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Immunity

Review

The cancer-immunity cycle: Indication, genotype, and immunotype

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SUMMARY

The cancer-immunity cycle provides a framework to understand the series of events that generate anti-cancer immune responses. It emphasizes the iterative nature of the response where the killing of tumor cells by T cells initiates subsequent rounds of antigen presentation and T cell stimulation, maintaining active immunity and adapting it to tumor evolution. Any step of the cycle can become rate-limiting, rendering the immune system unable to control tumor growth. Here, we update the cancer-immunity cycle based on the remarkable progress of the past decade. Understanding the mechanism of checkpoint inhibition has evolved, as has our view of dendritic cells in sustaining anti-tumor immunity. We additionally account for the role of the tumor microenvironment in facilitating, not just suppressing, the anti-cancer response, and discuss the importance of considering a tumor's immunological phenotype, the "immunotype". While these new insights add some complexity to the cycle, they also provide new targets for research and therapeutic intervention.

INTRODUCTION

In only 15 years, the advent of cancer immunotherapy has revolutionized both the clinical practice of oncology and our understanding of cancer biology. An increasing proportion of cancer patients now receive immunotherapeutic agents as standard-of-care in early and late disease. These patients represent an increasingly broad range of cancer indications and genotypes, attesting to the likelihood that the immune system plays a fundamental role in virtually all types and stages of cancer. This generality, combined with the potential for long-term benefit and safety, has driven the field's remarkable growth, and promises to do so for years to come. It also distinguishes cancer immunotherapy from almost all other therapeutic strategies, which usually rely on targeting tumor cells directly. Direct targeting creates selection pressures that typically drive rapid resistance and tumor progression. Although patients can and do become resistant to immunotherapies, by treating the immune system, the selection pressure on the tumor is indirect. Moreover, the anti-cancer immune response is inherently adaptive, presenting a greater challenge to the tumor and likely accounting for the extended overall survival benefit observed when immunotherapy is successful. Nevertheless, it is still the case that a majority of patients fail to achieve durable responses, a limitation that represents our greatest continuing therapeutic challenge.

Although the role of the immune system in cancer has been studied for decades, the current surge in interest was driven by results observed in the clinic, initiated from patients treated with antibodies to the immune "checkpoints" CTLA4 and PD-L1-PD-1.[1] Results from trials with these agents focused attention on the key role of T cells in anti-cancer immunity, and in the case of the PD-L1-PD-1 axis, the phenomenon of T cell exhaustion.[2–4] The introduction of the cancer-immunity cycle (CI cycle) in 2013 illustrated that T cells neither respond nor work on their own, but exist in the context of a series of steps, some of which are even extrinsic to the immune system and the cancer (Figure 1).[5] These steps are linked in a cycle, implying that (1) any individual step has the potential to be rate limiting for generating optimal immunity and (2) successful anti-cancer immunity has the potential to be self-reinforcing during the course of response. Even therapeutic strategies that create "synthetic immunity", such as adoptive cell therapy, the use of immune cell engaging antibodies, or CAR-T cell therapy, must work within the context of the CI cycle.

Over the past 10 years, greater attention has been paid to the mechanisms underlying each of the CI cycle's steps, with work in some cases altering some long-held assumptions (e.g., the significance of T cell exhaustion). Yet, basic understanding of these steps is only now beginning to catch up with the clinical data that both invigorated the field and provided significant mechanistic insights. The gap is also closing because the rate of progress in identifying effective therapeutic agents beyond the PD-1 axis has slowed. There is nevertheless exceptional potential for the discovery of new therapies, but the rate of discovery will be enhanced as we learn more about each of the cycle's steps and how they fit together. This review aims to summarize our progress in understanding each step and to identify key unknowns, challenges, and opportunities for the next decade.

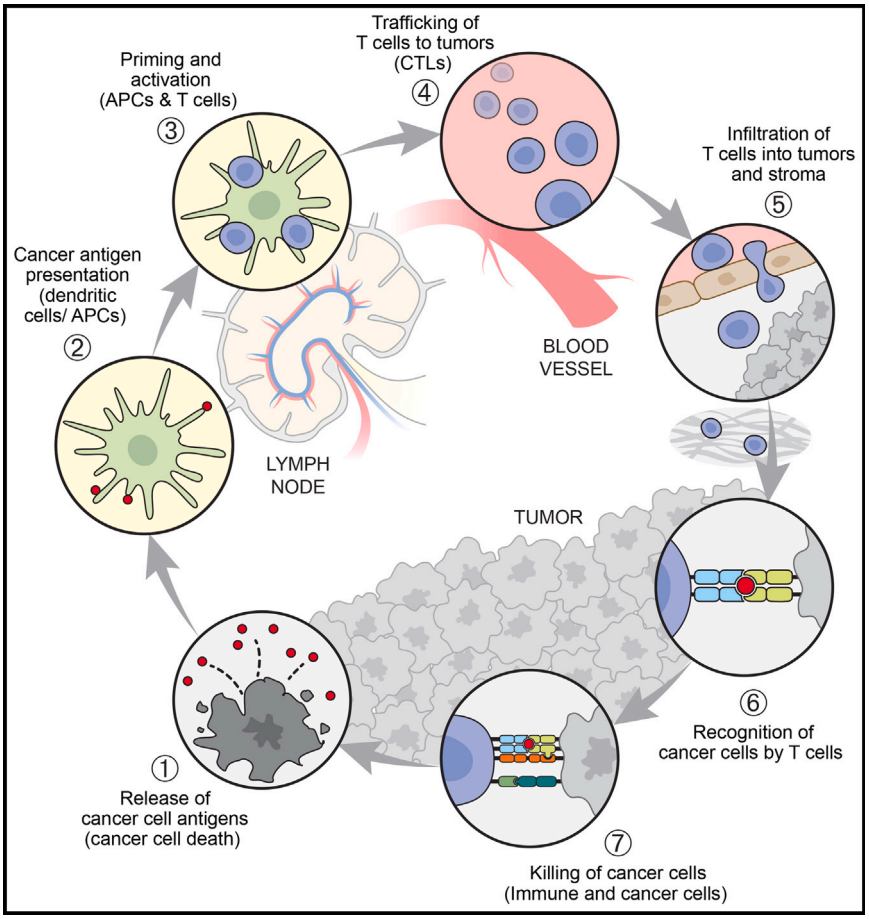

Figure 1. The cancer-immunity cycle
The seven steps of the cancer-immunity cycle as it was originally conceived and published in 2013.[5] These fundamental steps continue to serve as critical biologic steps in cancer immunity.

THE CI CYCLE FRAMEWORK AND THE TUMOR ENVIRONMENT

The basic framework of the CI cycle remains unchanged since its introduction, including a subsequent modification to emphasize that blood-derived T cells must often traverse a stromal barrier before reaching the tumor itself.[5] Based on recent work, however, a number of important new concepts require highlighting.

Even within individual cancer indications, tumors can still be viewed as assuming different immunological phenotypes, or "immunotypes". The three classical immunotypes, immune inflamed, immune excluded, and immune desert, are defined, respectively, as tumors containing abundant immune infiltrate, tumors where T cell infiltrate is limited to tumor stroma as opposed to the tumor parenchyma, and tumors that do not exhibit immune infiltrate (Figure 2).[6–8] Although the immunotypes are likely an oversimplification of what may to be a dynamic feature of tumors, which may also be altered during tumor evolution or by therapeutic intervention,[9] they do represent a useful, mechanism-based classification system. Immunotypes occur at different frequencies in different indications. For example, untreated prostate cancer, colon cancer, and melanoma most often exhibit desert, excluded, and inflamed phenotypes, respectively.[7] Nevertheless, it is critical to recognize that all three immuntypes also occur in different patients in any of these indications; this is true for all types of solid tumors, regardless of origin. As a result, immunotypes continue to represent a useful framework to understand the mechanistic basis of response and lack of response and to direct future investigation. Because most patient responses occur when a tumor exhibits the inflamed immunotype, uncovering the factors that contribute to the formation of the excluded or desert immunotypes will facilitate targeted discovery efforts and hopefully greatly expand the percentage of responsive patients. As the mechanisms responsible for these immunotypes are critical to developing better immunotherapies, it is an oversimplification and indeed misleading to refer to tumors as simply being hot (presence of T cells) or cold (absence of T cells). For example, immune excluded tumors have T cells, but the T cells are spatially restricted from the tumor cells and are therefore generally resistant to checkpoint blockade.

