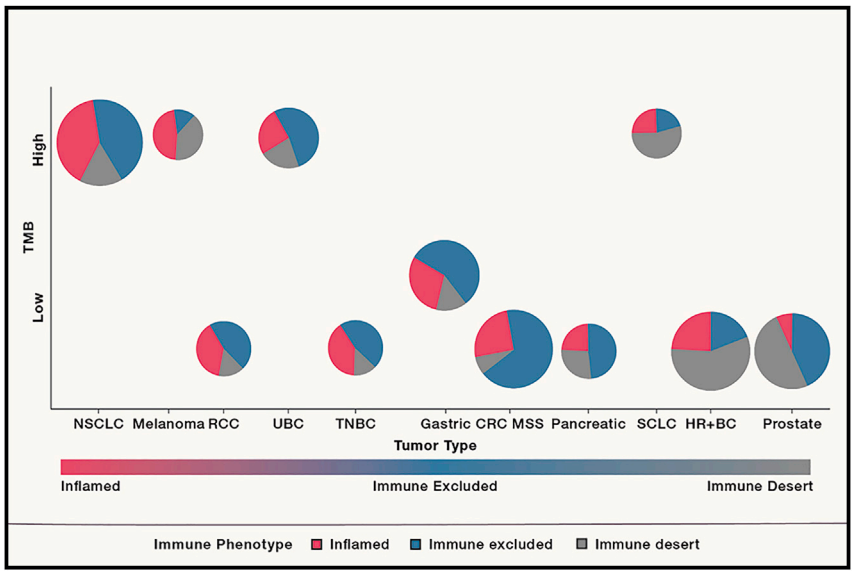

Figure 2. Qualitative Overview of Relationship Between Immune Phenotypes and TMB Across Cancer Types

Select tumor types are displayed on the immune phenotype continuum (x axis). Relative level of TMB is also depicted (y axis). The phenotype definitions have either been derived by assessment of CD8+ T cell immune contexture as in NSCLC, melanoma, and UBC (Herbst et al., 2014; Mariathasan et al., 2018) or by gene expression of the Cancer Genome Atlas datasets (George et al., 2015; Iglesia et al., 2016; Thorsson et al., 2018) interrogating gene signatures of IFNγ (as defined in McDermott et al., 2018) to define inflamed tumors and markers representing stromal biology for immune-excluded tumors (Mariathasan et al., 2018). Absence of both IFNγ and stromal signatures depicts immune deserts consisting of low expression of antigen presentation associated gene signatures and low expression of stromal genes. Circle sizes correspond to relative global incidence of each cancer (Bray et al., 2018).

CRC, colorectal cancer; MSS, microsatellite stable; HR+ BC, hormone-receptor positive breast cancer; NSCLC, non-small cell lung cancer; RCC, renal cell carcinoma; SCLC, small cell lung cancer; TMB, tumor mutational burden; TNBC, triple-negative breast cancer; UBC, urothelial bladder carcinoma.

unclear if agents such as aCSF1R deplete the "good" macrophages along with the "bad" immunosuppressive cells. Advances in single-cell RNA sequencing technologies will further help elucidate the phenotypic description of myeloid cell populations in human tumors, collectively aiding better specificity to combinations aimed to address immune suppression mediated by these cells.

Lastly, immune deserts are histologically represented by tumors devoid of immune infiltration, antigen presentation (low MHC class I), and high tumor cell proliferation as seen in cancers such as pediatric malignancies, hormone receptor positive breast cancer, prostate cancer, glioblastomas, and SCLC. With the exception of SCLC, these diseases tend to be genomically stable with low TMB and respond poorly to monotherapy CPIs (Hansen et al., 2018; Lukas et al., 2018; Dirix et al., 2018). Multiple distinct mechanisms are associated with the immune desert phenotype. Gajewski and colleagues (Spranger et al., 2015) were the first to describe a role for tumor-intrinsic WNT/β-catenin signaling in describing the underlying mechanism for immune deserts whereby the chemokine gradient required for recruiting CD103+ dendritic cells including CXCL1, CXCL2, and CCL4 is reduced, thereby impacting early T cell priming and infiltration into the TME. Neuroendocrine tumors including SCLC are dominated by low prevalence of TILs with unclear underlying drivers of the desert phenotype (Carvajal-Hausdorf et al., 2019). In addition to the lack of an immunogenic signal to promote immune surveillance, rapidly proliferating desert tumors alter the metabolic conditions in the TME to create a hostile environment for the function and proliferation of T cells (Sugiura and Rathmell, 2018). T cells depend on similar glycolytic pathways as tumor cells for survival (Wang et al., 2011). Increased hypoxia, lactic acid production, presence of an acidic TME, and lipogenesis all together alter the immunometabolism of T cells affecting TCR engagement, T-effector activation, differentiation, and proliferation, ultimately resulting in reduced TILs (Sugiura and Rathmell, 2018). Addressing the reprogramming of tumor cells via generic modulators of metabolic pathways is not trivial, as the same mechanisms will likely impact T cell function as well. Identifying tumors addicted to metabolic stress such as HIF1α-dependent tumors, tumors with mutations in *PI3K/AKT/FGFR3* signaling may represent ideal candidates for pathway-specific therapeutics that inhibit pathways these cancer cells depend on while leaving the immune cells unperturbed. This may create an immune permissive microenvironment in these tumors, in combination with CPIs. Patient selection for these combinations needs to consider the specific underlying mechanisms being investigated.

The inflamed, immune-excluded, and immune desert phenotypes described above can be prevalent at varying degrees within a given tumor type and across cancers (Hamid et al., 2019; Kowanetz et al., 2018; Mariathasan et al., 2018). In Figure 2, we illustrate and broadly classify the approximate phenotype prevalence within each cancer and place them across the tumor immunity continuum as they correlate to TMB (Alexandrov et al., 2013). This figure highlights the complexity of antigen presentation and cancer-type-dependent immune suppressive pathways for several human cancer indications.

The overall clinical challenge still remains to determine the threshold for defining an inflamed tumor or a tumor with high antigen load and determining the specific drivers associated with particular phenotypes. For instance, for TMB in inflamed tumors, inclusion of HLA LOH may be relevant. The cutoff for TMB in inflamed tumors may differ from the cutoff for TMB in non-inflamed tumors (Samstein et al., 2019). As noted above, clinical translation for combination therapies will require identification of the right patient populations to convert either deserts or excluded tumors to inflamed tumors. While CD8 immunohistochemistry (IHC) may be sufficient to broadly categorize tumors, identification of the specific pathway drivers such as TGFβ, myeloid biology, or WNT/β-catenin or altered tumor metabolism, will require equally complex biomarker approaches such as gene signatures developed to enrich for the specific and relevant biology.

