Reversing T-cell Exhaustion in Cancer: Lessons Learned from PD-1/PD-L1 Immune Checkpoint Blockade

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ABSTRACT

Anti–PD-1/PD-L1 immune checkpoint blockade (ICB) therapy has revolutionized the treatment of many types of cancer over the past decade. The initial therapeutic hypothesis underlying the mechanism of anti–PD-1/PD-L1 ICB was built around the premise that it acts locally in the tumor, reversing the exhaustion of PD-1^{hi}CD8^{+} T cells by "releasing the brakes." However, recent studies have provided unprecedented insight into the complexity within the CD8^{+} T-cell pool in the tumor microenvironment (TME). Single-cell RNA sequencing and epigenetic profiling studies have identified novel cell surface markers, revealing heterogeneity within CD8^{+} T-cell states classified as unique. Moreover, these studies highlighted that following ICB, CD8^{+} T-cell states within and outside the TME possess a differential capacity to respond, mobilize to the TME, and seed an effective antitumor immune response. In aggregate, these recent developments have led to a reevaluation of our understanding of both the underlying mechanisms and the sites of action of ICB therapy. Here, we discuss the evidence for the reversibility of CD8^{+} T-cell exhaustion after ICB treatment and its implication for the further development of cancer immunotherapy.

Introduction

CD8^{+} T cells play a crucial role in the control of both tumor growth and chronic viral infections. The hallmark of the CD8^{+} T-cell response in these settings is exhaustion, a dysfunctional state occurring as an adaptation to chronic antigen exposure (1–3). During exhaustion, persisting CD8^{+} T cells undergo a hierarchical loss of effector functions leading to a state of hyporesponsiveness and eventual clonal deletion (4–9). Notably, exhausted T cells exhibit enhanced and sustained expression of PD-1, primarily functioning to limit immunopathology in the setting of chronic T-cell receptor (TCR) stimulation (10–12). This feature of exhausted CD8^{+} T cells prompted the development of immune checkpoint blockade (ICB) therapy targeting PD-1 and its ligand, PD-L1 (13, 14). Antibody-mediated blockade of PD-1/PD-L1 interactions restores functional properties of CD8^{+} T cells in chronic infections and tumor models and is associated with improved control of viral and tumor load (15–17). Clinically, ICB has revolutionized the approach to cancer treatment as it enhances antitumor immunity and survival in multiple tumor indications (18–21).

Initially, the paradigm in the field was that anti–PD-1/PD-L1 ICB acts locally in the tumor microenvironment (TME) where it directly reverses exhaustion within PD-1^{hi}CD8^{+} T cells by opposing inhibitory signaling through PD-1. However, recent developments in the field have challenged this notion. Mechanistic studies have determined that the response to ICB is a complex process defined by heterogeneity in the functional characteristics and differentiation status within the intratumoral CD8^{+} T-cell pool (22, 23). In this revised mechanistic model, PD-1 blockade drives the expansion of a progenitor PD-1^{lo}CD8^{+} T-cell subset with self-renewal properties. These progenitor exhausted cells differentiate into PD-1^{hi}CD8^{+} cytotoxic effector-like cells that ultimately become exhausted. We now appreciate that ICB activity is not restricted to the TME and, at least in part, is a result of the mobilization of ICB-permissive, stem-like precursor CD8^{+} T cells that reside outside the tumor.

This review discusses the original seminal works supporting the concept that ICB reverts CD8^{+} T-cell exhaustion alongside emergent data giving rise to the new paradigm of vast intratumoral CD8^{+} T-cell heterogeneity and the existence of distinct ICB-permissive cell states. Although we note that a body of literature describes additional direct and indirect effects of ICB on multiple immune cells beyond CD8^{+} T cells, such as effector and regulatory CD4^{+} T cells, macrophages, and dendritic cells (DC), these topics exceed the focus of this review and have been recently addressed in detail elsewhere (24–30). Finally, we review the emergent body of work identifying immunologic niches outside of the TME (e.g., lymphoid tissue) as key sites of ICB action and discuss the significance of this knowledge for further improvement and development of immunotherapy approaches.

Reinvigoration of Exhausted CD8^{+} T Cells: Insights from Preclinical Models of Chronic Infection

Early evidence of anti–PD-1/PD-L1 ICB activity in the context of chronic antigen exposure was reported in viral infection models. Pioneering work by Barber and colleagues showed that treatment of mice chronically infected with lymphocytic choriomeningitis virus (LCMV) Clone 13 with anti–PD-L1 ICB leads to a functional reinvigoration of exhausted CD8^{+} T cells (15). In addition to a substantial increase in the number of LCMV-specific CD8^{+} T cells, ICB treatment also induces an increase in the production of effector cytokines and cytolytic function, leading to dramatically improved control of Clone 13 infection. Notably, this response originates from existing PD-1^{+}CD8^{+} T cells rather than *de novo* priming of naive PD-1^{-}CD8^{+} T cells (15). This early work provided data to support the idea that reversal of exhaustion in existing CD8^{+} T cells, featured by their