It seems likely that the tumor stroma, or more broadly the tumor microenvironment (TME), plays a key role in determining immunotype and the immune trajectory and fate of tumors. Not just T cells, but cells of the innate immune system (e.g., monocytes, granulocytes, natural killer [NK] cells) and non-immune cells (e.g., cancer associated fibroblasts or CAFs) are of exceptional importance.[10–13] These cell types collaborate to form collagen-rich fibrotic stroma that restricts T cell immunity by suppressing T cell function and physically restraining their migration into tumor nests.[14]

Somewhat paradoxically, the TME can also *promote* anticancer immunity, in part by generating peri-tumoral lymphoid aggregates or tertiary lymphoid structures (TLSs), which are associated with better T cell responses and clinical outcomes.[15] The composition and frequency of TLSs are emerging as key features that associate with response to immunotherapy, perhaps reflecting their role in amplifying the anti-tumor T cell response in the TME. These points will be considered further below.

Cancer and germline genetics are also important determinants of immunotherapy outcome and adverse events; they also represent potential drivers of immunotype. Tumors generate genetic diversity that relates to cell type of origin and tumor evolution. It is now appreciated, especially from human data, that this characteristic is intimately connected to the function of the CI cycle and must be considered as a determining factor.[16]

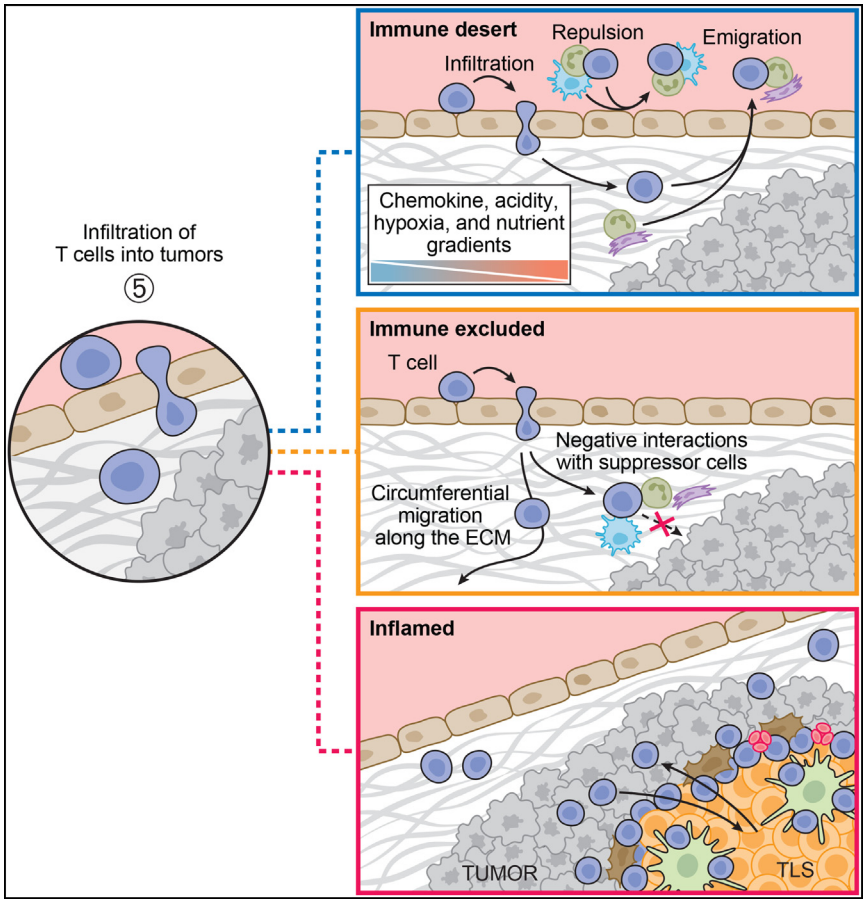

Figure 2. Immunotypes

Three primary immunotypes—immune desert (blue), immune excluded (yellow), and inflamed (red)—are described. In an immune desert, there is a clear paucity of any immune cells within the TME. This may relate to a repulsion or emigration of immune cells (or more passively, through a lack of attractive chemokines). In immune-excluded tumors, the presence of inhibitory stroma and ECM may prevent effective migration of T cells into direct contact with cancer cells, leaving them excluded from the actual cancer cell nests. In inflamed tumors, the presence of stimulatory immune cells, including peritumoral or intratumoral TLS, may provide additional stimulation to tumor infiltrating lymphocytes, increasing their functional capacity, survival and proliferation.

ECM, extracellular matrix; TLS, tertiary lymph node structure.

Perhaps the most dramatic conceptual alteration in our understanding of the cycle pertains to one of its most elemental features: the function of the T cell compartment and its regulation by dendritic cells (DCs). T cell dysfunction in tumors is often associated with the accumulation of exhausted T cells (Tex cells), cells that are alive but exhibit reduced effector activity.[2] First defined for T cells in chronic virus infection and later extended to tumors, Tex cells are thought to accumulate when the amount of antigen exceeds the ability for it to be cleared by antigen-specific T cells. Tex cells are characterized by the increased expression of various coinhibitory receptors, the most important of which is PD-1 but also includes LAG3, TIM3, and TIGIT; these receptors are also markers of T cell activation. Given their increased expression in the exhausted state, however, it was widely presumed that blocking the ability of coinhibitory receptors to bind their respective receptors would reverse exhaustion, reinvigorating anti-tumor activity.[17] This was especially true for PD-1 and TIGIT, whose ligands are often increased on tumor cells. Without this reversal, it was assumed that Tex cells would remain suboptimally active as effectors due to their low content of cytolytic factors (e.g., granzymes) and cytokines.

Over the past few years, however, views regarding the role of checkpoint blockade have evolved considerably. Tex cells acquire a heavily altered epigenetic state that cannot be easily reversed.[18–21] Therefore, they are likely to reflect a terminal differentiation path that is unlikely to be the only or most relevant target of checkpoint blockade. In addition, in the case of PD-1, perhaps the most important source of PD-L1 may not be the tumor cell, but rather the antigen-presenting DCs that stimulate tumor-specific T cells in the first place.[22] Thus, rather than acting to reverse exhaustion, checkpoint blockade may act to prevent the development of the exhausted phenotype and do so at a time earlier in the T cell terminal differentiation pathway. In addition, these findings emphasize that DCs may play critical roles not only in priming or activating T cell responses in draining lymph nodes (dLNs) but also in support of T cell responses after arrival in the tumor.[1]

Apart from checkpoint inhibitors, of which three biologic targets have been approved for clinical use (targeting CTLA4, PD-L1/PD-1, Lag-3) or are in late-stage clinical trials (targeting TIGIT), there have not been any true therapeutic breakthroughs in the past decade that act by modifying endogenous cancer immunity. Recent progress in cancer vaccines in the pre-metastatic setting may portend the next significant advance.[23,24]

Given that DCs are now seen as being key not only for initiating T cell responses early in the CI cycle (both endogenous and following vaccination) but also for maintaining them, the regulation of DC activation or "maturation" is re-emerging as a key element in driving the CI Cycle. To this point, type I interferons (IFNs) are probably the most important components, as are the various agents that induce the IFN response (e.g., STING, immunogenic lipids, certain TLR ligands, cytosolic sensors such as MDA5 and RIG-I, DNA damage response elements).[25–27]

In the area of synthetic immunity, CART cells as well as CD3-directed T cell engagers[28] have emerged as effective and widely approved approaches to modify the CI cycle in hematologic malignancies by bypassing the need to produce endogenous T cells responses.[29] These approaches must still negotiate the effector side of the CI cycle, being subject to mechanisms of immunosuppression and an apparent requirement, at least in the case of adoptive cell therapy, for DCs to optimize activity.[30] The

