BASIC AND CLINICAL CANCER REPORTS | Volume 1, Issue 2 | September 2026

T Cell Exhaustion in Cancer: From Progenitor to Therapeutic States

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Abstract

T-cell exhaustion is a specialized state of T-cell differentiation that arises during persistent antigen exposure and chronic inflammation and represents a major barrier to effective antitumor immunity. Rather than being a homogeneous population, exhausted CD8+ T cells comprise distinct functional and transcriptional states with different capacities for self-renewal, proliferation, cytotoxicity, and response to immunotherapy. Current models describe a developmental continuum from progenitor exhausted T cells (Tpex), through intermediate exhausted states (Texint), to terminally exhausted T cells (Texterm). Tpex cells, typically characterized by TCF1 expression and a stem-like phenotype, retain proliferative capacity and serve as a renewable source of differentiated exhausted cells. In contrast, Texterm cells display high levels of inhibitory receptors, severe functional impairment, and limited proliferative potential. The transcription factors TCF1 and TOX are key regulators of this trajectory. TCF1 promotes the maintenance and expansion of stem-like Tpex cells and is strongly associated with responses to checkpoint blockade, whereas TOX contributes to the establishment and stabilization of exhaustion-associated transcriptional and epigenetic programs. Importantly, TOX and TCF1 function within a complex regulatory network rather than as simple antagonists. Therapeutically, PD-1 blockade primarily expands Tpex populations, which subsequently generate differentiated progeny capable of mediating tumor control. Similarly, CAR-T efficacy is influenced by the differentiation state and exhaustion susceptibility of infused cells. Targeting Tpex preservation, epigenetic regulation, CAR signaling, and the tumor microenvironment may therefore improve the durability and efficacy of cancer immunotherapy.

Keywords: T-cell exhaustion; Tpex; Texint; Texterm; TCF1; TOX; PD-1; PD-L1; immune checkpoint blockade; CAR-T; cancer immunotherapy.

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Introduction

The immune system has the capacity to recognize and eliminate malignant cells, but established tumors frequently develop mechanisms that suppress or evade antitumor immunity. Among the most important mechanisms is the progressive dysfunction of tumor-reactive T cells exposed to persistent antigen, chronic stimulation, immunosuppressive cytokines, metabolic stress, and inhibitory receptor signaling. This state, generally referred to as T-cell exhaustion, was initially characterized in chronic viral infection and subsequently recognized as a central feature of the tumor microenvironment (TME) [1,2].

T-cell exhaustion should not be regarded simply as an irreversible failure of immunity. Rather, it represents a distinct differentiation state that develops through sustained antigenic stimulation and involves coordinated transcriptional, epigenetic, metabolic, and functional remodeling [1-4]. Exhausted CD8+ T cells commonly express multiple inhibitory receptors, including PD-1, TIM-3, LAG-3, TIGIT, and CD39, while displaying reduced proliferative capacity, altered cytokine production, impaired cytotoxic activity, and profound changes in chromatin accessibility [2,5]. However, the simultaneous expression of inhibitory receptors is not sufficient to define a single homogeneous exhausted population. Tumor-reactive T cells exist along a continuum of differentiation, and individual populations differ substantially in their ability to proliferate, persist, differentiate, and respond to therapeutic intervention.

The recognition of this heterogeneity has fundamentally changed the interpretation of immune checkpoint blockade. Earlier models suggested that PD-1 blockade primarily functioned by directly restoring the activity of dysfunctional terminally exhausted T cells. Increasing evidence instead indicates that a major component of the response originates from a stem-like or progenitor population of exhausted T cells. These cells retain proliferative potential, self-renewal capacity, and the ability to generate more differentiated exhausted progeny [6-9]. Their importance is particularly evident following PD-1 or PD-L1 blockade, during which progenitor-like populations undergo expansion and differentiation, producing effector-like descendants that contribute to tumor control [6,7].