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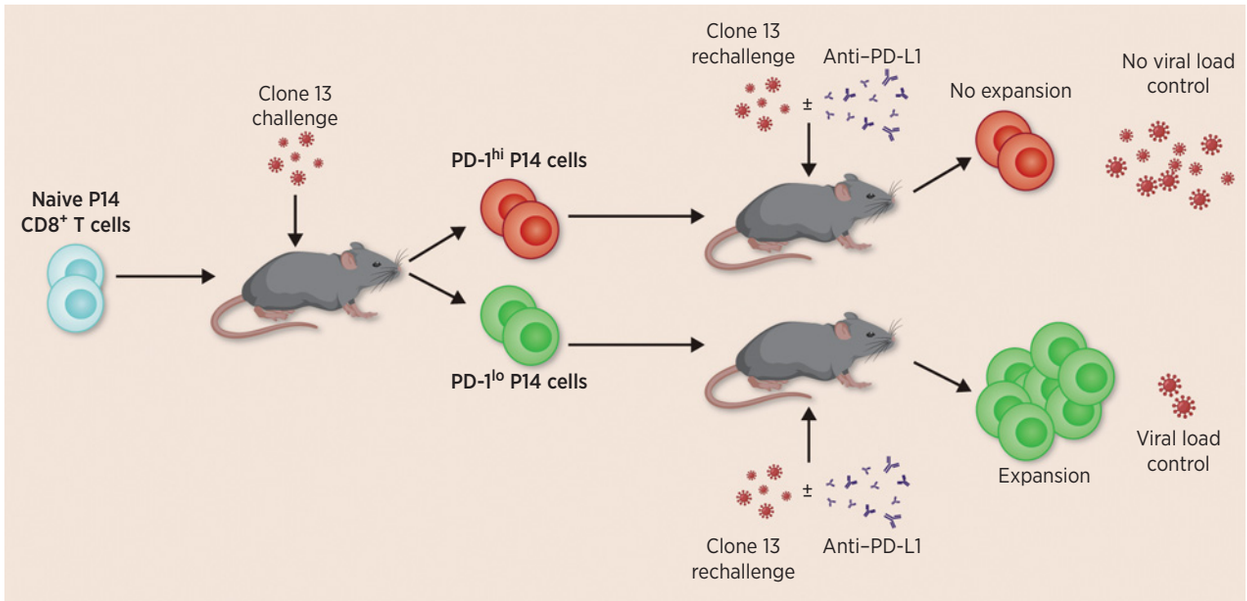

Figure 1.

PD-1^{lo}CD8^{+} T cells are susceptible to anti–PD-L1 ICB treatment. LCMV Clone 13 induces distinct CD8^{+} T-cell populations defined as PD-1^{lo} and PD-1^{hi}. These cells can be isolated by FACS. The PD-1^{lo} cells respond to anti–PD-L1 treatment, leading to robust expansion and viral control, whereas transferred PD-1^{hi}CD8^{+} T cells do not.

reacquisition of effector functions, is an underlying mechanism of action of anti–PD-1/PD-L1 ICB.

Several seminal studies revealing phenotypic and functional heterogeneity of exhausted CD8^{+} T cells followed the initial findings by Barber and colleagues. One of the key findings was that the exhausted PD-1^{+}CD8^{+} T-cell population is not uniform and can be divided into PD-1^{lo} and PD-1^{hi} subsets (31). Significantly, this PD-1$^{lo/hi}$ dichotomy is associated with dramatic differences in responsiveness to anti–PD-1/PD-L1 ICB (**Fig. 1**). In this study, PD-1^{lo} and PD-1^{hi} CD8^{+} T cells generated in the context of chronic LCMV infection were isolated and transferred into mice, followed by a rechallenge with LCMV Clone 13 in the presence or absence of anti–PD-L1 blocking antibodies (31). Only the PD-1^{lo} subset could respond to ICB treatment, and these cells imparted superior control of viral load compared with their PD-1^{hi} counterparts (**Fig. 1**). This finding was extended by Paley and colleagues, who introduced, for the first time, a concept of a developmental connection between PD-1^{lo} and PD-1^{hi} CD8^{+} T-cell states, which were further defined based upon differential expression of the transcription factors Eomes and Tbet (32). Specifically, PD-1^{lo}Tbet^{hi}Eomes^{lo} CD8^{+} T cells were shown to possess memory-like features characterized by slow homeostatic turnover. These cells respond to antigen leading to a proliferative burst that gives rise to the pool of PD-1^{hi}Tbet^{lo}Eomes^{hi} terminally differentiated progeny. Importantly, this conversion process seemed to be continuous, potentially leading to depletion of the precursor populations. Replenishing precursor exhausted cells through ICB-induced expansion or recruitment could prevent this depletion and ensure the persistence of the antitumor CD8^{+} T-cell response. The finding that PD-1^{hi} cells did not respond to anti–PD-L1 challenged the concept of ICB reversing the exhaustion of these cells and suggested that beneficial responses originate from a permissive PD-1^{lo} subset.

Transcriptional profiling of the PD-1^{lo} precursor population has further strengthened the connection of this population with stem-like or memory-like cells (33). In one study, it was shown that PD-1^{lo} precursors exhibit a protein expression signature resembling both follicular helper (Tfh) CD4^{+} and memory CD8^{+} T cells defined by expression of ICOS, CXCR5, Bcl-6, and TCF-1 (33). Moreover, transcription factor TCF-1, implicated in the maintenance of memory CD8^{+} T cells in the context of acute viral infections (34), was reported to play a crucial role in the maintenance of the memory-like precursor population in Clone 13 infection. TCF-1^{+} cells share some common features with *bona fide* memory CD8^{+} T cells, including pluripotency, homeostatic proliferation, and capacity to undergo recall response. However, unlike memory cells, they display some of the phenotypic features of exhaustion, such as enhanced expression of inhibitory receptors (35). These memory-like TCF-1^{+}CD8^{+} T cells were shown to respond to anti–PD-1/PD-L1 ICB treatment giving rise to effector cells required for successful viral control (35). The same was found to be true for CXCR5^{+}CD8^{+} T cells, unlike the CXCR5^{-} subset (33), pointing to a similar phenotypic assignment for these subpopulations in chronic viral infection. These early studies in viral models highlighted heterogeneity within the CD8^{+} T-cell exhaustion lineage and identified PD-1^{lo} precursor exhausted cells as the primary responders to anti–PD-1/PD-L1 ICB.