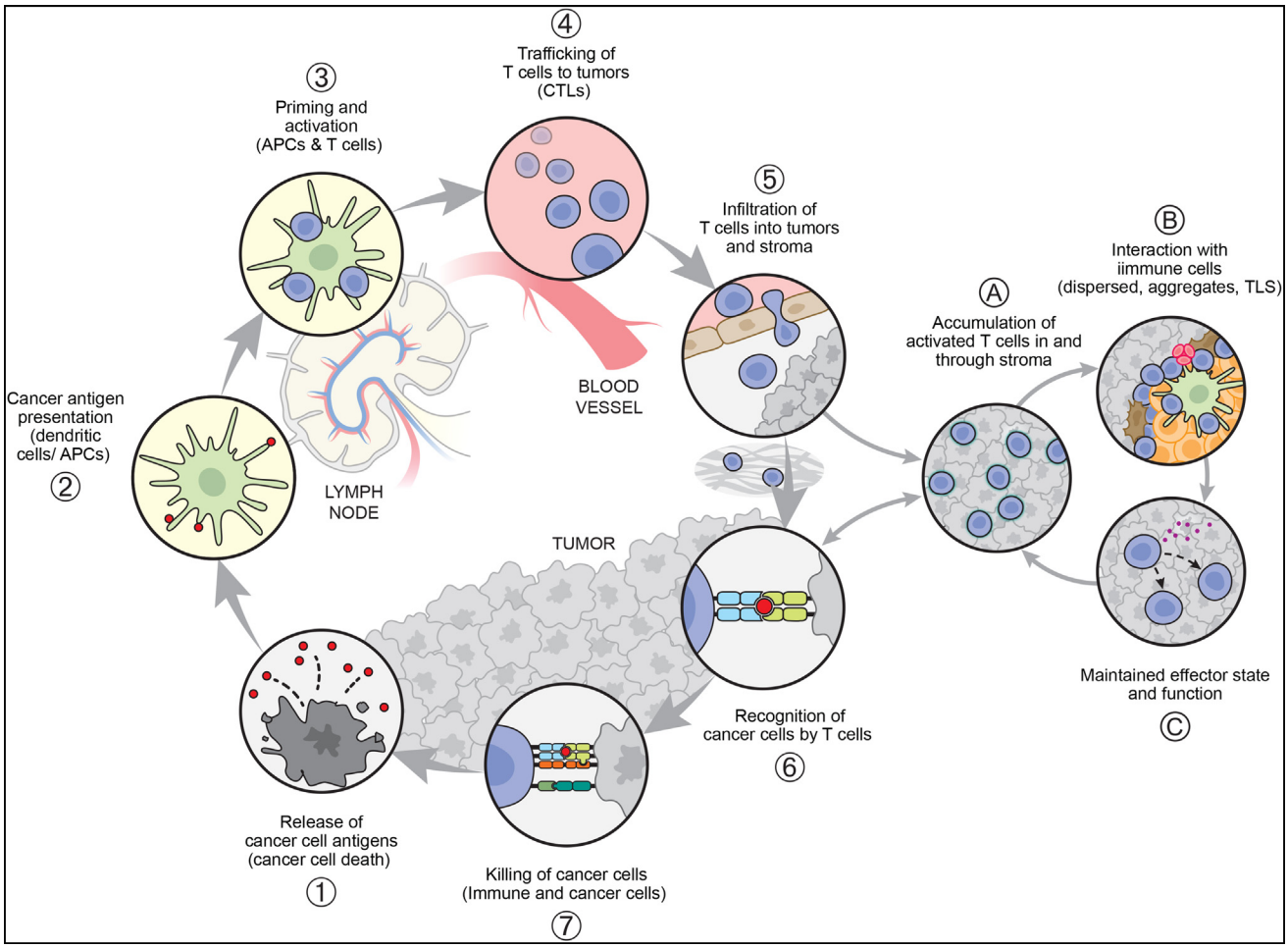

Figure 3. The cancer-immunity cycle and the tumor microenvironment cancer-immunity subcycle

Progress in the field of cancer immunity since 2013 has highlighted the importance T cell migration through tumor stroma, interaction with intratumoral immune cells, persistence, and function within the tumor microenvironment. T cells within the TME can respond in a series of steps that are a microcosm of what occurs systemically beyond the tumor. These subcycle steps represent an immunologic eddy in the TME, which we refer to as the cancer-immunity subcycle. When cancer immunity is active, stimulation, proliferation, and functional killing of cancer cells is possible. However, inhibitory immune cells and stroma, metabolic derangements, and loss of T cell function can occur within the TME, halting the cancer-immunity cycle.

APCs, antigen-presenting cells; CTLs, cytotoxic T lymphocytes; TLS, tertiary lymph node structure.

most impressive activity is currently limited to certain lymphomas, leukemias and myeloma although there are hints that solid tumors may also eventually yield to cell or engager therapies, particularly when targeted to cancer cells via tumor-specific T cell receptors.[31–33] Indeed, adoptively transferred T cells (in mouse models) can lead to the generation of endogenous T cell responses to antigens not specific to the injected cells ("antigen spreading").[34,35] This observation is consistent with a core prediction of the CI cycle: T cell killing leading to the persistence and priming of new or existing T cell responses.

In view of these major advances, we believe it is necessary to modify the initial view of the CI cycle to include a key role for the TME, particularly DCs, in regulating and sustaining the anti-tumor T cell response. As depicted here (Figure 3), this is best illustrated by a "subcycle" that occurs at the tumor site upon entry of dLN-derived T cells into the tumor (at step 5 of the CI cycle). We propose that these T cells encounter antigen-presenting cells (in particular DCs) interspersed within the tumor parenchyma, in tumor-associated lymphoid aggregates, or morphologically identifiable TLSs. The T cells may then expand and differentiate (e.g., effector, memory, or exhaustion) leading to direct tumor cell killing and perhaps initiating a local TME "eddy" of the CI cycle. This view emphasizes a far more important and complex role for the TME in both supporting and suppressing cancer immunity (CI cycle steps 5, 6, and 7). Conceivably, this role implies a range of new potential therapeutic targets. Figure 4 highlights some of the molecules or interactions known to influence T cell behaviors throughout the CI cycle and subcycle to exemplify the range of potential sites for intervention.

IMMUNOSUPPRESSION BY CANCER-ASSOCIATED FIBROBLASTS

Probably the most important conceptual advance is the appreciation of the key role likely played by the fibroblast compartment, cancer-associated fibroblasts or "CAFs". CAFs develop from fibroblasts upon exposure to activating signals from tumor cells

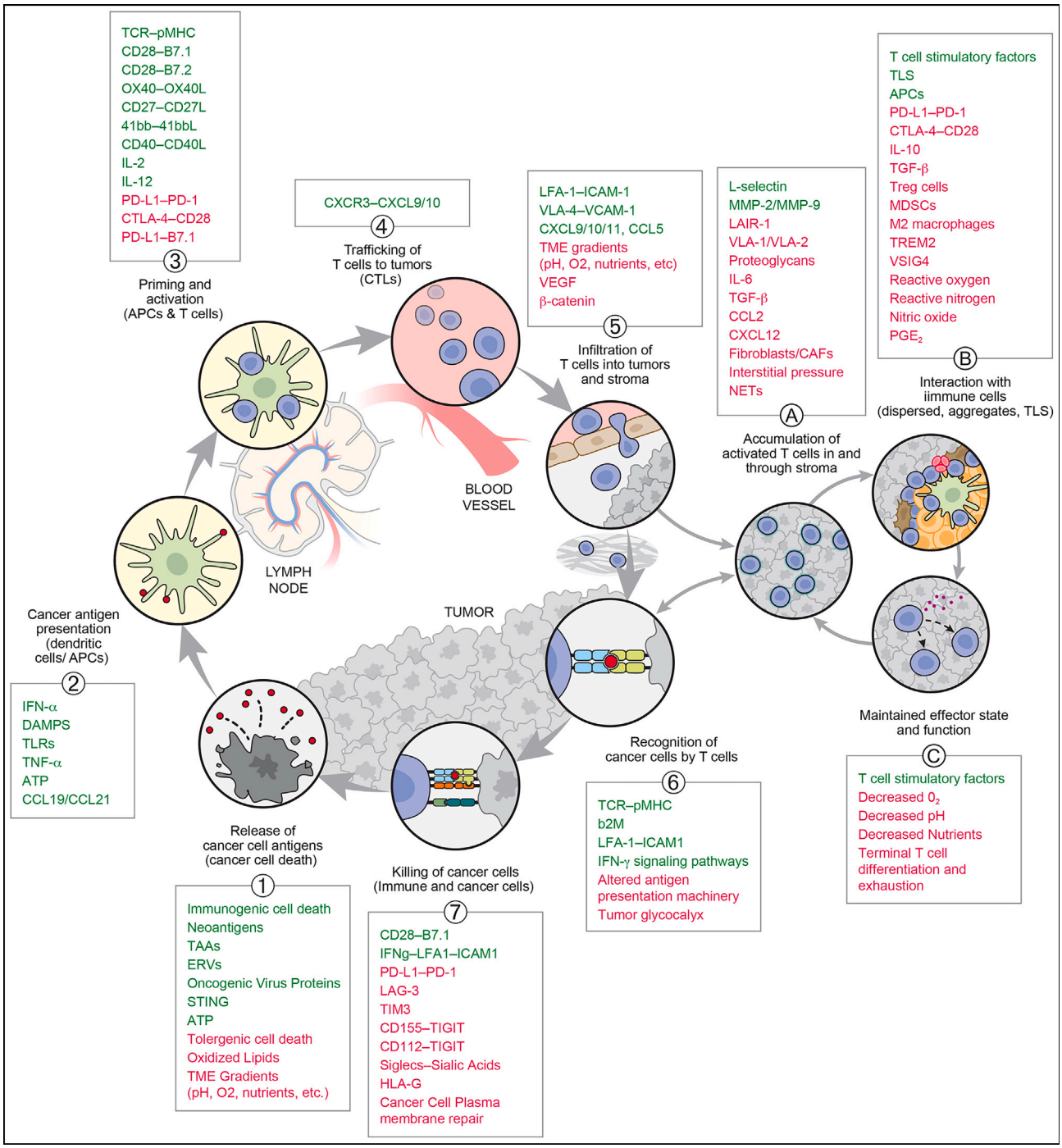

Figure 4. The cancer-immunity cycle with stimulatory and inhibitory factors

A multitude of stimulatory and inhibitory factors can influence success or failure of each step of the cancer-immunity cycle. Here, we provide selected examples at each step. Stimulatory factors shown in green promote immunity, whereas inhibitors shown in red help restrain the response. Factors shown in black may be either stimulatory or inhibitory. These listed factors do not represent a comprehensive list.