This concept has led to the development of the Tpex–Texint–Texterm model. Tpex cells represent the progenitor or stem-like compartment, typically characterized by TCF1 expression, whereas Texterm cells represent highly differentiated exhausted populations with reduced proliferative potential and more extensive functional impairment. Between these states lies an intermediate compartment that displays features of both progenitor and terminal exhaustion and may retain considerable plasticity [8-10]. Although this three-state model provides a useful framework, it should not be interpreted as an absolutely linear pathway in every tumor type. The tumor microenvironment, antigen load, tissue localization, inflammatory signals, and therapeutic intervention can modify the trajectory and phenotype of these populations **Figure1**.

At the molecular level, TCF1 and TOX have attracted particular attention. TCF1 is strongly associated with the stem-like properties of progenitor exhausted T cells and is required for maintaining a population capable of sustained immune responses during chronic antigen exposure [8,11]. TOX, in contrast, is a central regulator of the exhaustion-associated transcriptional and epigenetic program and allows T cells to adapt to persistent stimulation [12-15]. Importantly, TOX is not merely a detrimental factor. Its activity can facilitate T-cell persistence under chronic stimulation, indicating that exhaustion contains both maladaptive and adaptive components [13-15].

These concepts are particularly relevant to modern cancer immunotherapy. PD-1/PD-L1 blockade, adoptive T-cell therapy, and CAR-T therapy all depend on the ability of T cells to remain sufficiently functional and persistent within an antigen-rich and immunosuppressive environment. Understanding which T-cell states can respond to therapy, which populations are capable of long-term persistence, and which transcriptional programs impose terminal dysfunction may therefore provide a basis for improving therapeutic efficacy. This review summarizes the developmental continuum of exhausted CD8+ T cells, examines the roles of TCF1 and TOX, and discusses the implications of T-cell exhaustion for checkpoint blockade and CAR-T-cell therapy.

The Developmental Continuum of T-Cell Exhaustion

The classical view of T-cell exhaustion emphasized a progressive loss of effector function caused by chronic antigen stimulation. In this model, continuous T-cell receptor (TCR) engagement induces inhibitory receptors and gradually reduces proliferation, cytokine production, and cytotoxicity [1,2]. However, studies using chronic viral infection and tumor models have demonstrated that exhausted T cells are heterogeneous and contain populations with markedly different functional capacities [6,7,16].

One of the most important populations is the progenitor exhausted or Tpex compartment. Tpex cells typically express TCF1 and PD-1 and can display markers such as CXCR5, SLAMF6, and other features associated with stem-like or memory-like biology [8,11,17]. Unlike terminally exhausted cells, they retain substantial proliferative potential and can self-renew. They also maintain the capacity to differentiate into more effector-like exhausted descendants. In tumors, Tpex cells may be enriched in particular anatomical niches, including tumor-draining lymph nodes and specialized intratumoral microenvironments that provide signals supporting their maintenance [18,19].

The importance of Tpex cells was demonstrated particularly clearly by studies of PD-1 blockade. Miller and colleagues showed that exhausted CD8+ tumor-infiltrating lymphocytes contain a progenitor population capable of long-term persistence and differentiation into terminally exhausted cells [6]. Importantly, progenitor exhausted cells responded to anti-PD-1 treatment much more effectively than terminally exhausted cells. In melanoma, a higher proportion of progenitor exhausted cells was associated with a longer duration of response to checkpoint blockade [6]. These findings established that the exhausted T-cell compartment contains a therapeutically responsive reservoir rather than representing a uniformly dysfunctional population .

Tpex cells nevertheless differ from conventional memory T cells. They are continuously exposed to tumor-associated antigen and generally express inhibitory receptors such as PD-1. Their transcriptional and epigenetic programs are distinct from those of classical memory cells [8,20]. Consequently, the term "stem-like exhausted" is useful because it reflects their capacity for self-renewal and differentiation without implying that these cells are equivalent to conventional memory cells.