Reinvigoration of Exhausted CD8^{+} T Cells: Insights from Preclinical Cancer Models

It was recognized early on that CD8^{+} tumor-infiltrating lymphocytes (TIL) exhibit features of exhaustion (6, 36, 37). Thus, whether the paradigm of chronic antigen exposure–driven exhaustion derived from chronic viral infection models directly relates to the exhaustion observed in cancers has long been a topic of debate. In this regard, recent work has demonstrated that, despite disease-specific differences, CD8^{+} TILs share a common transcriptional program with

$CD8^+$ T cells in chronic infection (38). Progenitor exhausted $CD8^+$ TILs, defined as TCF-1^+SLAMF6^+Tim-3^-, are observed in multiple tumor models and share functional similarities with their counterparts induced by LCMV Clone 13 infection (38). These progenitor cells possess stem-like features, including long-term persistence and spontaneous differentiation into terminally exhausted TILs (TCF-1^-SLAMF6^-Tim-3^+). Notably, these stem-like progenitors were shown to respond to ICB by rapid expansion, leading to increased effector cells and enhanced cytotoxic activity. A meta-analysis of $CD8^+$ T-cell epigenomic footprints obtained from 12 independent studies comparing viral and bacterial infection alongside cancer models supports the hypothesis that phenotypic similarities between progenitor and terminally exhausted $CD8^+$ T cells in cancer and viral infection are governed by common molecular processes (39). In this work, Pritykin and colleagues compared Assay for Transposase-Accessible Chromatin using sequencing (ATAC-seq) profiles obtained from 166 biological samples comprising a wide range of $CD8^+$ T-cell states including naive, effector, exhausted, memory, and tissue-resident memory. An integrated analysis of these ATAC-seq signatures shows that dysfunctional $CD8^+$ T-cell states cluster together. Furthermore, assessing enriched transcription factor–binding sites identified a core set of exhaustion-associated transcription factor motifs including NFAT, AP-1, Myb, NR4A, and NF-κB (39).

Several studies have identified 'transitory' states between stem-like progenitors and terminally exhausted $CD8^+$ T cells. These cell states exhibit various degrees of susceptibility to ICB treatment and possess intrinsically different effector capacities (22, 40, 41). During Clone 13 infection, TCF-1^+ stem-like precursors transit through an effector-like CD101$^-$Tim-3^+ state before differentiating into CD101$^+$Tim-3^+ terminally exhausted $CD8^+$ T cells (22). Beltra and colleagues revealed further granularity in the composition of the exhausted $CD8^+$ T-cell pool in chronic viral infections and tumors, extending this developmental scheme into four distinct cell states (**Fig. 2**; ref. 23). They characterized stem-like PD-1^{lo}TCF-1^+CD8^+ T cells as two interconverting populations (progenitor 1, SLAMF6^+CD69^+; and progenitor 2, SLAMF6^+CD69^-) giving rise to a single intermediate (SLAMF6^-CD69^-) state that ultimately undergoes TOX-orchestrated conversion to a terminally differentiated state (SLAMF6^-CD69^+). This more granular appreciation of the stem-like/effector/exhaustion $CD8^+$ T-cell differentiation hierarchy paved the way to understanding how these distinct cell states contribute to immunity in chronic disease. In the four-cell subset scheme described by Beltra and colleagues (23), the progenitor 2 and intermediate populations respond to anti–PD-1/PD-L1 ICB treatment, as shown by their preferential accumulation following anti–PD-L1 treatment.

Similarly, the transitory CD101$^+$Tim-3^- population observed in Clone 13 infection expands following anti–PD-1/PD-L1 ICB treatment (22). This population resembles *bona fide* KLRG-1^+ effector $CD8^+$ T cells induced by acute LCMV Armstrong infection coexpressing Ki-67, granzyme B, S1PR1, and CX3CR1 and exhibits both proliferative and cytolytic functions (22). These recent findings cast a new paradigm in which anti–PD-1/PD-L1 ICB induces the proliferation of stem-like progenitors that differentiate into cytotoxic effectors before exhausting in the presence of chronic antigen.