TAAs, tumor-associated antigens; ERVs, endogenous retrovirus proteins; STING, stimulator of interferon genes; ATP, adenosine triphosphate; TME, tumor microenvironment; IFN, interferon; DAMPs, damage-associated molecular pattern; TLRs, toll-like receptors; TNF, tumor necrosis factor; CCL, CXCL, CCR, CXCR, chemokine ligands and receptors; TCR, T cell receptor; pMHC, MIC, MHC class I polypeptide-related sequence protein; PD-L1, programmed death-ligand 1; CTLA-4, cytotoxic T-lympocyte antigen-4; LFA, lymphocyte function-associated antigen; ICAM, intracellular adhesion molecule; VLA, very late antigen; VCAM, vascular cell adhesion protein; VEGF, vascular endothelial growth factor; MMP, matrix metallopeptidase; LAIR, Leukocyte-associated immunoglobulin-like receptor; TGF, transforming growth factor; CAF, cancer-associated fibroblast; NET, neutrophil extracelllar traps; TLS, tertiary lymphoid structure; APC, antigen-presenting cell; Tregs, regulatory T cell; MDSC, myeloid-derived suppressor cell; TREM, triggering receptor expressed on myeloid cells; VSIG4, v-set and immunoglobulin domain containing; PGE_2, prostaglandin E_2; B2M, b_2 microglobulin; LAG-3, lymphocyte-activation gene 3 protein; TIM-3, T cell immunoglobulin domain and mucin domain-3; TIGIT, T cell immunoreceptor with Ig and ITIM domains; HLA, human leukocyte antigen.

as well as alterations in oxygen and metabolite gradients and availability and play a key role in establishing the matrix architecture of the TME.[13,36–38] CAFs exhibit remarkable functional pleiotropy, influencing various hallmarks of cancer such as tumor initiation, metabolism, progression and metastasis, anti-cancer immunity, angiogenesis, drug penetration, and therapeutic responses.[39–45] These functions are due in part to their decisive roles in shaping the complex matrix milieu and mechanics of the tissue in which tumors grow and metastasize.

While CAFs have been studied for decades, the field still lacks a consensus framework that captures cell subsets and states, cell surface markers, lineage defining transcription factors, developmental origins, localization patterns, and functions. Single-cell omics technologies, particularly the advent of single-cell RNA sequencing (scRNA-seq), has rapidly advanced our understanding of CAFs, providing granularity on new markers, subset identities, and tool generation for mechanistic studies. Three major classes of CAFs are observed across most human solid tumors: myofibroblastic CAFs (myCAFs), inflammatory CAFs (iCAFs), and antigen-presenting CAFs (apCAFs).[13] MyCAFs comprise a prominent CAF subtype in most human solid cancers and produce large amounts of extracellular matrix (ECM) and other fibrosis-associated molecules, particularly in late-stage tumors. CAF patterning of matrix architecture affects cancer cell invasiveness, immune cell infiltration, vascularization, organ stiffness and drug penetration.[46–50] MyCAFs are also immunomodulatory, exhibiting potential to suppress and compartmentalize CD8 T cells and other immunocytes.[51,52] Producing a breadth of cytokines and chemokines, iCAFs secrete interleukin (IL)-6 and the chemokine ligands CCL2 and CXCL12 and may play an immunosuppressive role; these cells dominate the CAF compartment in select metastatic settings.[53–56] Antigen-presenting CAFs are similar to iCAFs in expressing immunomodulatory factors but also express relatively high levels of major histocompatibility complex class II (MHC class II) molecules and induce recruitment regulatory T (Treg) cells.[57,58]

MyCAF development is dependent on fibroblast-intrinsic TGF-β signaling, mechanical force-driven activation, and increased contractility, whereas IL-1 and TNFα are thought to induce iCAFs. Interestingly, iCAFs may arise from mesothelial cells through a mesothelial-to-mesenchymal transition.[58] Paradoxically, the same CAF subsets may exhibit tumor promoting and tumor-restricting functions in distinct settings, emphasizing the need for additional mechanistic research to systematically elucidate the underlying functional and developmental complexities of these cells.[13,59–62]

In clinical data, myCAF-specific gene signatures associate with reduced patient survival and poor response to chemo-, immune- and tumor-targeted therapies. iCAFs and apCAFs are more difficult to study in the context of cancer patient outcome due to a lack of discrete markers and robust gene signatures. Nevertheless, CAFs generally associate with both promotion of tumor progression and lack of response to cancer therapeutics although anti-tumor associations have also been observed.

A hallmark of immune excluded tumors is the densely packed, highly aligned network of matrix fibers organized circumferentially around tumors together with myCAFs and CD8 T cells.[63,64] Within this stromal niche, CD8 T cells migrate along collagen fibers and exhibit functional deficiencies that impede their ability to effectively respond to checkpoint blockade inhibitors and infiltrate into direct tumor contact.[14,63,65] Furthermore, CAF-deposited matrix is associated with reduced lung tumor infiltration by T cells and DCs as well as alterations in TAM states.[66] Preclinical studies suggest that lack of therapeutic response to immune checkpoint blockade and chemotherapy is driven at least in part by the effects of TGF-β signaling.[9,14,66–68] Further mechanistic work is needed to understand how TGF-β and myCAFs modulate CD8 T cells in the peritumoral stromal niche.[69,70] While many questions remain unanswered regarding the immune-excluded immunotype, a few features are clear. First, CD8 T cells are neither immobile in this niche nor obstructed by a wall of matrix.[63] Second, excluded CD8 T cells can be rescued by interventions that disturb stromal architecture, enabling the T cells to infiltrate directly into tumor cell contact and eradicate cancer cells[14]; thus, the excluded T cells do not represent a dysfunctional or terminal state. The CD8 T cell "problem" in immune-excluded tumors relates more to features of the peritumoral microarchitecture that favor their retention in the stromal compartment rather than a strictly intrinsic and irreversible rewiring of CD8 T cell physiology.

IMMUNOSUPPRESSION BY THE MYELOID COMPARTMENT

Myeloid cells are the most abundant cell type in solid cancers beyond the cancer cells themselves, with macrophages, monocytes and immature myeloid cells (also referred to as myeloid-derived suppressor cells or MDSC's) comprising nearly half of all cells the tumor microenvironment.[71–73] Neutrophils and DCs are also present in most human solid cancers but represent a much smaller fraction (<10%) of the tumor myeloid compartment. Myeloid cells thrive in the TME due in part to an abundance of growth factors, nutrients, cytokines, and chemokines secreted by tumor cells (e.g., M-CSF/CSF-1, IL-6, GM-CSF, G-CSF, CCL2, CCL5).[71] Tumor-associated myeloid cells associate with reduced patient survival and lack of response to anti-cancer therapies although associations with better outcomes have also been reported.[74]

Tumor-associated macrophages, or TAMs, are a mixture of embryonically derived tissue-resident macrophages as well as macrophages derived from circulating bone marrow originating monocytes.[75,76] Tumor-infiltrating myeloid cells as well as tissue-resident monocytes and macrophages co-evolve with cancer cells, adopting distinct features in response to factors derived from cancer cells harboring diverse mutations and undergoing changes with tumor progression, metastasis and response to therapy. In addition, developing myeloid progenitors in a tumor-bearing subject are often exposed to tumor-cell-derived factors that act remotely on myeloid progenitors in the bone marrow long before their differentiated progeny reach blood and tumor.[71,77]

Comprising a plethora of subsets and states, the TAM compartment is more heterogeneous than macrophages of the surrounding normal tissue. Single-cell atlases of human tumors demonstrate myeloid diversity with 5–10 macrophage subsets and 2–4 monocyte subsets depending on clustering methodology.[72,73] These subsets, largely identified based on transcriptional profiles, may represent developmentally discrete subsets,

interconvertible activation states or a mix of both. The life cycle of myeloid cells, which can involve continuous migration from blood into tissues, from tissues into lymph, or residency within diverse tissue niches, requires a physiologic adaptability in order to thrive. Within tumors, monocytes, neutrophils, and macrophages adapt to hypoxic, acidic, and nutrient-poor gradients, resulting in metabolically distinct phenotypes from those of macrophages in more hospitable conditions of healthy non-tumor tissue.[78–80] Furthermore, TAM subsets differ from one another in their metabolic profiles and nutrient dependencies.