As Tpex cells encounter persistent antigen and environmental signals, they can transition toward intermediate exhausted states. Texint cells occupy a biologically important position between the progenitor and terminal compartments. They generally show reduced stem-like characteristics compared with Tpex cells while retaining greater functional and proliferative capacity than fully terminally exhausted cells. Their precise phenotype varies between models and studies, but intermediate states frequently show changes in transcription factors, inhibitory receptor expression, metabolic programs, and cytotoxic machinery [3,9,10].

The intermediate compartment is particularly important because it illustrates that T-cell exhaustion is better understood as a dynamic differentiation continuum than as a binary exhausted/non-exhausted state. Some intermediate cells may progress toward terminal exhaustion, whereas others may maintain cytotoxic potential or respond to therapeutic signals depending on the tumor microenvironment. The transition is influenced by the duration and intensity of antigen exposure, inflammatory cytokines, metabolic availability, co-stimulatory signals, and transcriptional regulators.

At the terminal end of the continuum are Texterm cells. These cells generally exhibit high expression of inhibitory receptors and exhaustion-associated genes, together with reduced proliferative potential and diminished capacity to generate durable progeny [6,9]. Terminally exhausted cells can retain considerable cytotoxic activity in some contexts, and therefore "terminal" should not be interpreted as completely inactive. Indeed, some Texterm cells may contribute directly to short-term tumor cell killing. Their principal limitation is their restricted capacity for long-term self-renewal and therapeutic reinvigoration.

The distinction between Tpex and Texterm is therefore clinically important. A tumor containing a large population of highly differentiated exhausted cells may still display abundant PD-1-positive T cells but respond poorly to PD-1 blockade if the reservoir of responsive progenitor cells is limited. Conversely, tumors containing a substantial Tpex compartment may possess greater capacity for therapeutic expansion following checkpoint inhibition [6,7].

Spatial organization further complicates this model. Tpex cells are not randomly distributed throughout tumors. Their maintenance may depend on specialized niches involving dendritic cells, lymphoid structures, chemokine gradients, and interactions with other immune populations [18,19]. The localization of these cells can therefore influence whether they receive the signals required for survival and expansion. Tumors lacking appropriate immune niches may fail to maintain an effective Tpex reservoir even when tumor-reactive T cells are present.

The developmental model also provides a useful explanation for why checkpoint blockade produces a proliferative burst rather than an unlimited restoration of T-cell function. Following PD-1 inhibition, Tpex cells can expand and differentiate, increasing the number of tumor-reactive effector-like cells. However, continued antigen exposure and the tumor microenvironment can subsequently drive these cells toward more differentiated exhausted states [6,7]. Thus, checkpoint blockade may partially reshape the exhausted compartment rather than completely erase exhaustion.

An additional layer of complexity is the relationship between Tpex cells and T-cell receptor clonotypes. Tumor-reactive clones can be distributed across different differentiation states, and clonotype tracking suggests that progenitor populations can serve as sources of differentiated populations [6]. This observation supports a developmental hierarchy in which long-term tumor control depends not only on the number of tumor-reactive T cells but also on the availability of clonotypes capable of maintaining a renewable progenitor pool.

Overall, the Tpex–Texint–Texterm model provides a biologically meaningful framework for interpreting T-cell exhaustion. Tpex cells function as a renewable reservoir, Texint cells represent a transitional compartment with substantial plasticity, and Texterm cells represent highly differentiated states with limited proliferative capacity. The relative abundance and spatial distribution of these populations may be as important as the total number of CD8+ T cells within a tumor.