Challenge: Understanding Organ-Specific Tumor Immune Contexture

At a rudimentary level, the dominant forces that promote tumor growth include tumor cell intrinsic properties and the organ in which the tumor resides (Salmon et al., 2019). In metastatic urothelial carcinoma for instance, two starkly divergent patterns of response emerge when considering the location of the metastasis. Tumors that metastasize to the liver are more likely to resist therapy, whereas those that metastasize to the lymph nodes are more likely to demonstrate complete responses to CPI (Balar et al., 2017; Massard et al., 2016; Rosenberg et al., 2016; Tumeh et al., 2017; Pillai et al., 2016). Thus, location is an important component of the equation associated with an anti-tumor immune response, or lack thereof.

The concept of tissue-specific immunity is well appreciated in the contexts of infection and autoimmunity, and increased consideration of the distinct immune compartments and requirements within different tissues in cancer immunology, especially as related to the anti-tumor response, is warranted (Pao et al., 2018; Oliver et al., 2018). The liver, for example, is associated with the induction of immune tolerance through various mechanisms (Tiegs and Lohse, 2010; Crispe, 2014; Zhou et al., 2019). Myeloid-derived suppressor cells, Kupffer cells, and liver dendritic cells predominantly promote a network of active immunosuppressive pathways, dampening the activation of CD8+ and CD4+ effector T cells. The relevance of these tolerogenic mechanisms in cancer is evidenced by responses to liver metastases. Patients afflicted with melanoma or NSCLC with metastasis to the liver exhibit reduced responses to pembrolizumab and shorter progression-free survival (PFS) (Tumeh et al., 2017), and this is associated with reduced marginal CD8+ T cell infiltration leading to the inability of the immune system to effectively migrate to, interact with, and specifically recognize and respond to the cancer cells. The liver is also a highly vascularized organ with MDSCs and hepatic stellate cells that constitute the architecture of the liver. These, along with cancer-associated fibroblasts in the liver, provide a high threshold for resident T cells to get effectively activated and kill tumor cells (Gentilini et al., 2018; Pao et al., 2018; Crispe, 2014).

HCC, a disease that arises via chronic liver disease inflicted by cirrhosis caused by hepatitis C or hepatitis B infections, is now showing promising responses to CPIs in combination with VEGF-targeted therapies (Lee et al., 2019; Ikeda et al., 2019). The role of VEGF-targeted therapies in reversing the myeloid inflammation as seen in unresectable RCC, in addition to normalizing the tumor vasculature, could be hypothesized as a mechanism by which these therapies work in concert. It remains to be seen, however, if these combinations will prove to be effective in liver metastases of cancers that arise elsewhere. For example, MSS CRC commonly metastasizes to the liver and is poorly responsive to CPI. To date, studies of VEGF-targeted therapy and CPI in MSS CRC have not clearly demonstrated enhanced efficacy for the combination (Hochster et al., 2017).

Current clinical therapeutic paradigms generally do not incorporate organ-specific treatment approaches. This is despite the clear association between different sites of metastases and prognostic outcome (Tumeh et al., 2017; Botticelli et al., 2019; Reck et al., 2019). While the reasons for liver metastases being associated with poor survival outcomes and lymph node metastases being associated with favorable survival outcomes across multiple diseases and treatment modalities have been speculated upon, it is possible that the immune context of these different anatomic sites has a strong influence upon the reactive immune response of any anti-cancer therapy and is responsible for the poor outcomes. While our understanding of liver-specific immunity has benefited from many of these recent results, organ-specific immunity is certainly not limited to the liver. Anti-cancer immune responses also appear specifically limited in the bone microenvironment as well (Landi et al., 2019; Xiang and Gilkes, 2019), potentially due to the unique stromal interactions (e.g., collagen), cytokines present (e.g., TGFβ), and increased presence of MDSCs. Additionally, sites such as lymph nodes, lung, and skin may generally present as a more favorable site for anti-cancer immunity, potentially due to the relatively high presence of immune cells in these specific organs (Lavin et al., 2017; Zheng et al., 2017; Azizi et al., 2018; Guo et al., 2018; Sade-Feldman et al., 2018). Again, recent data suggest that an organ-specific therapeutic approach could be taken, and that additional studies of combination therapy may elucidate specific benefit for patients presenting with immune mechanism matched therapy (Chen and Hurwitz, 2018).

Challenge: Understanding the Molecular and Cellular Drivers of Primary versus Secondary Immune Escape to Checkpoint Blockade Immunotherapies

Clinical experience has shown that even when particular patients are well enabled to respond to CPIs (e.g., high tumor PD-L1 expression), a large percentage of them (>50%) do not respond to these agents (Reck et al., 2016). Conversely, some patients experiencing clinical responses lasting years can still ultimately experience progression of their cancer (Hamid et al., 2018). The former phenomenon is termed "primary immune escape" and the latter, "secondary immune escape" (Kim and Chen, 2016). While the mechanisms involved in these two different immune escape phenomena (sometimes termed resistance) can overlap, they do not overlap temporally. Multi-omics analyses on tissues from patients on CPIs such as atezolizumab, pembrolizumab, and nivolumab have now shed light on the many factors that contribute to immune escape (Anagnostou et al., 2017; Mariathasan et al., 2018; Wang et al., 2017; Zaretsky et al., 2016). As noted earlier, Mariathasan et al. showed that immune-excluded urothelial cancer tumors with heightened TGFβ signaling prior to initiating treatment fared poorly on monotherapy CPI. These tumors exhibit dense collagen fibrils that trap T cells in the stromal compartment and prevent them from engaging and killing tumor cells. At least preclinically, combining anti-PD-L1 with anti-TGFβ antibodies can partially overcome this escape mechanism, thus readily changing the immune milieu from immune-excluded to inflamed in concert with improving the functionality of infiltrating T cells. It is important to note here that TGFβ signaling was associated with lack of clinical benefit to atezolizumab just in tumors exhibiting the immune-excluded phenotype. Thus, mechanisms of immune escape may be associated with specific immune phenotypes. For example, in RCC, the mechanism of immune escape appears to be different. Here, inflamed TMB low tumors, defined by the presence of a pre-existing immune response, are often immune suppressed by the presence of myeloid inflammation and respond poorly to monotherapy CPI. However, addition