Of note, the discussed findings are primarily focused on the molecular and cellular mechanisms of action of anti–PD-1/PD-L1 ICB. Extrapolation to other immunotherapeutic agents (including ICB) or combinations, therefore, should be carefully considered. For example, high-dimensional profiling of murine and human tumors treated with anti–PD-1 or anti–CTLA-4 reveal distinct cellular mechanisms induced by these individual ICB agents (42). Although both antibodies were able to induce substantial expansion of distinct exhausted-like $CD8^+$ T cell populations, only anti–CTLA-4 treatment could numerically enhance a population of $ICOS^+$ Th1-like $CD4^+$

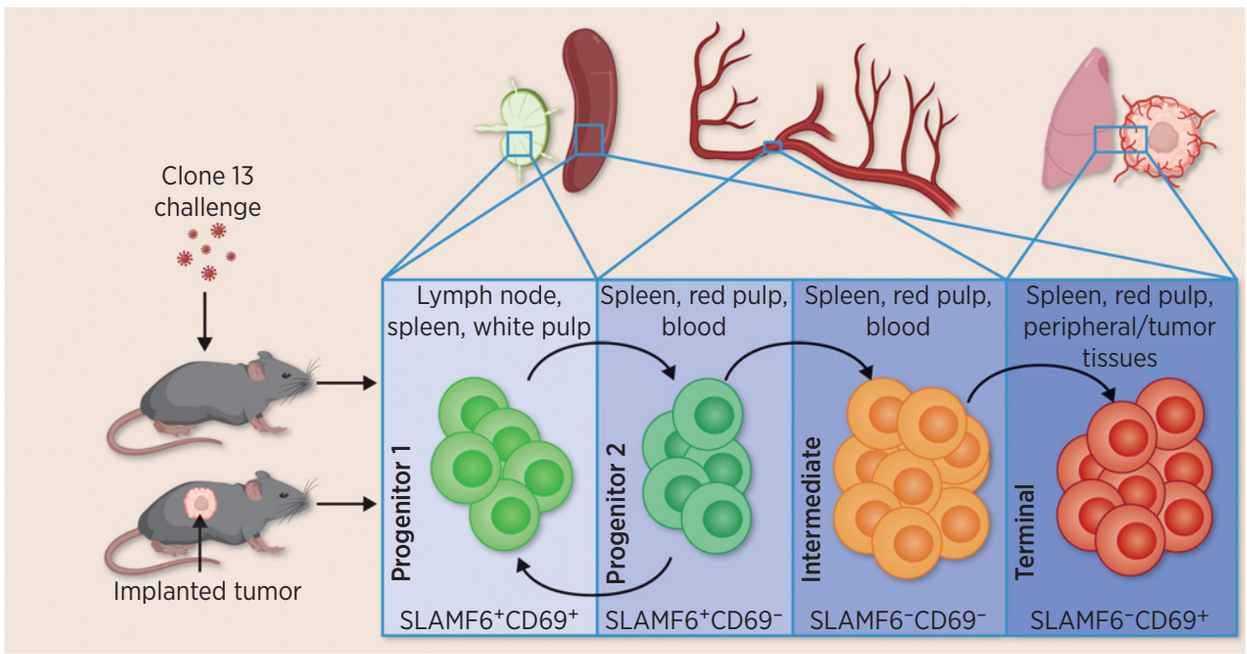

Figure 2.
Heterogeneity of $CD8^+$ T-cell states in the context of chronic viral infections and tumors. The pool of $CD8^+$ T cells induced by chronic viral infection or tumorigenesis is composed of cells in distinct cellular states. These cells therefore exhibit different phenotypic, functional, differentiation, and tissue residence properties.

T cells. Although the functional importance of individual populations is not fully clear, their potential contribution to tumor control cannot be ruled out. Finally, treatment based on the combination of anti–PD-1 and anti–CTLA-4 exhibits synergistic activity in both murine tumors and peripheral blood of patients with melanoma, as demonstrated by induction of partially distinct cellular mechanisms (43).

The Site of ICB Activity: Tumor versus Lymph Nodes

Several studies support the notion that secondary lymphoid tissues, such as lymph nodes (LN), are important sites of action for anti–PD-1/PD-L1 ICB (**Fig. 3**; refs. 44–47). In one of these studies, immuno-PET was used to track $CD8^+$ T-cell kinetics following anti–PD-1 treatment in syngeneic mouse colorectal MC38 tumors to characterize features that discriminate resistance from response to the anti–PD-1 (44). The authors found that robust accumulation of $CD8^+$ T cells within the TME following anti–PD-1 was required for a therapeutic response. Evidence that these $CD8^+$ T-cell populations were mobilized from peripheral lymphoid tissues was provided by the observation that the S1P receptor antagonist FTY720 prevented the accumulation of intratumoral $CD8^+$ T cells following anti–PD-1 ICB, leading to enhanced tumor growth (44). Additional studies support the involvement of tumor-draining LNs (tdLN) in response to anti–PD-1/PD-L1 ICB. In one study, Fransen and colleagues investigated $CD8^+$ T-cell responses in mice bearing syngeneic murine colon cancers (MC38 and CT26) treated with anti–PD-1/PD-L1 ICB and found a substantial increase in the frequencies of $CD8^+$ T cells within tumor-draining rather than nondraining LNs (45). Surgical removal of the tumor-draining but not nondraining LNs compromised the efficacy of anti–PD-1/PD-L1 ICB. Direct evidence of a requirement for anti–PD-1 action within tdLNs was provided by a study using the AC29 mesothelioma model that tdLNs contain a high abundance of tumor-specific stem-like PD-$1^{lo}CD8^+$ T cells (**Fig. 3**; ref. 46). Taking advantage of the pleural cavity lymphatic drainage in the AC29 model, the authors used low dose intrapleural anti–PD-1 treatment to show that PD-1/PD-L1 interactions within the LN are crucial for effective mobilization of $CD8^+$ T cells following anti–PD-1/PD-L1 ICB. Thus, $CD8^+$ T-cell priming within the tdLNs is an essential component of the response to ICB (**Fig. 3**).