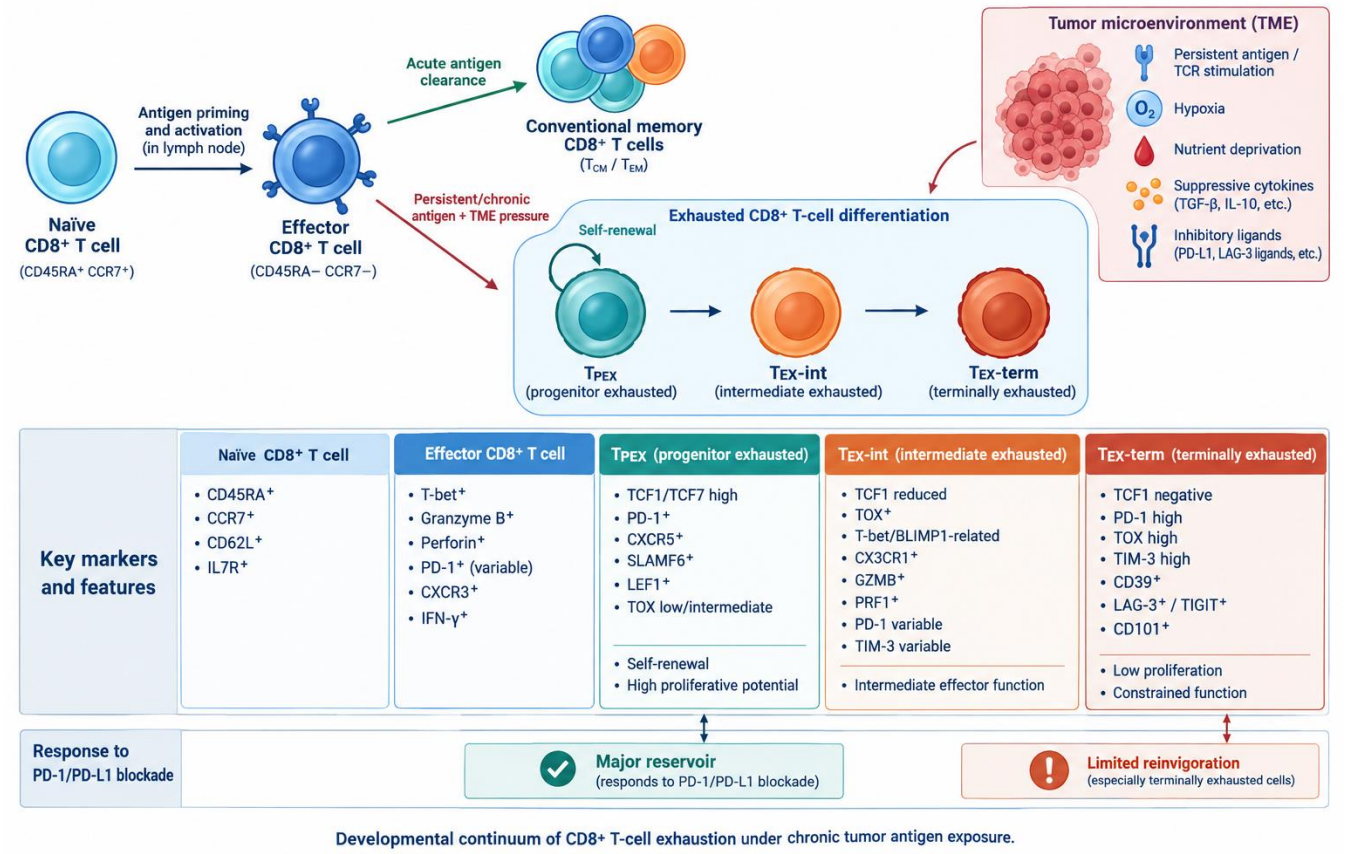

Figure1; *Origin of T cells exhausted and key markers*

Molecular Regulation of Exhaustion: TCF1, TOX, and the Epigenetic Landscape

The differentiation of exhausted T cells is governed by a complex network of transcription factors rather than by a single molecular switch. Among the most extensively studied regulators are TCF1 and TOX. These factors have complementary but interconnected roles in maintaining the exhausted T-cell state and determining the balance between self-renewal, differentiation, persistence, and effector function.