TAMs play both pro-tumor and anti-tumor functions and contribute to multiple hallmarks of cancer.[71] Furthermore, TAMs exhibiting pro-tumor properties appear to far outnumber those with anti-tumor function, and yet the precise phenotypic identity and functional contributions of TAM subtypes remain incompletely understood. In general, anti-tumor functions of TAMs include killing and phagocytosis of tumor cells, MHC class II antigen presentation, and expression of proinflammatory cytokines. Pro-tumor functions of TAMs include expression of factors that promote angiogenesis, ECM remodeling, and suppression of anti-tumor immunity by inducing Treg cells.[81] TAMs express PD-L1 and other molecules that restrain T cell responses to tumors.[82,83] TAM expression of MHC class II can also serve to drive tolerance rather than immunity depending on their expression of costimulatory molecules and cytokines. TAMs also secrete factors that promote blood vessel growth and tumor cell metastasis. New genetic and pharmacologic tools that target discrete myeloid subsets will markedly advance our understanding of this lineage and its significance in tumor progression and therapeutic response. Preclinical studies in mouse tumor models have generated diverse TAM targeting approaches that overcome their pro-tumor and immunosuppressive functions.[71,78,84]

Given the sheer size, developmental complexity, and functional impact of the macrophage compartment on cancer cells, the TME, and anti-tumor immunity, it is reasonable to think that breaking the efficacy ceiling for cancer treatments may require strategies that target myeloid cells. A number of approaches have been evaluated in clinical trials, such as total macrophage depletion, without success to date.[71,85] However, additional new therapeutics that aim to selectively deplete pro-tumor TAMs, directly inhibit their pro-tumor functions or reprogram TAM subtypes away from pro-tumor states and toward anti-tumor states are in development. One attractive idea is to utilize potent innate activators such as type I IFNs, either by targeted delivery or approaches that induce *in situ* formation in the tumor.[27] Since innate activation is key to firing up the CI cycle both systemically and intratumorally (in the case of the subcycle), this strategy should garner considerable interest.

IMMUNOSUPPRESSION BY THE TUMOR

In addition to the myriad of suppression mechanisms attributable to the TME, tumor cells themselves harbor the ability to restrict T cell immunity. While several enticing mechanisms have been described, mainly in preclinical models, few have been validated in the clinic or provided new therapeutic targets. For example, in melanoma models, activation of β-catenin signaling associates with immune deserts and resistance to checkpoint inhibitors.[86] This effect has been attributed to a paucity of T cell chemokine secretion, although whether this is a tumor-specific defect or a failure of NK cell infiltration is unclear.[87] The release of prostaglandin E2, a regulator of T cells and other immune cells, associates with the activation of the cyclooxygenase pathway in tumors and resistance to immunotherapy.[88,89] Similarly, tumors (especially gliomas) that harbor mutations in IDH1 or IDH2 overproduce 2-hydroxyglutarate, which suppresses T cell function.[90]

Other suppressive metabolites such as kyneurenine (due to overexpressed Indoleamine 2,3-dioxygenase 1 (IDO) and tryptophan 2,3-dioxygenase (TDO) by tumor cells) and adenosine (produced extracellularly from ATP released by dying tumor cells) are also released by tumors, but the impact of these metabolites requires clinical validation. Conceivably, the tumor (and TME) releases a panoply of such metabolites or facilitates the depletion of amino acids such as tryptophan that are essential for T cell function. Together, these metabolic alterations would create creating a distinctly immunosuppressive environment,[91] suggesting that therapeutic targeting any one component may prove ineffective. Indeed, inhibitors of IDO or adenosine signaling have not yet proved successful in the clinic. The release of oxidized lipids by many tumors, especially after cell death, presents an interesting paradigm with some of these being suppressive to T cells[92] while others are strong activators of innate immunity and anti-tumor responses.[4,93] Understanding these features will be key to understanding factors regulating the progression of the CI cycle.

TGF-β release by many tumors can also be expected to be immunosuppressive given that this cytokine promotes a T cell exclusionary stromal reaction,[14,67] facilitates Treg cell differentiation,[94] and restricts the expansion of T stem-like memory cells.[68,95] Several TGF-β antagonists have been evaluated in the clinic without obvious benefit, perhaps reflecting the attendant toxicities associated with the sequestration of this pleiotropic cytokine family or the incomplete reversal of inhibitory factors that are the result of prolonged TGF-β signaling. In addition, it is unclear whether the three TGF-β cytokines have interchangeable or even antagonistic functions in the tumor context, making it difficult to know whether one, two, or all three isoforms should be targeted.

Activation of oncogenic pathways may also directly or indirectly oppose T cell immunity. For example, increased Ras/MAPK signaling reduces the expression of MHC class I gene products, which would reduce a tumor cell's susceptibility to T cell attack.[96,97] There are also rare instances where loss of type II IFN signaling by tumors confers protection, presumably by limiting the cytotoxicity of IFN release by T cell effectors.[98] Finally, tumor cells may protect themselves from T cell killing by rapidly repairing the plasma membrane pores created by perforin upon T cell granule release.[99] Other cell autonomous defense mechanisms likely await to be discovered.

IMMUNOSTIMULATION IN THE TME: DCs

DCs remain indispensable in the CI cycle due to their unparalleled ability to prime and expand antigen-specific CD4 and CD8 T cell responses.[1,100] Over the past decade, there has been great progress in the definition and functional characterization of various DC subsets and populations.[101–103] The conventional DC1 (cDC1) population continues to be the most important

initiator of CD8 T cell tumor immunity, reflecting at least in part their ability to traffic from the tumor bed to dLNs, their ability to cross present internalized tumor antigens on MHC class I, and their capacity for stimulating naive CD8 T cells. It also remains possible that these or other migrating cells somehow "hand off" tumor antigens to dLN resident DCs, representing a second option for antigen cross-presentation to T cells on both MHC class I and class II molecules.[104,105] Two other general classes of DCs also exist, although their roles are a bit less well defined. As reviewed by Pittet and colleagues,[106] cDC2's are typically associated with presentation on MHC class II molecules and stimulation of CD4 responses. cDC3's, also known as CCR7 DCs or mRegDCs, are also found intratumorally as well as in dLN, can be migratory and may mediate immunostimulatory or regulatory functions depending on context.

The positioning of DCs in tumors is clearly a primary determinant of the anti-cancer immune response. Patients whose tumors are "immune deserts" are almost totally unresponsive to immunotherapy and lack any T cell infiltrate, suggesting the absence of an ongoing immune response. These tumors also lack DCs, which may be the primary reason for the lack of response. This possibility that has received some experimental support in mouse models.[9,86] If a failure of DC infiltration is the culprit for producing the immune desert immunotype, then understanding the reasons for this failure and possible mechanistic solutions should reveal potential paths for therapeutic intervention.

DCs maintain a balance between immunity and tolerance,[107,108] a dual responsibility that may be a double-edged sword in the cancer context. DCs, regardless of subset, must receive an activating signal to initiate a terminal differentiation process of "maturation" that converts DCs from antigen accumulation mode to antigen presentation mode.[100,109] When this is a proinflammatory or inflammatory stimulus, the mature DCs promote immunity, tuned to the precise nature of the stimulus; when it is not, DCs promote tolerance. To generate an effective anti-cancer response, therefore, antigen-accumulating DCs must receive an appropriate activating signal, or adjuvant.[1,4] If the TME is insufficiently inflammatory, the DCs will be less likely to mature or to produce anti-tumor T cells. Although the identity of the toleragenic DCs remains uncertain, maturation does occur at the steady state even in the absence of overt inflammatory stimuli as phenotypically mature DCs (elevated MHC class II and CD86) can be found in dLNs and the spleen. These DCs may lack certain features (cytokine production, high costimulatory receptor ligands) that result in tolerance. The discovery of a mature DC with immunoregulatory properties (mRegDCs, CCR7 DCs) may be of particular interest in this regard.[106,110]

It has become increasingly clear that DCs, especially those in the TME, provide an additional essential function in the tumor, namely the stimulation and expansion of antigen-committed memory or effector T cells. Early evidence came from adoptive cell transfer experiments in mice, where anti-tumor efficacy was substantially diminished in animals whose DCs were conditionally ablated.[30] Similarly, a DC-directed mRNA "vaccine" encoding a CART target (claudin-6) enhances CART function.[111] *In situ* approaches reveal a close association of intratumoral DCs with CD4 and/or CD8 T cells (or all three) in both humans and mice.[112,113]

In the immunotherapy context, it also appears to be the case that PD-L1 expression by DCs plays a disproportionately important role in controlling T cell responses[22,114] and also serves as a more effective predictor of response in human cancer patients than total PD-L1 expression (including tumor cell expression).[115] Finally, the onset of T cell exhaustion in tumors may be controlled in the tumor itself as a consequence of antigen presentation by DCs.[116] Taken together, these considerations strongly suggest that the role of DCs in the TME is not limited to the transfer of antigens from tumor to dLNs but also to ensure the activation and expansion of antigen-specific T cells in the tumor itself.