Relating these findings to the discussion of heterogeneity within the T-cell exhaustion lineage, Beltra and colleagues have shown distinct tissue distribution profiles of the four exhaustion-lineage cell states discussed above (**Fig. 2**; ref. 23). The least differentiated progenitor 1 population ($SLAMF6^+CD69^+$) is quiescent and resident within the splenic white pulp. In contrast, the anti–PD-1 responsive progenitor 2 ($SLAMF6^+CD69^-$) and intermediate ($SLAMF6^-CD69^-$) populations are associated with egress from lymphoid tissues and occupation of the blood compartment (23). Terminally exhausted cells ($SLAMF6^-CD69^+$) are associated with widespread tissue distribution, not only in the lungs and liver, but also in highly vascularized splenic red pulp after Clone 13 infection. Thus, exhaustion lineage cell subpopulations that respond to anti–PD-1/PD-L1 ICB are predominantly located within the blood and lymphoid tissue. However, it should be noted that TCF-1^+CD8^+ progenitor exhausted cells are observed within the TME. The frequency of these cells is a favorable

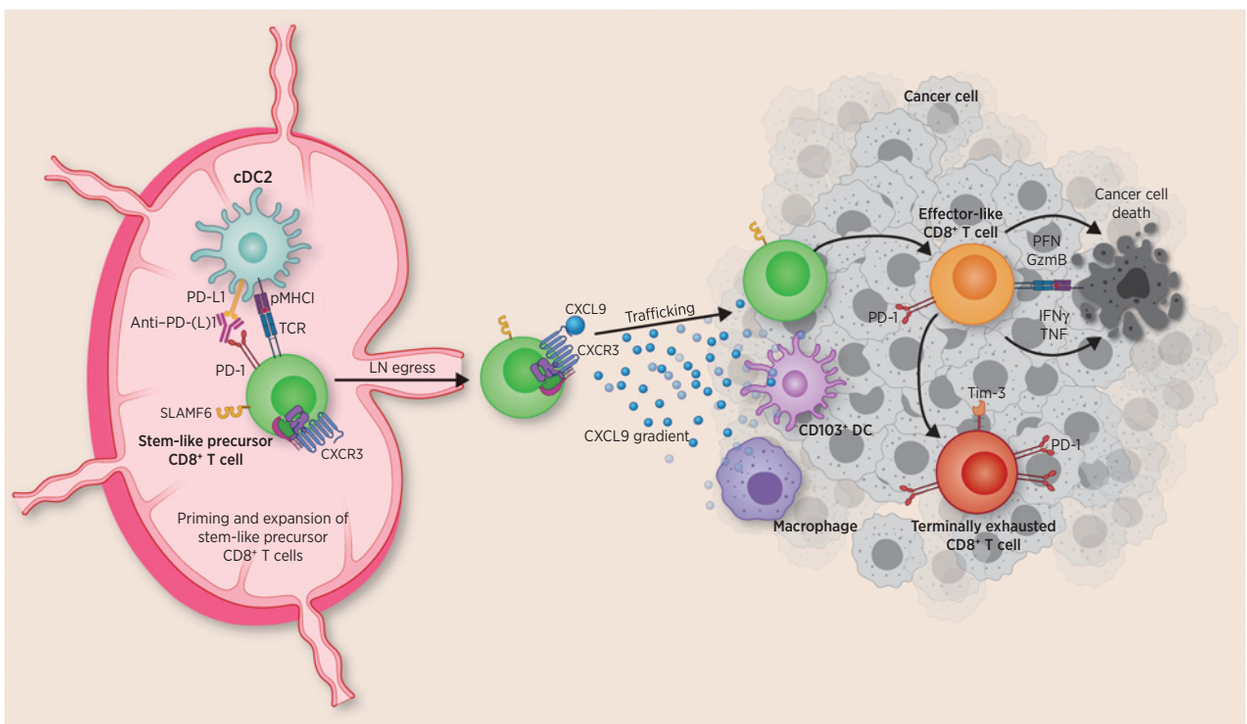

Figure 3.

tdLNs and the CXCR3–CXCL9 chemokine axis are key mediators of anti–PD-1/PD-L1 ICB activity. ICB enables *de novo* priming of stem-like precursor exhausted $CD8^+$ T cells in the tdLN. Primed $CXCR3^+CD8^+$ T cells traffic to the tumor following the CXCL9 gradient established by tumor-resident macrophages and $CD103^+$ DCs. Within the tumor, stem-like precursors undergo further differentiation to a transient effector-like state before finally becoming terminally exhausted $CD8^+$ T cells.

prognostic indicator for response to anti–PD-1/PD-L1 ICB (48, 49), highlighting the potential that these agents may act on progenitor-exhausted cells within the TME.

Thus, recent evidence points toward tdLNs as the essential players in response to ICB by enabling *de novo* priming of tumor-specific stem-like $CD8^+$ T-cell responses. This supports a new concept of ICB not (only) acting locally within the TME but preferentially mobilizing cells from niches outside tumors. It also poses an important question: which mechanism(s) are involved in the trafficking of tdLN-primed $CD8^+$ T cells to the TME?