TCF1, encoded by TCF7, is a transcription factor strongly associated with stem-like CD8+ T cells during chronic infection and cancer. TCF1-positive cells retain characteristics associated with self-renewal, proliferation, and memory-like differentiation and are critical for sustaining long-term immune responses under conditions of persistent antigen exposure [8,11]. Utzschneider and colleagues demonstrated that TCF1-expressing CD8+ T cells sustain immune responses during chronic viral infection and are particularly important for the proliferative response to inhibitory receptor blockade [11]. Subsequent work extended these observations to cancer and established the relevance of TCF1-positive populations for tumor immunity and immunotherapy [17,18].

TCF1 should therefore be viewed as a marker and functional regulator of the progenitor compartment rather than simply a marker of conventional memory. TCF1-positive Tpex cells can simultaneously express inhibitory receptors and exhibit characteristics of exhaustion while maintaining a capacity for self-renewal. This apparent paradox reflects the unique biology of the progenitor exhausted state: these cells are sufficiently differentiated to adapt to chronic antigen exposure but retain sufficient developmental plasticity to generate new effector populations.

The absence or reduction of TCF1 is associated with progression toward more differentiated exhausted states. As Tpex cells differentiate, their transcriptional landscape changes, with increasing expression of genes associated with effector differentiation and terminal exhaustion. Thus, TCF1 helps preserve the cellular reservoir from which therapeutic responses can emerge.

TOX represents another central component of the exhaustion program. TOX is induced under conditions of persistent TCR stimulation and downstream calcium–calcineurin–NFAT signaling [12-15]. In contrast to acute activation, where T-cell stimulation is generally transient, chronic stimulation can maintain TOX expression and establish a stable transcriptional and epigenetic program characteristic of exhaustion [12]. Khan and colleagues demonstrated that TOX

is critical for the formation of exhausted CD8+ T cells and contributes to a distinct exhaustion-associated chromatin landscape [12].

The role of TOX is more nuanced than the simple interpretation that increased TOX equals impaired T-cell function. Several studies have shown that TOX can promote persistence of exhausted T cells under chronic antigen exposure [13-15]. Without an appropriate exhaustion-adaptation program, chronically stimulated T cells may undergo excessive effector differentiation, dysfunction, or deletion. TOX therefore appears to help T cells survive conditions that would otherwise be incompatible with long-term persistence.

This concept is particularly important when considering therapeutic targeting of TOX. Complete suppression of TOX may increase short-term effector function in some experimental settings but could simultaneously reduce the persistence of tumor-reactive T cells. Consequently, therapeutic strategies designed to manipulate TOX must distinguish between pathological stabilization of terminal exhaustion and the adaptive functions of the exhaustion program.

The relationship between TCF1 and TOX is similarly complex. TCF1 is associated with the maintenance of stem-like progenitor cells, whereas TOX contributes to the establishment and persistence of the exhaustion-associated program. Nevertheless, TOX can be expressed in progenitor-like populations and may participate in maintaining their persistence [14]. Thus, these factors should not be regarded as simple molecular opposites. Instead, their relative activity, together with other transcriptional regulators, determines whether a T cell remains within a progenitor-like state or progresses toward more differentiated exhaustion.

Other transcription factors also contribute to this network. NFAT signaling is particularly important because persistent antigenic stimulation can activate NFAT in the absence of appropriate AP-1 activity, favoring an exhaustion-associated transcriptional program [12,15]. NR4A family transcription factors, including NR4A1, NR4A2, and NR4A3, are induced by chronic TCR signaling and can reinforce dysfunctional states [21]. In CAR-T models, simultaneous disruption of NR4A factors improved antitumor activity, suggesting that these pathways may represent targets for engineering more resistant therapeutic T cells [21].