of bevacizumab (anti-VEGF) appears to reverse this immune suppression by extending PFS from a median of 6.1 months for CPI monotherapy to 11.7 months for the doublet (McDermott et al., 2018). A similar enhancement of benefit has been reported with CPI when combined with a small-molecule VEGF receptor tyrosine kinase inhibitor, such as axitinib (Atkins et al., 2018; Motzer et al., 2018a). Given the wealth of data on the role of anti-VEGF in reprogramming MDSCs, the addition of targeting VEGF to overcome myeloid-mediated immune suppression seems logical (Hegde et al., 2018; Oyama et al., 1998). As previously noted, loss of heterozygosity at the HLA locus in inflamed TMB high NSCLC tumors and epigenetic repression of neo-antigen transcripts is another mechanism by which inflamed tumors evade immunity (McGranahan et al., 2017; Rosenthal et al., 2019). The impact of these aberrations with outcomes to CPIs is yet to be determined. Different still is MSI-high CRC, in which the activation of Wnt-β-catenin signaling has been associated with immune escape in the large majority of cases (Grasso et al., 2018). In summary, multiple primary immune escape mechanisms may be associated in an immune phenotype specific manner presumably dependent on the underlying drivers of the phenotype.

Studying the drivers of secondary escape has been more challenging, mainly due to lack of systematic tumor tissue collection both prior to treatment initiation and at progression. Like primary resistance, the mechanisms associated with secondary resistance may be equally diverse. There is anecdotal evidence to suggest that tumors may induce genetic changes in the antigen presentation machinery including mutations in JAK-STAT signaling, loss of β2 microglobulin, downregulation of MHC expression, and loss of immunogenic mutations, or antigen loss, to overcome immune pressure (Shin et al., 2017; Zaretsky et al., 2016). Secondary escape has also been well documented in the fields of CAR T cell therapies and neo-antigen-specific T cell therapies. In the former, loss of tumor antigen targeted by the CARs has been documented to be associated with loss of clinical benefit as seen with CD19 CAR Ts in hematological malignancies (June et al., 2018; Grupp et al., 2013). It is unclear if tumor regrowth eventually occurs due to downregulation of target expression or if antigen-expressing cells get depleted resulting in growth over time of subclones that do not express these antigens. In the case of adoptive T cell therapies in melanoma and CRC, loss of functional β2-microglobulin (Restifo et al., 1996) and loss of specific HLA haplotypes to the neo-antigens introduced (Tran et al., 2016) resulted in secondary escape.

The drivers of primary immune escape are likely to have evolved over months and/or years and are the product of the endogenous host immune response. This makes primary immune escape easier to study from pre-existing tumor specimens and may make these mechanisms more dependent on the afflicted organs and the biology of the cell type that the cancer arose from, such as immune exclusion in GI malignancies. This may differ from secondary immune escape, which likely arise over shorter intervals following active immunotherapy. These mechanisms may be more associated with the specific type of CIT used, as is the case for CD19-directed CAR T treatment. Data from different types of effective CIT from different cancer types over time are needed to better understand the variety of different mechanisms for secondary immune escape.

Challenge: Elucidating the Benefit of Endogenous versus Synthetic Immunity

The development of PD-L1/PD-1 inhibitors has enabled a highly effective endogenous anti-cancer immune response to eliminate cancer cells in many patients. This endogenous immune response largely relies on previously primed CD8+ T cells that specifically recognize cancer cells. These immune cells can either be re-invigorated or expanded by PD-L1/PD-1 inhibition, leading to recognition and killing of cancer cells, as well as further proliferation of the T cells (Chen and Mellman, 2013; Herbst et al., 2014; Tumeh et al., 2014).

However, not all cancers may present appropriately immunogenic antigens that endogenous T cells can effectively recognize. Additionally, some cancers employ loss or downregulation of class I MHC to evade immune destruction (Aptsiauri et al., 2018; Garrido et al., 1993). Synthetic immune responses are the result of therapeutics that artificially bind T cells to cancer cells that might not normally do so based on their cognate binding of a T cell receptor to a specific peptide-MHC complex. Examples include engineered CAR T cells and CD3 bispecific antibody approaches (June et al., 2018; Zhukovsky et al., 2016). While these two distinct approaches both represent synthetic immunotherapy, they differ in many important ways (Figure 3). CAR T cells represent genetically modified T cells isolated from a patient and manipulated to introduce an artificial binding domain to a cancer-associated antigen linked most often to TCR signaling proteins and T cell co-stimulatory proteins prior to re-infusion in a cancer patient. Effectiveness of CAR T-based therepeutics relies on CAR T cell infiltration into all anatomic locations harboring cancer, specific binding and killing of malignant cells, and often, survival and/or proliferation of the CAR T *in situ* until all malignant cells are eliminated. CD3 bispecific antibodies generally encompass a group of infused therapeutic proteins that incorporate a binding domain to CD3 and a binding domain to a cancer-associated antigen in a fashion that specifically binds T cells to cancer cells and mediates elimination of the cancer cell utilizing T cell cytotoxic function. CD3 bispecific antibodies rely on naturally cancer-infiltrating T cells. Further proliferation and survival of these T cells may be beneficial but not required as long as the patient continues to produce T cells that infiltrate into cancer sites.

Unlike adaptive immunity, synthetic immunity promotes proliferation of both tumor-resident and non-specific T cells recruited to the tumor (Bacac et al., 2016; Bargou et al., 2008; Hoffmann et al., 2005; Kobold et al., 2018; Porter et al., 2011). Synthetic immune approaches may enable the generation of an initial anti-cancer immune response to cancers that are poorly immunogenic, leading to stimulatory cytokine upregulation, immunogenic cancer cell death, and further activation of endogenous anti-cancer immune responses. However, synthetic immune responses generally lead to IFNγ secretion and increased expression of PD-L1, which can at least partially dampen the synthetic immune response (Köhnke et al., 2015; Bacac et al., 2016). Inhibition of PD-L1/PD-1 can thereby relieve any immune suppressive effect of PD-L1 on T cell function, further enhancing the synthetic immune response (Chong et al., 2017).