Trafficking and Positioning of Stem-like $CD8^+$ T Cells within the TME

Trafficking of $CD8^+$ T cells within the lymphoid compartment and to inflamed peripheral tissues is a complex, highly orchestrated process enabled by sets of context-dependent chemokines and chemokine receptors (50, 51). Several studies have identified a critical role for CXCR3 in $CD8^+$ T-cell trafficking to the TME and anti–PD-1/PD-L1 ICB efficacy (29, 51–55). For example, interactions between CXCR3 and CXCL9 are critical for the trafficking of adoptively transferred $CD8^+$ T cells into murine B16 melanoma by enabling intravascular adhesion and extravasation (52). In this study, deletion of CXCR3 in transferred $CD8^+$ T cells was shown to impair accumulation within the TME and prevent control of tumor growth (52). Spranger and colleagues also identified a role for the CXCR3 axis in tumor control (53). Immunization with a model antigen could induce substantial growth inhibition only of inflamed and not noninflamed antigen-expressing murine melanoma tumors (53). Investigation of mechanisms of tumor rejection revealed that noninflamed tumors are associated with defective migration of antigen-specific $CD8^+$ T cells and an inability of the infiltrate to effectively clear tumors. The authors determined that the CXCR3 ligands, CXCL9 and CXCL10, are critical mediators of intratumoral $CD8^+$ T-cell trafficking and further showed intratumoral *Batf3*-dependent DCs are a significant source of these chemokines (**Fig. 3**; ref. 53).

Our group's recent publication reported on underlying mechanisms of the response to anti–PD-L1 treatment using a predictive bilateral tumor model coupled with single-cell RNA sequencing (scRNA-seq; refs. 29, 56). This analysis showed that the baseline presence of an $F4/80^+MHCII^+Ly6C^{lo}$ tumor-associated macrophage population could predict response to anti–PD-L1 (avelumab). Phenotypic characterization of this subset revealed an inflammatory IFNγ gene response signature characterized by abundant CXCL9 expression (56). In addition, we observed that CXCL9 or CXCR3 blockade could abrogate the efficacy of avelumab treatment. Thus, interactions between CXCR3 and its ligands regulate the trafficking of $CD8^+$ T cells in response to anti–PD-1/PD-L1 ICB. This finding is supported by a meta-analysis of clinical responses to anti–PD-1/PD-L1 ICB showing that intratumoral CXCL9 expression is one of the few universal predictors of response to therapy and correlates tightly with *Cd8a* mRNA levels (57).

Whether the CXCR3–CXCR3 ligand axis solely is the sole chemokine axis supporting intratumoral $CD8^+$ T-cell trafficking in response to anti–PD-1/PD-L1 ICB treatment is an open question. Using *Cxcr3*-deficient mice, Chow and colleagues demonstrated that the CXCR3–CXCL9 axis represents a limiting factor of anti–PD-1 efficacy in the murine MC38 model (54). In this study, mechanistic analyses revealed that CXCR3-expressing $CD8^+$ T cells (many of which possess a progenitor exhausted phenotype) localize within $CXCL9^+$ tumor niches. Furthermore, CXCL9 was found to be frequently expressed by $CD103^+$ DCs (**Fig. 3**), leading the authors to conclude that CXCL9-mediated positioning of $CXCR3^+$ stem-like $CD8^+$ T cells within the tumor microenvironment is required for optimal antitumor activity.

This hypothesis is consistent with observations in various human tumors, such as kidney, prostate, and bladder cancer, where stem-like $CD8^+$ T cells preferentially reside in microniches rich in $MHCII^+$ antigen-presenting cells (APC) (58). The intratumoral presence of such niches strongly correlates with the response to anti–PD-1/PD-L1 ICB, and their absence is associated with disease progression. The authors of this publication proposed that interactions with APCs facilitate the differentiation of stem-like precursors and the acquisition of effector-like functions (58). The functional relevance of this intratumoral compartmentalization is still not fully clear. Although the lack of longitudinal data in this study poses some interpretational restrictions that may be addressed in the future, these APC-rich microniches appear to be important for providing as yet undefined cues required for the survival and persistence of stem-like $CD8^+$ T cells within the TME.

In addition to the CXCR3–CXCL9 axis, CCL5–CCR5 interactions seem to play an important role in the initial recruitment of $CD8^+$ T cells and strongly correlate with response to anti–PD-1/PD-L1 ICB (59). Through enabling initial infiltration of $CCR5^+CD8^+$ T cells, CCL5-expressing tumor cells trigger a cascade of events ultimately leading to the production of IFNγ by tumor-reactive $CD8^+$ T cells upon their encounter with antigen within the TME. This initial surge of IFNγ further amplifies CXCL9 production and consequently drives recruitment and intratumoral positioning of $CXCR3^+CD8^+$ T cells (59).

It has been suggested that CXCR3 ligands may directly promote $CD8^+$ T-cell maturation toward an effector-like state (60). In support of this hypothesis, a stimulatory effect of CXCL11 (the third known CXCR3 ligand) on $CXCR3^+$ stem-like $CD8^+$ T cells has been reported in muscle-invasive bladder cancer (55). In this study, *in vitro* exposure of CXCR3-expressing stem-like $CD8^+$ T cells to CXCL11 was found to induce effector-like properties and enhance migration in culture (55). CXCR3 biology, therefore, seems to play a multifaceted role in shaping the response to anti–PD-1/PD-L1 ICB.