The exhaustion program is also strongly shaped by epigenetic remodeling. Exhausted T cells develop a chromatin landscape distinct from conventional effector and memory populations [20,22]. These changes influence accessibility of genes involved in cytokine production, cytotoxicity, inhibitory receptors, metabolism, and differentiation. Importantly, checkpoint blockade does not completely reset this epigenetic landscape. Pauken and colleagues demonstrated that PD-1 blockade can reinvigorate exhausted T cells without fully restoring the chromatin configuration of conventional effector cells [20]. This finding provides a mechanistic explanation for why checkpoint blockade may produce substantial but incomplete functional recovery.

The epigenetic stability of exhaustion has major therapeutic implications. If exhaustion-associated chromatin states become relatively fixed, simply blocking one inhibitory receptor may be insufficient to restore complete T-cell function. Combining checkpoint blockade with epigenetic modulation, metabolic interventions, cytokine support, or strategies that preserve Tpex cells may therefore be more effective than attempting to reverse terminal exhaustion directly.

Metabolic regulation is another important component. Tumor-infiltrating T cells encounter glucose deprivation, hypoxia, altered amino acid availability, lactic acid accumulation, and mitochondrial stress. These conditions can limit ATP production and biosynthetic capacity while reinforcing dysfunctional states. Because Tpex and Texterm populations have distinct metabolic requirements and capabilities, metabolic interventions may need to be tailored to the differentiation state of the target population rather than applied uniformly.

The tumor microenvironment additionally contributes through suppressive cytokines, regulatory cells, abnormal vasculature, and persistent antigen. Transforming growth factor-β, suppressive myeloid populations, regulatory T cells, and altered nutrient availability can all affect T-cell persistence and differentiation. Consequently, exhaustion should be viewed as the result of a continuous interaction between intrinsic transcriptional programs and extrinsic environmental signals.

This integrated model explains why T-cell exhaustion cannot be reduced to PD-1 expression alone. PD-1 is an important therapeutic target and useful phenotypic marker, but it does not uniquely identify terminal exhaustion. Tpex cells can express high levels of PD-1 while remaining highly relevant to therapeutic responses [6,11]. Conversely, terminally differentiated cells may express several inhibitory receptors simultaneously but possess limited capacity for durable reinvigoration. Functional state, developmental history, transcriptional identity, epigenetic configuration, and spatial localization must therefore be considered together.

Therapeutic Implications for PD-1/PD-L1 Blockade

The discovery of Tpex cells has changed the conceptual framework for immune checkpoint blockade. PD-1/PD-L1 inhibitors are among the most successful cancer immunotherapies, but their clinical activity varies substantially among

tumor types and patients. The presence of PD-1-positive T cells alone does not reliably predict response because PD-1 is expressed across multiple exhausted states [3,6].

The classical explanation for PD-1 blockade proposed that PD-1 signaling suppresses TCR and costimulatory pathways and that antibody-mediated inhibition releases this brake, allowing exhausted T cells to recover effector function. This mechanism remains biologically relevant, but current evidence suggests that the cellular source of the therapeutic response is particularly important. Tpex cells appear to represent a major reservoir capable of proliferating after PD-1 blockade [6,7,11].

Upon PD-1 inhibition, Tpex cells can undergo proliferation and differentiation, generating downstream populations with enhanced effector function. Therefore, the response to checkpoint blockade is not simply a process of transforming terminally exhausted cells into normal memory or effector cells. Instead, it resembles activation of a renewable progenitor population followed by production of differentiated descendants [6].

This model explains several clinical observations. First, patients with tumors containing a greater abundance of stem-like or progenitor-like T cells may have greater potential to respond to checkpoint inhibition. Second, treatment may increase the number of differentiated tumor-reactive T cells without permanently eliminating exhaustion. Third, durable responses may depend on preservation of the Tpex reservoir rather than maximization of immediate cytotoxic activity alone.