The factors that drive immune suppression in immune-excluded and immune desert tumors are not yet fully understood, and immunotherapies that can generate effective endogenous immunity in patients with these tumors are lacking. Furthermore, many

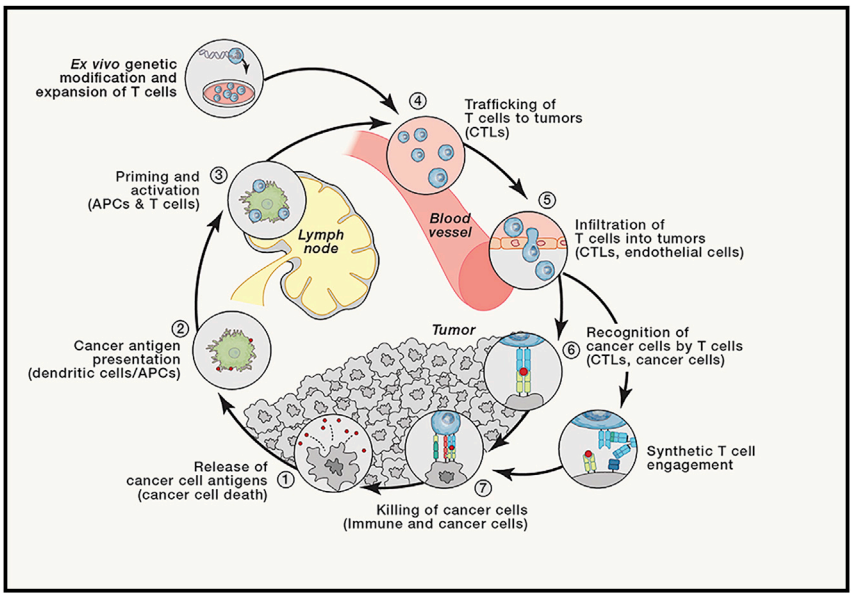

Figure 3. The Relationship Between Synthetic Immunity and The Cancer-Immunity Cycle

Endogenous cancer-immunity has been depicted as a self-propagating cyclic process, referred to as a series of steps known as the cancer-immunity cycle. These seven steps include release of cancer antigens, antigen presentation, immune activation, trafficking, infiltration, specific recognition of cancer cells by T cells, and killing of cancer cells. Synthetic immunity, primarily based on *ex vivo* manipulated cellular therapy and T cell engagers that bind CD3 and a cancer-cell surface expressed antigen, can also feed into, be influenced by, and impact the cancer-immunity cycle as shown.

APCs, antigen-presenting cells; CTLs, cytotoxic T cells. Adapted from Chen and Mellman, 2013.

inflamed tumors also present with multi-factor immune suppression—often specifically driven by IFNγ signaling, which may not be successfully overcome with treatment that only incorporates one or two CPIs (Kowanetz et al., 2018). In addition, downregulation or loss of MHC and/or β2 microglobulin may also prevent endogenous immune responses from binding to and eliminating cancer cells (Perea et al., 2017; Pereira et al., 2017). The synthetic approach to binding a T cell to a cancer cell can lead to not only non-endogenous T cell engagement with a cancer cell but also a potent T cell activation signal, resulting in a powerful cytotoxic immune response that can potentially overcome and/or overwhelm the immune suppressive signals that may be present (Topp et al., 2015; Gökbuget et al., 2018). The ability to treat patients with these tumors using synthetic immunotherapy as monotherapy or in combination with checkpoint inhibition may enable effective treatment long before we fully understand how to reverse the plethora of redundant layers of immune suppression and immune regulatory feedback loops.

Engagement of synthetic immunity leading to log-kill of cancer cells (defined as the ability of a therapeutic to cause a log increase in cell kill as is typically seen with chemotherapy in hematologic malignancies and solid tumors such as testicular cancer) has been clinically validated in hematologic malignancies and solid tumors. Examples of synthetic immunity in the clinic include bispecific T cell engagers for acute lymphoblastic leukemia (Kantarjian et al., 2017), CD19-directed CAR T cells for large B cell lymphoma (Neelapu et al., 2017; Schuster et al., 2018), carcinoembryonic antigen T cell bispecific antibody for CRC (Argiles et al., 2017), and New York esophageal squamous cell carcinoma-1 SPEAR T cells for myxoid liposarcomas (Robbins et al., 2015). It remains to be determined if monotherapy with a synthetic immunotherapy agent will result in the formation of an endogenous anti-cancer immune response. Endogenous immune activation could include a diverse tumor-antigen-specific immune response and robust memory T cells required for long-lasting durable anti-tumor responses in cases where direct log-kill is not enough to fully eradicate all cancer cells. Antigen-independent co-stimulatory signal 2 on antigen-presenting cells and their receptors on T cells may be required in some cases to drive memory responses with this platform (June et al., 2018). Moreover, synthetic immunity is likely to be highly dependent on target expression on cancer cells. For this reason, a personalized approach that incorporates target expression level may be required for maximizing benefit from engagement of synthetic immunity.

Given the above, therapies that combine endogenous and synthetic immune approaches may be particularly synergistic. In situations where monotherapy endogenous or synthetic immunity are curative alone, combination therapy may not be warranted; for example, PD-L1/PD-1 targeted therapies in patients with high PD-L1 and high TMB (Carbone et al., 2017) or CAR T therapy in certain CD19 expressing hematologic malignancies (Neelapu et al., 2017; Schuster et al., 2018). However, the majority of cancer neither expresses a strong enough endogenous immune response to eradicate cancer nor is sensitive enough to synthetic immune approaches alone to achieve curative outcome. The intersection of these two therapeutic areas is represented by cancers where cancer-associated antigens can be targeted by synthetic immune approaches and an endogenous anti-cancer immune response exists or can be generated. Determining the ideal combination of synthetic immune and endogenous immune approaches at this intersection will require additional studies. However, certain principles can be examined. How do we optimize synthetic immune approaches to engender, preserve, and/or stimulate an endogenous immune response rather than optimization only on cancer cell kill? How do the acutely proliferative signals and broad systemic levels of cytokines secreted impact endogenous anti-cancer T cell responses? How might we improve endogenous immune approaches for antigen spread and memory response? Are T cell agonistic signals, T cell survival factors, vaccination, or checkpoint inhibition approaches that should be prioritized? How do we safely combine these two modalities? In terms of this last question, it is clear that current synthetic immune approaches generate high levels of systemic cytokine secretion. These high levels can lead to cytokine release syndrome, a potentially fatal event. Further work is likely necessary to mostly dissociate the synthetic stimulation of cytokine secretion from the cytotoxic activity, which may be possible given

the differential T cell signaling threshold necessary to fully trigger these different T cell functions (Faroudi et al., 2003; Purbhoo et al., 2004; Varadarajan et al., 2011; Li et al., 2019). If these fundamental questions can be addressed, the unique strengths of both synthetic and endogenous immunotherapy may be most successfully combined.