TLSs IN CANCER

TLSs are essentially proto-LNs containing germinal-center-like structures that have long been appreciated to occur in tumors, as has the presence of poorly organized lymphoid aggregates. Only over the past few years, however, has their likely role in tumor immunity become clear.[5] Human clinical studies have documented the fact that response to checkpoint therapies generally associates with the presence of TLSs in the TME.[117–120] Especially given the accumulating evidence that DCs in the TME may work *in situ*, the clinical data suggest that there is also a functional association. By providing an organized, LN-like structure for T cell stimulation, TLSs may be the site at which T cells are activated and expanded by tumor-associated DCs.

This association has also invigorated interest in the role of B cell and anti-tumor antibodies in cancer immunity, as well as in understanding the role of the CD4 T cell response. Recent work has implicated both possibilities, with CD4 T cells now seen as possibly having their own cytotoxic properties or as harboring the ability to provide "help" to the generation of anti-tumor CD8 responses.[121] These considerations also provide an interesting mechanistic basis for understanding the function of the coinhibitory receptor Lag-3, whose presumed ligand is the MHC class II molecule.[122]

The relevance of TLSs has enhanced the concept that the TME can be immunostimulatory in addition to immunosuppressive and that T cell stimulation by DCs is not limited to secondary lymphoid organs (e.g., dLN) but has an essential component in the tumor itself. This activity is likely not limited to the TLS but to DCs (and perhaps other antigen-presenting cells) found distributed throughout the TME and intratumorally. This model indicates that DCs can work to stimulate T cells *in situ*, in addition to their well-established role after lymphatic migration to dLN.

THE CI CYCLE DIRECTS T CELL DIFFERENTIATION AND FUNCTION AT MULTIPLE STEPS

The likelihood that T cells can be primed and further stimulated in both dLN and the TME raises important questions regarding the control of T cell differentiation and trajectory. The original assumption that T cell activation and expansion occurred only in dLN (step 3 of the CI cycle) suggested that all subsequent features of T cell function were determined at that site. Thus, whether a T cell was destined for the exhaustion, effector, or memory pathways would be specified by the conditions of antigen presentation in dLN. As this simple assumption no longer seems correct, it is possible that only priming or activation is initiated in dLN, while terminal differentiation occurs at the tumor site (the "subcycle" at step 5). It is also possible that all of these

activities can occur in both sites, with TLS perhaps functioning as a site for T cell priming in the TME, in certain cases.

The possibility that T cell stimulation by DCs in the tumor plays a key role in T cell function has received support from recent experiments in mouse models.[116] It is also consistent with work in human cancer showing that expanded T cell clonotypes found in the blood are also found in the tumor bed, albeit distributed among different T cell phenotypes.[123] Unpublished work in mouse has provided further support for this interpretation by showing that dLN-derived CD8 T cells are polyclonal with respect to their TCR specificities, but are contained within a single cell state that differentiates after tumor arrival (K. Nutsch, K. Banta, T. Wu, E. Chiang, and I.M., unpublished data). Further, studies of human cancer have demonstrated that T cells (CD4 and CD8) can form clusters or "triads" together with DCs in the tumor.[112,113] In all of these studies, T cells can be shown to achieve terminal differentiation (e.g., exhaustion) only after reaching the tumor.

Although a detailed consideration of T cell differentiation and trajectory cannot be considered here, the development of Tex cells is obviously relevant to the function of the CI cycle. The revised view would suggest that T cells become committed to the exhaustion pathway at the level of the tumor, and not at the time of initial stimulation in dLN (K. Nutsch, K. Banta, T. Wu, E. Chiang, and I.M., unpublished data).[116,124] As discussed earlier, the fact that terminal Tex cells are characterized by a largely irreversible epigenetic state itself strongly suggests that therapeutic checkpoint inhibition does not act to reverse but rather to prevent the development of the exhausted phenotype within the tumor. In the case of the coinhibitory receptors PD-1 and TIGIT, their biochemical mechanism appears to involve the inhibition of costimulatory signaling via CD28 and CD226, respectively.[125] This in turn suggests that blockade of PD-1 (and TIGIT) may prevent exhaustion by promoting costimulation. DCs may provide the most relevant source of PD-L1 as well as of the CD28 ligands B7.1 and B7.2 (CD80 and CD86, respectively) and reside in both dLN and the tumor. Lag-3-mediated coinhibition may act in a distinct fashion but is triggered by binding MHC class II, which is also abundantly expressed by DCs. (Figure 4).

Recent evidence has suggested that these two geographically separate populations of DCs can play distinct roles in T cell development and exhaustion. Although the identities and precise trajectories of the T cell populations involved remain poorly characterized, one attractive model might be that tumor-specific T cells leave the dLN in a relatively multipotent state that undergoes final differentiation in the tumor, including the formation of tissue resident memory T cells and central memory T cells that may then recirculate.[126] Regardless of the model, we predict that T cells are directed by intratumoral DCs to differentiate along the effector, memory, or exhaustion pathways. Much additional work will be required to fully understand the issue, but the early evidence is sufficiently compelling to incorporate a subcycle to step 5 of the CI cycle that captures this second stage of DC-dependent T cell differentiation in the periphery.

THE DETERMINATIVE ROLE OF TUMOR IMMUNOTYPE

It remains the case that far fewer than half of patients have durable outcomes with immunotherapy, even in combination. Understanding features to predict response or understanding mechanisms of resistance continues to be a major focus of investigation both pre-clinically and in the clinic. These factors may be intrinsic to the tumor, the TME, or a reflection of patient genetics, microbiome, metabolism, or pharmacologic status but in each case must reflect the site of a rate limiting step in the CI cycle.[8] The expression of PD-L1 on tumor cells or on immune cells (DCs in particular) continues to be the most useful parameter for patient selection, but it is one that is incompletely predictive perhaps because it may not necessarily be indicative of a particular rate limiting step in the CI cycle. Mechanistically, PD-L1 expression is thought to denote patients harboring an ongoing anti-tumor response, with IFN-γ released by effector T cells in the tumor bed causing increased expression of PD-L1 by surrounding cells, especially DCs that are likely involved in directing the terminal differentiation of newly arrived or locally generated T cells. Even assuming that this idea is correct, and PD-L1-positive patients do have a pre-existing immune response, it does not necessarily follow that blockade of coinhibitory receptors such as PD-1 will overcome the CI cycle's rate-limiting step in a given patient.

All tumors, regardless of origin, exhibit a basic immunotype: immune inflamed, immune excluded, or immune desert. It seems likely that these classifications will prove useful in identifying the factors that limit or promote T cell responses to tumors.[8] For example, in immune excluded tumors, the proliferation of immunosuppressive stromal investments around a tumor has focused attention on the role of peritumoral collagen-rich fibrotic matrix, the role of CAFs and their regulation by TGF-β signaling: blockade of TGF-β signaling can alter stromal architecture and permit T cell entry in preclinical models.[14] Excluded tumors can be scored as PD-L1-positive, yet they respond poorly due to their ability to limit T cell infiltration.

Although individual indications express all three phenotypes, their ratio can vary in characteristic ways: colorectal cancers generally exhibit up to 70%–75% immune excluded tumors and only 10% immune inflamed, while non-small cell lung cancer (NSCLC) can exhibit 30%–35% inflamed and only 40% excluded.[7] Further, immune exclusion in colorectal cancer may differ from immune exclusion in an inflamed tumor such as NSCLC, and the small percentage of immune inflamed in colon cancer may reflect the MSI^{hi} population. Indication-related immunologic context is relevant. Although response to therapy may not be predicted more accurately by revealing a tumor's immune phenotype, the point is that the mechanistic basis of response or lack thereof may be hiding in plain sight. If the basis for these phenotypes can be understood, the path to future potential therapeutic targets may become clearer.

What does appear certain, however, is that the different phenotypes define immunologically distinct tumor populations that, in turn, help determine response to therapy better than a consideration of indication or tumor genetics alone or combined. Therefore, in the age of immunotherapy, it makes sense to take these immunological classifiers into account when describing tumors. The term immunotype captures this aspect, representing a feature that for precision guiding of immunotherapies may be more relevant than "indication" or "genotype" alone. We propose, therefore, that immunotype be considered for inclusion as a new and informative classifier when characterizing a patient's tumor, as

each immunotype by definition must reflect the location of rate limiting steps on the CI Cycle for each patient's tumor.