As discussed in this review, there is substantial evidence supporting a critical role for the CXCR3 axis in intratumoral trafficking of anti–PD-1/PD-L1 ICB–induced stem-like cells and possibly their persistence within the TME. However, the signals that shape the same properties of other $CD8^+$ T-cell differentiation states (e.g., effector-like cells) are less well understood. Given the heterogeneity of the populations forming the intratumoral $CD8^+$ T-cell pool and the complexity of developmental connections between them, it is crucial to better understand cues defining the basic biology of these different cell types. A study by Di Pilato and colleagues represents one of the first steps toward addressing this critical issue (61). Combining the D4M.3A-OVA mouse melanoma model with genetic approaches and multiphoton intravital imaging, the authors demonstrated that, similar to stem-like $CD8^+$ T cells, effector-like $CD8^+$ T cells occupy distinct niches within the TME. In contrast to their stem-like counterparts, whose intratumoral positioning seems to be mainly controlled by the CXCR3–CXCL9 axis, effector-like $CD8^+$ T-cell accumulation within perivascular niches of the tumor is driven by their expression of CXCR6 and its interaction with CXCL16 expressed by a subset of $CCR7^+$ DCs. This localized interaction of $CXCR6^+$ effector-like $CD8^+$ T cells with CXCL16-expressing DCs supports their survival and proliferation through receiving IL15 signals presented *in trans* by the DCs.

In aggregate, these data show that anti–PD-1/PD-L1 ICB elicits stem-like $CD8^+$ T-cell expansion and mobilization within the tdLNs. It also emphasizes a nonredundant role of the CXCR3–CXCL9/CXCL10 axis in the trafficking of stem-like cells into CXCR3 ligand–expressing microniches within the TME. However, the precise role of peripheral LN and intratumoral niches in coordinating antitumor immunity remains to be determined. Do CXCR3-expressing progenitor exhausted $CD8^+$ T cells solely originate in or also persist within these microniches? Is the migration of progenitor exhausted $CD8^+$ T cells between peripheral and intratumoral niches unidirectional? How do interactions between CXCL9/CXCL10-expressing APCs and $CXCR3^+CD8^+$ T cells within the TME shape the quality and persistence of the antitumor immune response? Finally, do different $CD8^+$ T-cell states (e.g., stem-like versus effector-like) occupy distinct microniches within the TME specialized in supporting their individual developmental and persistence requirements? Also, what is the role of complex chemokine networks in orchestrating this compartmentalization? These are just some of the critical knowledge gaps remaining to be addressed to advance our understanding of the mechanism and site of action of anti–PD-1/PD-L1 ICB.

Lessons Learned from the Clinic

Here, we have reviewed new findings derived from preclinical models that have reshaped our understanding of anti–PD-1/PD-L1 ICB mechanisms of action. Understanding whether these findings are recapitulated in human tumors has long been of interest. Single-cell technologies pairing transcriptomics with TCR clonotype analysis have enabled researchers to probe the heterogeneity and developmental connections between $CD8^+$ TIL populations, assess how this heterogeneity changes in response to anti–PD-1/PD-L1 ICB treatment, and gain insights into the anatomical partitioning of the human anti–PD-1–responsive $CD8^+$ T-cell subsets.

Stem-like progenitor ($TCF\text{-}7^+$, $TCF\text{-}1^+$ in a murine system) and exhausted progeny ($TCF\text{-}7^-$) $CD8^+$ T cells have been observed in multiple human tumor types, including melanoma, non–small cell lung carcinoma (NSCLC), and kidney cancer (38, 41, 58). Using high-dimensional single-cell analysis of $CD8^+$ T cells derived from treatment-naive NSCLC samples, Brummelman and colleagues identified a population of stem-like, cytolytic $CXCR5^+Tim\text{-}3^-CD8^+$ T cells alongside both exhausted and activated T-cell subsets (62). Similar to stem-like $CXCR5^+$ cells observed in the Clone 13 infection model (33), these cells exhibit enhanced homeostatic proliferation and polyfunctionality compared with their terminally differentiated counterparts, and their abundance negatively correlates with disease progression (62).

Given the overall similarities between the differentiation hierarchy of TILs in preclinical models and human tumors, it is essential to understand whether similar $CD8^+$ T-cell populations are involved in the actual response to anti–PD-1/PD-L1 ICB treatment. In this respect, it has been shown that tumor profiles associated with favorable response to anti–PD-1/PD-L1 ICB in multiple indications are enriched for gene signatures associated with stem- or memory-like precursor exhausted TILs (49, 58). Furthermore, in patients with melanoma, a high abundance of stem-like progenitor exhausted $CD8^+$ TILs is associated with the efficacy of anti–PD-1 treatment (38). Specifically, the frequency of these cells correlates with the duration of the response in the responder patient population. Moreover, $TCF\text{-}7^+CD8^+$ T-cell frequencies, rather than total $CD8^+$ T cells, are associated with progression-free and overall patient survival (38).