Challenge: Effective and Efficient Assessment of Cancer Immunotherapy Combinations in Early-Phase Clinical Studies

With over 1,000 CIT combinations in the clinic (Tang et al., 2018), there is a critical need to be able to assess these combinations as early as possible. Given the multiple steps involved in anti-cancer immunity, the potential to enhance CIT via rational combinations by modulating different biological steps in immunity simultaneously or in rapid sequence is quite broad (Chen and Mellman, 2013; Kim and Chen, 2016). However, the daunting biological complexity and combinatorial possibilities are at odds with the need to deliver the most effective immunotherapy to patients as quickly as possible. Clinically active CIT regimens that are not as effective as other immunotherapy approaches should be deprioritized early to enable more potent approaches to be pursued. Improved integration of preclinical and clinical studies—particularly with improved preclinical models and/or a better understanding of where models translate well (e.g., a particular model translates to a human patient with strong Treg infiltration rather than broadly to human cancer), would greatly assist in matching combination approaches to patient populations to be tested. This also relies on a clear understanding and clinical demonstration of the combining partner's mechanism of action. Immune-based therapies may often exhibit both direct and indirect effects on different biologic steps. Despite the intensive study of PD-L1/PD-1 inhibitors, we still do not clearly understand the differential effects of such agents on lifting inhibition of exhausted effector T cells in the TME versus removing restraints from cancer-specific T cell proliferation in draining lymph nodes versus effects on T cell priming and activation. Attention to these different effects of CIT will be required and likely will tie into any comprehensive biomarker strategy that is to be incorporated into such trials, including both pharmacodynamic and predictive biomarkers. Utilization of biomarkers that are only detectable or changed when the combination is acting synergistically (or additively) is desperately needed.

In early phase studies, monotherapy response rate remains the most useful endpoint for the initial assessment of CIT efficacy. The vast majority of approved therapeutic anti-cancer agents demonstrate such single agent activity. However, combinations, particularly those that include at least one agent that has single agent activity, are notoriously complicated to assess in Phase I combination studies. This is due to patient heterogeneity, potential patient selection bias, and lack of randomization. Further incorporation of randomization into Phase I oncology studies may be necessary, as has been utilized in non-oncology Phase I studies (Lee et al., 2019). Additionally, specific imaging approaches that can provide insights into immune biology of different compartments, microenvironments, and organs may represent useful opportunities available to accelerate particular CIT combinations (Chau et al., 2018; Mellman et al., 2016; Shields et al., 2018).

To date, validated effective combination immunotherapy regimens have incorporated combination agents that can independently induce cancer cell shrinkage and response (Antonia et al., 2017; Gandhi et al., 2018; Hodi et al., 2018b; Jotte et al., 2018; Motzer et al., 2018b; Paz-Ares et al., 2018; Rini et al., 2017). Immunologically, such combinations should not require individual response activity from each component, as some combination components should be capable of only sensitizing cancer to a second agent, such as in the case of a vaccine combined with a CPI. However, it is possible that the most potent combinations will rely on agents that are individually active, and such combinations would benefit from prioritization during regimen development.

Challenge: Full Characterization of the Impact of Steroids and Immune Suppression on Cancer Immunotherapy and Autoimmune Toxicities

Corticosteroids have a direct effect on human immunity, affecting T cells more than B cells. The effects on T cells are pleomorphic and vary by subset, with the net result being a reduction in the total number of T cells in circulation (Olnes et al., 2016). As such, corticosteroids are routinely used in the treatment of autoimmune disease. They are also used to treat graft-versus-host disease (Martin et al., 2012) and to reverse autoimmune adverse events triggered by checkpoint inhibition (Sosa et al., 2018; Wang et al., 2018) or cytokine release syndrome (CRS) in synthetic immune approaches. Autoimmunity following PD-L1/PD-1 inhibition appears to be particularly sensitive to systemic corticosteroids, which can often rapidly reverse it. Given the overlapping mechanisms between anti-cancer immunity and autoimmunity resulting from checkpoint inhibition, it seems likely that corticosteroids would negate the very T-cell-based mechanisms that also lead to efficacy with CIT. However, clinical studies have suggested that patients experiencing autoimmune toxicities following CIT who are treated with corticosteroids can still experience durable responses and/or survival (Freeman-Keller et al., 2016; von Pawel et al., 2017; Weber et al., 2017). Such evidence supporting the benefit of immunotherapy despite systemic corticosteroid treatment is weak at best. In such cases, it is not known how much of the durable response or survival observed was driven by the anti-cancer immune response before or after steroid administration. Additionally, patients who experience these autoimmune adverse events may have more responsive immune systems and an immune set point that tends toward an inherently stronger immune response (Chen and Mellman, 2017), making these patients a biased population on which to conduct such non-controlled retrospective analyses. Further, anti-cancer immune responses may be more refractory to steroid-related immune suppression after a response has been initiated, as is observed in early versus later autoimmune disease. Hence, assessment of the impact of steroids on anti-cancer immunity likely needs to incorporate timing of steroid administration (e.g., at or before initiation of CIT versus many months or even years after initiation of immunotherapy). Some Phase I studies have been conducted on patients receiving platinum-based doublet chemotherapy, including either paclitaxel (which requires pre-treatment steroids, generally dexamethasone 20 mg every 3 weeks prior to paclitaxel administration) or *nab*-paclitaxel (which does not

require pre-treatment steroids with or without PD-L1/PD-1 inhibitors (Liu et al., 2018). However, while randomized studies of CIT with and without steroids would best define the impact of steroids, such studies would also likely be unethical. The closest such studies have come to examining this question is in studies of carboplatin + paclitaxel or nab-paclitaxel. For example, in the Phase III KEYNOTE-407 study examining patients with metastatic squamous NSCLC, patients receiving carboplatin + paclitaxel + pembrolizumab achieved a similar OS benefit from the addition of pembrolizumab as did patients receiving carboplatin + *nab*-paclitaxel + pembrolizumab versus those receiving carboplatin + paclitaxel or *nab*-paclitaxel (Paz-Ares et al., 2018). Based on the totality of clinical data, the best current assessment for the impact of steroids on cancer immunity is that systemic steroids can weaken the anti-cancer immune response that we are trying to achieve with CIT. However, that impact often does not completely abolish the anti-cancer immune response. How much the steroids actually weaken the anti-cancer immune response is most likely driven by the timing of administration (early versus late), dose, schedule, and specific steroid administered (high- versus low-dose steroids, continuous versus intermittent versus single-episode steroid dosing, and potency of steroid administered), and strength of the anti-cancer immune response at the time of administration. Further study on other forms of immune suppression are even more limited.