HOST-RELATED FACTORS INFLUENCE TUMOR IMMUNITY

Host and environmental factors are likely to influence the CI cycle and response to immune therapy therapy.[8] High vitiligo or psoriasis polygenic risk scores, derived from germ-line SNPs, are associated with longer OS under anti-PD-L1 monotherapy as compared to chemotherapy. This indicates the host response to tumorogenesis is relevant in predicting outcomes. These are also likely to epigenetic factors, such as chromatin structure regulating expression of key immune related proteins. Finally the influence of the gut microbiome on the immune repertoire is well established, but working to understand where in the CI Cycle the microbiome plays a role (positive or negative) will greatly assist in understanding underlying mechanisms.

Concomitant medications also play a role in determining the outcome of immunotherapies. Apart from the predicted effects of lympho-ablative chemotherapies, prior treatment with antibiotics that deplete the gut microbiota also have a generally negative effect.[127] The antibiotic effect presumably attests to a positive influence of the microbiome on anti-cancer immune responses. Certain classes of benzodiazapenes, which are often described as palliatives to cancer patients, associate with poor response to immunotherapy.[128] This effect may reflect the mobilization of the neurotransmitter GABA, which has intrinsic immunsuppressive properties.

On the other hand, various oncogene-targeted therapies, such as Ras-MAPK inhibitors and Cdk4/6 inhibitors, may enhance anti-cancer immune responses by increasing antigen presentation by tumors or by facilitating T cell function.[96,129] Understanding where on the CI cycle these various manipulations work should prove most useful in understanding the basis for these effects.

CLINICAL APPLICATION OF THE CI CYCLE AND ITS MODIFICATIONS

Immune checkpoint inhibition (ICI), especially with PD-L1/PD-1 therapy, has achieved success across a broad spectrum of cancer, with many patients benefitting from durable remissions. These agents have successfully moved from the advanced setting into the perioperative setting, reducing relapse rates after surgery and transforming outcomes in specific tumor types. Their activity in the perioperative setting is under intense investigation with randomized trials. In melanoma, it appears the neoadjuvant approach is preferable to adjuvant therapy.[130] Although the mechanistic basis for this effect has not been studied, applying the logic of the CI cycle might predict that neo-antigen load at the time of therapy allows checkpoint blockade to facilitate T cell responses, with subsequent surgery reducing the overall tumor burden thereby enabling the T cell numbers that were insufficient to yield a durable response in the neoadjuvant setting to control the growth in the adjuvant setting. In this example, tumor burden may be seen as being rate limiting prior to surgery and T cell activity rate limiting after surgery.

Issues around the optimal duration of ICI therapy and immune memory after cessation of therapy have not been addressed adequately. Existing T cell immunity prior to starting therapy appears crucial in predicting response and while dynamic changes to the TME occur with ICI therapy, their relevance remains uncertain and will require further study both pre-clinically and in patients.[131] Rechallenge with PD-(L)1 therapy after recent progression on ICI therapy does not appear to be associated with clinical benefit, suggesting that the loss of response reflected the development of another rate limiting step in the CI cycle.[132]

The only established ICI combination is PD-1 and CLTA4 inhibition, although it has only shown efficacy in specific cancers and is associated with higher toxicity that cannot be tolerated by many patients. CLTA-4's mechanism of action remains uncertain and can act either to facilitate the priming of new T cell responses or remove Tregs, which are high in CTLA4 expression and would be expected to suppress anti-cancer T cells. Thus, anti-CTLA4 could function at two sites on the CI cycle (Figure 5).

Targeting different points in the CI cycle with combination is an established strategy, although the results have been mixed. The second generation of immune therapies, alone or in combination, have not yet successfully built on the initial success of PD-L1/CTLA-4 based therapy. There are a few exceptions to this, one of which is LAG-3, which has recently attracted attention in melanoma with a progression-free survival advantage and FDA approval.[133] LAG-3 is expressed on a spectrum of immune cells including DCs. Its major ligand is MHC class II, further implicating T helper immunity in cancer immunity and the modifications to the immune cycle suggested in this article. TIGIT is a second area of interest attracting renewed attention.[134]

Other areas for optimism include personalized cancer vaccines (usually mRNA) with encouraging combination data in melanoma and pancreatic adenocarcinoma, the latter being a cancer type that is generally refractory to ICI.[24] Interestingly, both of these positive results have been in the adjuvant (post-surgical) setting, suggesting that the vaccines alone cannot generate sufficient T cell responses to exert clinical benefit under conditions of high tumor burden or entrenched non-permissive immunotypes that may be re-programmed at least transiently following surgery. Other less specific or potent vaccine platforms have struggled in solid tumors previously, due to tumor heterogeneity, manufacturing challenges, and possible inhibition by the TME.[135] Combinations that address the TME, potentially by re-wiring the inhibitory myeloid compartment may potentially address this limitation.[136] Understanding the nature of the steps of the CI cycle that limit vaccine efficacy is important to maximize the chances for this potentially curative approach.

Single-agent CAR-T cell therapy or T cell engagers have had excellent success in hematopoietic tumors, where the target is relatively clear: CD19 or CD20 in lymphoma and certain leukemias and BCMA in myeloma. However, in more heterogeneous solid tumors, where the targets are often expressed on host tissues and the TME can be immunologically challenging, results are less impressive. CAR-T cells in solid tumors may require novel targeting, more sophisticated cell engineering, and combination-based approaches. Success will likely be predicate on attention to the relevant steps of the CI cycle. As mentioned above, preclinical data have shown that programming the expression by DCs of a CAR-T target antigen (claudin-6) increases the efficacy of the cognate cell therapy, presumably reflecting the role of DCs in

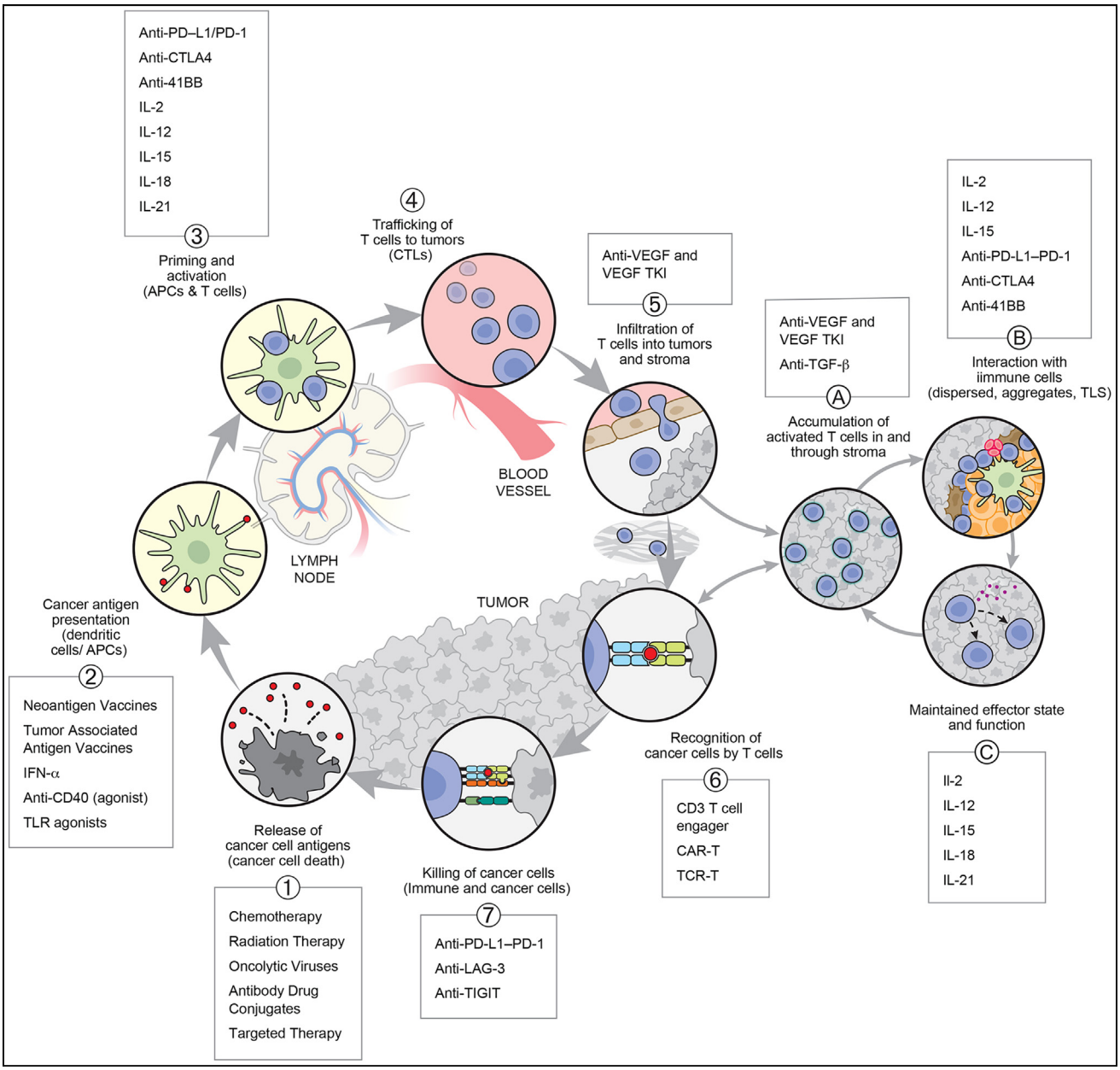

Figure 5. Approved and selected investigational therapies that target the cancer-immunity cycle