In another study, Sade-Feldman and colleagues profiled biopsies of metastatic melanoma tumors from patients treated with anti–PD-1/PD-L1 ICB (49). scRNA-seq phenotyping of $CD8^+$ T cells identified two major $CD8^+$ T-cell clusters associated with response. The CD8_B "bad" cluster possessed a terminal exhaustion transcriptional signature, whereas the CD8_G "good" cluster that correlated with a favorable response had a transcriptional signature characterized by the expression of the TCF-7 transcription factor associated with stem-like $CD8^+$ T-cell states. Here, the $TCF\text{-}7^+CD8^+$ T cells detected in melanoma tumor samples were also predictive of a beneficial response to anti–PD-1/PD-L1 ICB treatment (49). To evaluate the clonal evolution of the $CD8^+$ T-cell pool in response to treatment, the authors performed TCR clonotyping of $CD8^+$ T cells derived from matched pre- and posttreatment samples. Identical TCR clonotypes were detected in both memory-like and exhausted $CD8^+$ T cells, strongly suggesting a developmental connection and transition between the two cell states. However, there was minimal overlap of $CD8^+$ T-cell TCRs between pre- and posttreatment samples, suggesting that anti–PD-1/PD-L1 ICB–induced $CD8^+$ T-cell clones did not originate from the TME but rather from outside the tumor (e.g., tdLN). Furthermore, the intratumoral stem-like $CD8^+$ T-cell clonotype turnover observed in this study suggests that this population has a limited capacity to persist in response to anti–PD-1/PD-L1 ICB (49). Similar observations were reported in a different study evaluating the effect of anti–PD-1 treatment in patients with basal or squamous cell carcinoma (63). Combined transcriptional phenotyping and TCR clonotyping of samples isolated pre– and post–anti–PD-1/PD-L1 ICB allowed Yost and colleagues to follow treatment-induced numerical and functional changes of T-cell clones (63). The study's key finding was that TCR clones present in the tumor before anti–PD-1/PD-L1 ICB treatment were not enriched in posttreatment samples. The posttreatment expanded clones were not solely derived from preexisting $TCF\text{-}7^+$ TILs, but mostly from novel clonotypes originating outside the TME. This phenomenon referred to as "clonal replacement" has been attributed to the limited capacity of preexisting $CD8^+$ TILs to respond to anti–PD-1/PD-L1 ICB. In addition, a comparison of TCR clonotypes derived from tumor tissue and blood revealed that a substantial proportion of the new clones originated from the circulation. Moreover, a substantial fraction of these clonotypes were found even in the pretreatment blood samples (63).

The idea that anti–PD-1/PD-L1 ICB–induced T-cell clones originate from the periphery is further strengthened by observations reported by Wu and colleagues (64). Paired scRNA-seq and TCR clonotyping were used to assess the clonal properties of T cells isolated from lung, endometrial, colorectal, and renal tumors; healthy adjacent tissue; and the blood of patients treated with anti–PD-L1. An expansion of effector-like $CD8^+$ T-cell clones associated with response to anti–PD-1/PD-L1 ICB treatment was observed in both tumor and healthy adjacent tissue. The authors confirmed that these expanded tumor- and healthy adjacent tissue–associated clonotypes were present in the blood, further emphasizing the importance of the peripheral blood compartment for anti–PD-1/PD-L1 ICB activity and efficacy. A similar approach was used to profile the differentiation status and TCR clonality of $CD8^+$ T cells isolated from untreated tumors, healthy adjacent tissue, and blood of a cohort of patients with NSCLC (65). In addition to tissue-resident precursors (CD8-XCR1), the intratumoral memory-like precursor $CD8^+$ T-cell pool contained a sizeable population of circulating precursors originating from the blood (CD8-GZMK/CD8-KLF2), suggesting a dual origin of $CD8^+$ TILs. Both precursor states progressed through a common GZMH-expressing transitional

state characterized by substantial clonal expansion before transitioning into terminally differentiated cells (CD8-LAYN; ref. 65).

These findings thus offer a view in which anti–PD-1/PD-L1 ICB activity critically depends on communication between the tumor, blood, and lymphoid immune compartments. This concept suggests that liquid biopsy samples (e.g., blood) can potentially enable the identification of clinical biomarkers, facilitate early prediction of anti–PD-1/PD-L1 ICB treatment response and improve stratification of patient populations, all of which remain significant clinical challenges. The translational value of monitoring peripheral blood samples to assess immunotherapy response has recently been shown (66, 67). Two independent studies showed that anti–PD-1/PD-L1 ICB induced immunomodulation of the $CD8^+$ T-cell pool within the blood of patients with responding, but not nonresponding, metastatic melanoma. The presence of expanded clones of effector-memory $CD8^+$ T cells 3 weeks after the treatment was identified as a robust prognostic marker.

Final Remarks

The findings from clinical studies support the paradigm derived from preclinical models. Anti–PD-1/PD-L1 ICB activity is not restricted to the TME and instead depends upon the orchestration of systemic and localized intratumoral immune responses. This mechanistic understanding, which was derived from chronic viral infection and tumor models, provides a new hypothesis-driven framework for developing novel cancer immunotherapies. However, essential questions remain to be addressed. Can a population of progenitor exhausted $CD8^+$ T cells persist long-term in response to anti–PD-1/PD-L1 ICB? Given the treatment-induced turnover of intratumoral TCF-7^+ cells, it is important to understand whether the same anti–PD-1/PD-L1 ICB–induced clonotypes persist throughout therapy. Moreover, what additional factors and pathways govern the self-renewal of progenitor exhausted cells and their differentiation into functional, antitumor effector cells? What factors expressed by tumors of anti–PD-1/PD-L1 ICB–nonresponsive patients repress the differentiation of stem-like $CD8^+$ T cells to effector cells or accelerate their terminal exhaustion, and do these provide therapeutic opportunities? Given the role of the tdLN in progenitor exhausted $CD8^+$ T-cell development, are tumor-targeted treatment approaches warranted, and what opportunities exist for DC agonist therapies?

The main challenge moving forward will be to convert this new understanding of how PD-1/PD-L1 antagonists mediate their therapeutic effects to identify additional rate-limiting steps in the antitumor immune response. Drugs designed to reinvigorate and support antitumor immunity in anti–PD-1/PD-L1 ICB nonresponsive patients hold great therapeutic potential.

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