Synthetic immune approaches such as CAR T and T cell engaging antibodies can also lead to highly acute toxicity most commonly manifesting as CRS. This is likely due to overstimulation of the engaged T cell or CAR T due to high density T cell receptor engagement and signaling. Given the much higher expression of targeted cancer antigens (e.g., CD19 or CD20, CEA, etc.) on the surface of cancer cells versus the expression level of specific endogenous antigens expressed in accordance to MHC I (e.g., a specific neoantigen peptide fragment bound MHC I) (Ebrahimi-Nik et al., 2019), engaged T cells are exposed to much denser clustering of TCR and also secrete much higher levels of cytokines than observed in the vast majority of endogenous immune responses. This appears to be true even when the efficiency of T cell mediated target cell elimination is similar. The early recognition of CRS and the aggressive administration of steroids is important in preventing grade 5 events. Steroid use to treat CRS doesn't appear to overtly impact the ability to generate responses to synthetic immune therapy. However, the relationship between steroid administration and long-term outcomes such as durable survival over years is not yet clear. While understanding the mechanisms of immune toxicities and optimizing approaches to managing these toxicities is an important part of cancer immune therapeutics, an even more important challenge relates to developing more effective and specific CITs that are able to eliminate cancer cells without generating autoimmunity or immune-mediated toxicity.

Challenge: Maximizing Personalized Approaches Through Composite Biomarkers

There are over 3,000 ongoing clinical trials of immunotherapy agents either alone or in combination with standard of care or other targeted agents (Tang et al., 2018). This may result in the standard of care rapidly evolving over the coming years. The question for patients and their physicians then becomes, "What is the right therapeutic approach for their specific disease?" Today, diagnostic testing for cancer patients is not fully embedded into clinical practice; testing rates range from 7% to 50% depending on the type of cancer (Chawla et al., 2018). While some of this variability is due to lack of education and access to diagnostic testing platforms, much of it has to do with the lack of clinical evidence to support informing a physician's decision. There are challenges associated with biomarker development in CIT. Unlike targeted therapies where the diagnostic is usually a genetic aberration in the target itself (e.g., Her2 amplification, EGFR mutations, ALK translocations) which is often defined as a binary (yes or no) assay, biomarkers in CIT are commonly faced with the issue of a gradation of association and a continuous variable as is the case with PD-L1 expression, TMB, and tumor-derived IFNγ gene signatures, each of which can be studied at multiple different biomarker cutoffs in different indications. This often results in challenges in *a priori* identification of a cutoff to define biomarker high versus low populations for the design of clinical trials. Statistical methodologies such as receiver operating characteristic (ROC) curves, which plot sensitivity versus 1-specifity, can be helpful in such situations, but are most commonly employed early in clinical testing where the endpoints being measured may or may not be tightly associated with long term outcomes. In addition, given that the biomarkers in some instances are expressed in the TME, tissue sampling and time of sampling (archival tissue versus fresh pre-treatment tissue) may have an impact on defining biomarker positivity. These issues have resulted in the design of large clinical trials to define the cutoff for the biomarker in Phase III trials and may contribute to patients with diagnostic negative tumors responding or benefiting from therapy (Herbst et al., 2016; Rittmeyer et al., 2017; Spigel et al., 2018). This could be due to sampling bias during study conduct (small biopsies unable to capture the TME adequately), important unmeasured immunologic variables (Chen and Mellman, 2017), or the dynamic nature of immunity.

Conduct of clinical trials with statistical rigor applied to the biomarker hypothesis, even if conducted retrospectively, may allow for the development of treatment algorithms. As seen with PD-L1 IHC (Hirsch et al., 2017; Tsao et al., 2018), the challenge here again will be in ensuring consistent definitions of biomarkers and assays to enable equivalent interpretation of data across drug developers. Regulators are increasingly embracing the concept of retrospective analyses of trials for the interrogation of biomarkers (Lemery et al., 2017), and diagnostic manufacturers are developing tools that allow for the rapid turnaround of molecular and/or cellular data, all of which will make actionable information affordable, feasible, and, importantly, useful for patients. Epidemiological evidence on the impact of diagnostic-based treatment on healthcare cost-effectiveness and patient quality of life increasingly favors diagnostic-based treatment decisions (e.g., oncotype diagnostic references in breast cancer). There are some hurdles, however, particularly related to the reimbursement of diagnostic tests, that urgently need to be addressed. The utility of algorithms for defining patient benefit and enabling patients and treating physicians to make evidence-based decisions may become the norm as more and more trials use comprehensive platforms with pre-specified analysis plans to inform diagnostic strategies with the potential to register trials

based on these endpoints. These approaches may be particularly applicable to CIT, given the complexity of the interactions required for effective treatment. Ongoing accumulation of factors that impact an individual's cancer-immune set point (Chen and Mellman, 2017) and the approaches that may encompass a personalized CIT paradigm (Kim and Chen, 2016) will likely represent only the first step necessary in enabling such approaches. These complex biomarker approaches are unlikely to be straightforward. However, these efforts will be important in optimizing CIT benefit.