Since 2013, thousands of clinical trials testing cancer immunotherapy agents have been conducted. This has led to numerous approvals of immunotherapy and immunotherapy regimens in many different cancer indications, highlighting the most efficacious immunotherapeutic approaches. These approved agents and several select others that are in clinical testing are shown at the step of the cancer-immunity cycle where their primary action occurs.

dLN and intratumorally in supporting T cell responses (even after adoptive cell therapy).

Attempting to transform tumors into immune responsive cancers by altering the TME with non-immune therapy should be an effective approach, but the limited attempts thus far have had mixed results. VEGF targeted therapy has had some success in altering the immune infiltrate and possibly favoring DC maturation, but the mechanism of this strategy is poorly understood.[137] There has been much interest in using TGF-β antagonists (anti-TGF-β antibodies, inhibitors of the TGF-β receptor kinase), although the therapeutic hypothesis in these trials may not have directly addressed the immune excluded phenotype. Moreover, TGF-β is highly pleiotropic with its pan inhibition being associated with a variety of toxicities that have limited the dose. Attempts thus far that have targeted all three TGF-β isoforms or the receptor have proved unsuccessful in cancer indications. Most notably, a large trial using a soluble TGF-β receptor (TGF-β "trap") fused to an anti-PD-L1 antibody failed to exhibit efficacy without much toxicity, although the distribution and pharmacodynamic activity at relevant sites was not reported.[138] It is also possible that inhibiting two or more isoforms simultaneously may itself have negative consequences for efficacy. TGF-β may be important at several

sites on the CI cycle beyond controlling stromal architecture such as Treg and Tscl production,[139] so further study would appear warranted despite the lack of success thus far.

Chemotherapy/PD-L1 combinations have had success, potentially by targeting immune resistance within the TME, but results have been inconsistent across tumor types.[140,141] There is a rationale for exploring new agents such as PARP inhibition or CDK4/6 inhibition or antibody-drug conjugates (ADCs) in combination with immune therapy. Many trials are ongoing and should be explored not only with efficacy goals but also to learn more about the immune modulatory effects of these agents.

There has also been preliminary success in targeting the microbiome, which adjusts the hosts immune repertoire. The principle of the host immune fitness is gaining momentum. The link between this fitness, the gut microbiome, and improving immune therapy efficacy is being clinically tested. Encouraging randomized phase II data showed enhanced activity of immune combinations, by altering the microbiome with oral agents such as CBM-588 have been published.[142]

Many novel immune combinations have failed. They have been tested in various cancer types with distinct immunological features but without attention paid to the immunotypes under investigation. This has led to the hypothesis of immune responsive and resistant histological tumor (melanoma vs. pancreas). While this is true at one level, it is an over generalization that could be refined by considering the immunotypes of the patients under investigation. Tumor and TME heterogeneity show immune repertoire variability even in classic non-immune responsive cancers, such as prostate cancer, suggesting that indeed immune responses have been generated but rendered ineffective. The randomized trials for PD-(L)1-based therapy in prostate cancer are negative in unselected patients, but those rare patients with tumor immune infiltration exhibited increased response rates.[143] Moreover, experiments in mice, and possibly humans, have demonstrated the immunosuppressive aspects of androgens on (male) CD8 T cells.[144–146]

Together, these considerations suggest that the overall mechanism of response is multifactorial but biologically similar across tumor types. An important step would be to categorize patients according to immunotype (e.g., immune excluded vs. immune inflamed can both be PD-L1 positive) although it is likely that there is further heterogeneity even within immunotype that could contribute to response variability.

Over the last decade, there has been ample clinical research to show that innate, adaptive and immune independent biomarker (such as stromal biomarkers) all play a role in response.[147] This is in addition to tumor related factors such as oncogene alleles and tumor mutation burden. The multifactorial mechanisms of sensitivity and resistance mean that no single biomarker such as PD-L1 or tumor mutational burden (TMB) will account solely for response.[148] As we develop newer immune therapies at different points of the CI cycle, alternative biomarkers will be needed. Indeed, the modified cycle increases the chances to discover unified biomarkers as it now calls out additional critical activities (e.g., the requirement for T cell stimulation by DCs or other antigen-presenting cells in the tumor) that had not been previously considered. Clearly, these will go beyond PD-L1 expression or TMB and may even be specific to the class of drug under study, as different drugs address different stages of the CI cycle.

A further challenge that has limited progress in the clinic is that many combinations have been tested in suboptimal circumstances, in small single arm trials with heterogeneous patient populations previously exposed to immune therapy. Many combinations, potentially active in specific clinical settings, may have been discarded prematurely. However, examples of unsuccessful drug development such as IDO inhibition, which progressed quickly from phase I to phase III combinations without single agent activity, genetics, or activity in pre-clinical models highlights the difficulty associated with unbridled enthusiasm.[149] Robust initial testing is highly desirable, and if drugs are to be developed absent single agent activity, there must be a testable therapeutic hypothesis that one can evaluate during a trial, so that important mechanistic and pharmacodynamic information can be obtained regardless of the trial's efficacy outcome. This returns us, again, to the development of concepts such as the CI cycle: having a clear framework within which one can view the steps that must occur to mount and sustain an effective anti-cancer response is essential to interpreting complex clinical outcomes.

CONCLUDING REMARKS

A decade after its publication, the basic features of the CI cycle remain an accurate reflection of our understanding of the immune response in cancer. Yet, understanding the cycle's individual steps and how they interconnect does not by itself ensure an understanding of their mechanisms of action. We have noted how initial mechanistic assumptions, even of successful therapies, such as exhaustion reversal by checkpoint inhibitors, have changed as a consequence of detailed study. We have also noted new information that T cell activation can be influenced not only in dLN but in the tumor and tumor-associated lymphoid structures such as TLSs. Such insights should impact how we think about objectives for sculpting the most effective T cell responses: quality, trajectory, and persistence may be as important as quantity. Similarly, such considerations should impact our understanding of T cell-based immune-related toxicities.

The fact that only about one-third of patients respond to immunotherapy remains a major challenge, one that is even more daunting than acquired resistance to therapy. Given the importance of the TME and, especially, of tumor immunotypes in regulating T cell responses, far more attention needs to be paid to these factors when searching for ways to further leverage T cell immunity in cancer. Although next-generation checkpoint inhibitors are likely to bring some benefit, it seems unlikely that they alone will overcome the barriers endemic to the immune excluded and immune desert immunotypes. Solving the basis for these immune restrictive situations and generating therapeutics that render these immunotypes more permissive to T cell activity represent the greatest opportunities for the next transformative step forward: perhaps as many as 60%–70% of all cancer patients have tumors that exhibit immune-restrictive phenotypes, and this large group contains the bulk of individuals who prove refractory to ICI.

Transferring immunotherapies to early disease or the adjuvant setting where immunotypes may be less restrictive and possibly

more plastic could also represent a chance for significant clinical advances. But here, too, mechanistic understanding will be key.

In the end, the challenges of developing immune therapies reflect the complexity of human immunity, specifically the array of mechanisms responsible for creating rate limiting steps at each successive step of the CI cycle. This consideration goes beyond even the existence of permissive or restrictive immunotypes and can include immunotype-agnostic features that can best be described as mechanisms of *shared immune escape*. Such mechanisms would include the involvement of both cancer intrinsic and extrinsic factors, such as class I loss or downregulation, neoantigen loss, an accumulation of multiple immune checkpoints, mounting populations of suppressive cells in the TME, and the loss of the appropriate cell populations. Early disease settings may avoid at least some of these mechanisms; machine learning models informed by relevant biomarker data may help mitigate them or suggest new therapeutic combinations when they do occur. Whatever the approach, the goal will remain taking appropriate steps to ensure the continued revolution of the cancer-immunity cycle.Declaration of interests

The authors declare no competing interests.

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