Challenge: Developing Improved Regulatory Endpoints for Cancer Immunotherapy

Traditional statistical and approvable regulatory endpoints for PD-L1/PD-1 inhibitors have been serviceable, enabling rapid and broad assessment and approval for many of these agents (e.g., pembrolizumab, nivolumab, atezolizumab, durvalumab, and avelumab) (Tang et al., 2018). However, such endpoints, including ORR, PFS, and OS, have not been designed or well-matched for the assessment of CITs. The goal for most patients with metastatic cancer and their treating physicians has been to achieve durable response and survival with CIT. This is perhaps best measured by the "tail of the curve" when examining Kaplan-Meier survival curves (Chen, 2013). However, current clinical trial methodologies for measuring this benefit are lacking, with the analyses being underpowered and requiring prolonged follow-up often lasting multiple years (Gauci et al., 2019). Furthermore, ORR assessment does not take into account the potential for successful CIT to lead to either a delayed response (e.g., continued tumor growth followed by tumor shrinkage) or "pseudoprogression" (e.g., apparent tumor growth driven by tumor inflammation followed by tumor shrinkage) (Hodi et al., 2016; Wolchok et al., 2009). PFS assessment is similarly affected by the above. As such, collaborative attempts to define and validate better CIT endpoints have been undertaken, including the development of immune-related response criteria (Wolchok et al., 2009), immune response evaluation criteria in solid tumors (RECIST) (Seymour et al., 2017), immune-modified RECIST, and immune-modified PFS (Hodi et al., 2018a). Unfortunately, validation requires large datasets and iterative modifications and ultimately may be optimized only for the CIT on which it was validated. OS assessment is well suited to the assessment of benefits from CIT, although it can take a protracted amount of time to complete, requires patients treated on the control arm to die from their cancer to enable the assessment, and is impacted by control arm crossover to immunotherapy and differential time to benefit in a given patient population (e.g., crossing OS Kaplan-Meier curves). Additional possibilities for endpoints that may address some of these issues include the use of model-based estimates of on-treatment growth rate constants to predict OS benefit (Claret et al., 2018), landmark OS, weighted log-rank OS, or OS derived using a Cox-model-based time-varying treatment effect estimate (Hodi et al., 2018a; Lin and León, 2017). Continued collaboration and optimization of CIT endpoints is warranted.

Challenge: Optimizing Long-Term Survival With Multi-agent Cancer Immunotherapy Combination Regimens

Developing the optimal CIT regimen may require multiple therapeutics to modulate each step required to generate an anti-cancer immune response (Chen, 2017; Chen, 2017, conference). Using a multipronged approach, a complete curative regimen might contain agents that have the ability to kill cancer cells, drive log-kill, reduce tumor burden, sensitize the cancer to immunotherapy, activate an endogenous anti-cancer immune response, drive immune cell infiltration, and provide maintenance to drive long-term memory response, thereby prolonging the tail of the survival curve. Such regimens could include anti–PD-L1/PD-1 agents along with other agents that can remove negative regulators; activate, prime, and/or generate new immunity in patients without an existing strong immune response; overcome immune exclusion; increase class I MHC molecules; increase T cell survival; and/or drive T cell memory. Additionally, cytotoxic agents such as chemotherapy and radiation therapy and synthetic immunity agents, which are some of the most powerful anti-cancer agents, can be incorporated. These types of regimens will require the optimization of drug dosing, timing due to the potential for transient effects, and/or sequencing to achieve maximal benefit. Some drugs might only need to be given a few times over the course of a regimen or infrequently during the regimen. The overall goal is to avoid overlapping toxicities, maximize any synergistic effects, and minimize the possibility for overlapping resistance. While such regimens are complicated and pose challenges to regimen development, most therapeutic regimens with curative potential are made up of multiple agents. Cyclophosphamide, doxorubicin, vincristine, and prednisone in non-Hodgkin lymphoma; bleomycin, etoposide, and cisplatin for testicular cancer; and pediatric acute lymphoblastic leukemia chemotherapy regimens are such examples in oncology (Cary et al., 2018; Czuczman et al., 2004; Pui et al., 2015). Beyond oncology, highly active antiviral regimens also incorporate multiple anti-viral treatments that, while not curative for HIV infection, are very effective in preventing mortality (May et al., 2014).

To rapidly prioritize and accelerate the development of such regimens, novel clinical trial approaches are required (Nass et al., 2018). These trial "platforms" include umbrella trials, which focus on tumor histology but with treatment determined based on prespecified biomarkers. Platform trials are similar to traditional combination trials but incorporate multiple biomarkers and drugs (Simon, 2017). Additionally, there are basket trials, which organize a clinical trial around a genomic alteration or other intrinsic tumor characteristics, including those of the immune system (Tao et al., 2018). These trial designs are adaptable, allowing for new combinations, dosing, and/or schedules to be quickly added to the trial, as well as providing the flexibility for patients to switch combinations. Overall, these clinical trial designs can promote faster and more confident decision making and also enable successive addition of therapeutics to a regimen or, conversely, successively deconstruct the components of a complex regimen.

An early example of adaptive clinical trials is the BATTLE platform, which explores targeted therapies in NSCLC (Zhou et al., 2008). Another early adaptive trial is I-SPY, which examines neoadjuvant treatments for breast cancer (Barker et al., 2009). The neoadjuvant setting represents another opportunity to explore immunotherapy combinations, and the combination of durvalumab with olaparib is currently part of I-SPY (NCT01042379). More focused on anti-cancer combinations are the MORPHEUS and FRACTION studies (Chau et al., 2018; Simonsen et al.,

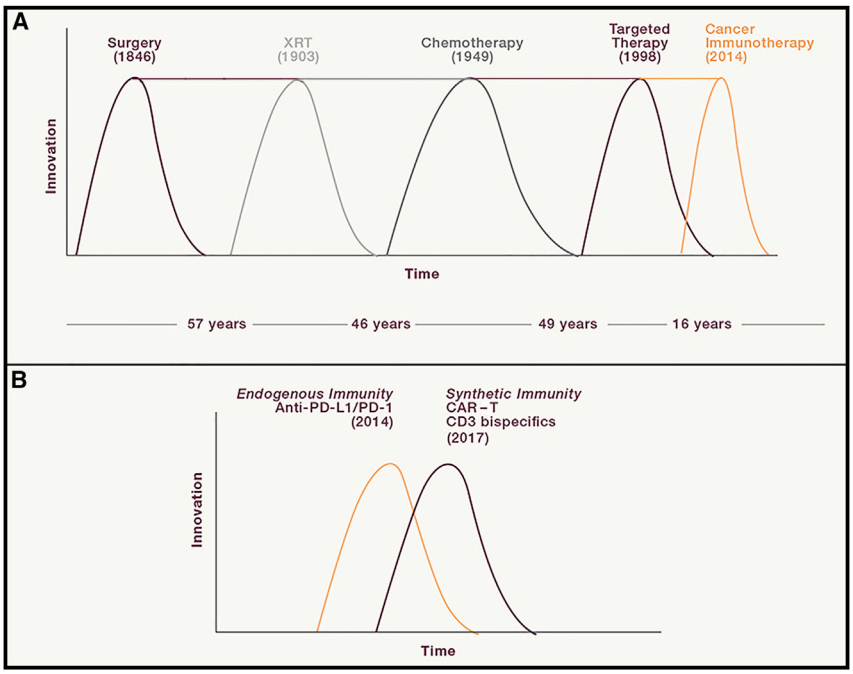

Figure 4. The Intervals Between Disruptive Therapies in Oncology

(A) Relative timings of innovation and impact in the treatment of cancer.

(B) The development of immune checkpoint inhibitors and synthetic immunity for the treatment of cancer represents two overlapping and potentially disruptive treatment paradigm shifts.

CAR, chimeric antigen receptor; CIT, cancer immunotherapy; PD-1, programmed death-1; PD-L1, programmed death-ligand 1; XRT, external radiation.

2018). Both of these adaptive platforms explore novel therapeutic combinations across multiple types of cancers. Another example is the adaptive efficacy-seeking QuEST1 trial in patients with metastatic castration-resistant prostate cancer. In this trial, sequential study arms each add an additional immunotherapy agent with the goal of determining whether a 2-, 3- or 4-drug regimen is required to stimulate anti-tumor immune responses (Redman et al., 2018).

There is also the possibility to develop appropriate "non-study" control arms for clinical trials, based on real-world data. These control arms could be developed using the same inclusion and exclusion criteria as the randomized controlled trial, incorporating patients treated by the standard of care. These types of control arms could help to accelerate the understanding of the bar that a new therapeutic drug or regimen needs to achieve to become a new standard of care (Chau et al., 2018).

Concluding Remarks

These top ten challenges will need to be overcome to move the field of CIT forward. Further technology development will also play a key role (Riley et al., 2019). Within this list of challenges, additional prioritization may still be required. Recognizing that such prioritization is subjective, one perspective could focus on the importance of better evaluation of CIT combinations in Phase I clinical studies. With well over 1,000 combination studies underway, and an order of magnitude more patients enrolled on such studies, clear conclusions are likely to be difficult. Additionally, roughly 100 of these combination studies would be expected to demonstrate a 40% response rate by chance alone, due to inclusion of a highly effective therapy in PD-L1/PD-1 pathway inhibitors in the majority of these studies. From false positive results that lead to large negative confirmatory studies to the inability to distinguish differentiated combination approaches due to underpowered statistics, patient immune set point heterogeneity, and efforts that are not deprioritized due to personal stakes involved, this challenge is likely to impact the ability to effectively and efficiently advance the CIT combinations that significantly benefit survival. Improvements in addressing this challenge would likely require advances in other challenges, from improved pre-clinical models to clinical biomarkers and organ-specific immunity. Beyond this, prioritizing the relationship between synthetic and endogenous immunity should be critically important. Both of these approaches have separately resulted in some patients that no longer die of their disease. Biologically, both approaches should be enhanced or restrained by similar mechanisms. And both approaches have resulted in rapid regulatory approvals. Yet little is understood to date regarding how these approaches might be optimally combined. Further, leading scientists and investigators working in these areas tend to specialize in one approach over the other—with limited overlap and dialog. Bridging these two areas of CIT will likely lead to major advancement in the field.

Historically, there have been extended periods of time between shifts in treatment paradigms; however, these intervals have been shrinking (Figure 4A). Today, there is tremendous growth within the field of CIT (Tang et al., 2018) consisting of largely independent efforts utilizing either endogenous immunity or synthetic immunity approaches (Figure 4B). As such, collaborative approaches are required and currently include partnerships across academic institutions, government agencies, and industry partners. These partnerships within the CIT community are notable and include many separate organizations that intentionally bridge across the above groups, including the Society for Immunotherapy of Cancer (SITC), the Cancer Research Institute (and specifically the Cancer Immunotherapy Consortium), the Parker Institute for Cancer Immunotherapy, and the National Cancer Institute's Cancer Moonshot. Additional efforts through Stand Up to Cancer (SU2C), Friends of Cancer Research (FoCR), American Society of Clinical Oncology (ASCO), American Association for Cancer Research (AACR), European Organization for Research and Treatment of Cancer (EORTC), the National Cancer Institute (NCI), individual cooperative groups, and health authorities including the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA), add additional connections for collaboration. Efforts to bring together

the CIT community could immediately and directly address open challenges, such as the need to better understand secondary immune escape—a specific area where the collection and analysis of late at-time-of-cancer-progression samples is necessary. However, individual investigators and institutions have been limited in their ability to collect enough specimens to reliably establish and validate conclusions.

These collaborative efforts have already successfully led to CIT-specific assessment of response, publication guidelines, adverse event management and diagnostic approaches (Haanen et al., 2017; Hodi et al., 2018a; Tsimberidou et al., 2018). However, as the field has grown over the past several years, so has the number of different efforts that seek to bring different components of cancer research together. These entities help generate different ideas and perspectives for the field, both of which are much needed. The concept of consilience, a unity of knowledge—but not necessarily thinking—is a particularly apt description of what is now necessary: bridging scientists, collaborative groups, and ideas (Graeber, 2019). Much as the "moonshot" originally galvanized research efforts on reaching the moon and more recently has been used as an effort to cure cancer, largely through CIT, our ability to galvanize the global CIT community not around specific solutions but around broad challenges that we need to face, focus on, and solve for, will likely go a long way toward successfully developing what amounts to "the cure."

ACKNOWLEDGMENTS

We recognize Jean-Charles Soria for the original concept of a top-ten manuscript. We thank Fabiola Rivas and members of the imCORE network for providing additional thoughts and input. We thank the cancer immunotherapy committee and cancer immunotherapy biomarker group at Genentech/Roche for ideas, discussion, and ongoing work on the challenges, and the larger CIT community as represented by many of the groups listed in this manuscript for the degree of collaboration and scientific discourse over the past several years. Histologic images and staining for CD8 T cells representing cancer immune phenotypes are provided by H. Koeppen MD PhD (personal communication). Medical writing assistance for this manuscript was provided by Jessica B Bessler, PhD, of Health Interactions and funded by F. Hoffmann-La Roche Ltd.

DECLARATION OF INTERESTS

P.S.H. is an employee and stockholder at Genentech/Roche, and D.S.C. is an employee and stockholder at IGM Biosciences.

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