The distinction between Tpex and Texterm also explains why combination checkpoint blockade may have variable effects. Blocking additional inhibitory receptors such as LAG-3 or TIGIT may enhance T-cell function, but the effectiveness of such combinations can depend on which differentiation state is targeted. If the tumor lacks a sufficient progenitor compartment, removing additional inhibitory signals from terminally exhausted cells may have limited capacity to generate durable responses.

Another implication is that treatment timing may influence efficacy. Prolonged exposure to immunotherapy may repeatedly stimulate Tpex populations and drive differentiation into downstream states. The optimal balance between expansion and differentiation remains incompletely understood. Future therapeutic strategies may therefore aim not simply to maximize T-cell activation but to maintain an appropriate equilibrium between self-renewal and effector differentiation.

The spatial distribution of Tpex cells is also therapeutically relevant. Stem-like CD8+ T cells can be maintained in specialized niches containing antigen-presenting cells and supportive signals [18,19]. Therapies that enhance the formation or function of these niches could theoretically increase the reservoir of checkpoint-responsive cells. This possibility provides a rationale for combining checkpoint blockade with vaccines, dendritic-cell-directed therapies, radiation, or other interventions capable of improving local antigen presentation.

Epigenetic resistance presents another barrier. Although PD-1 blockade can enhance T-cell proliferation and function, it does not completely erase the epigenetic program of exhaustion [20,22]. This observation has stimulated interest in combining checkpoint inhibitors with agents that modify chromatin states. However, because epigenetic programs also support T-cell identity and persistence, nonspecific disruption could be detrimental. The goal should therefore be selective modulation of pathological exhaustion while preserving the characteristics of Tpex cells.

The concept of T-cell exhaustion also has implications for biomarkers. Traditional biomarkers such as PD-L1 expression and tumor mutational burden provide useful information but do not fully capture the state of the immune compartment. Future biomarker strategies may incorporate TCF1 expression, progenitor exhausted-cell frequency, clonotype architecture, spatial organization, and transcriptional signatures. A tumor with abundant PD-1-positive cells but few TCF1-positive progenitors may have a different therapeutic potential from a tumor containing a substantial TCF1-positive reservoir.

Ultimately, the success of checkpoint blockade may depend on the ability to maintain a functional source of tumor-reactive T cells. Rather than asking only whether a tumor contains exhausted T cells, it may be more informative to determine whether it contains an adequate reservoir of renewable exhausted T cells capable of responding to therapy.

T-Cell Exhaustion and CAR-T-Cell Therapy

CAR-T-cell therapy has transformed the treatment of several hematological malignancies, but its efficacy in solid tumors remains substantially more limited [23,24]. One of the major barriers is the development of T-cell dysfunction within the tumor microenvironment. CAR-T cells experience repeated antigen stimulation, inhibitory receptor signaling, metabolic stress, and immunosuppressive cytokines, creating conditions that resemble the exhaustion observed in endogenous tumor-reactive T cells [21,25].

The differentiation state of the cells used to manufacture CAR-T products is therefore critically important. T cells with stem-like or memory-associated characteristics generally show superior persistence and expansion after infusion compared with highly differentiated effector cells. Similar principles have been observed in adoptive transfer of tumor-infiltrating lymphocytes, where stem-like T-cell populations were associated with better persistence and clinical response [26].

This finding has influenced CAR-T manufacturing strategies. Rather than maximizing immediate cytotoxic activity during ex vivo expansion, modern approaches increasingly attempt to preserve less differentiated T-cell populations. Such cells may have greater proliferative capacity after infusion and may generate functional progeny over time. The balance between immediate killing and long-term persistence is therefore a central consideration in CAR-T design.

One mechanism that can promote CAR-T exhaustion is tonic signaling. CAR molecules can cluster or signal in the absence of antigen, producing chronic activation through CD3ζ and other intracellular signaling pathways. Long and colleagues demonstrated that tonic CAR signaling can induce early exhaustion and that the choice of costimulatory domain influences this process [25]. In their model, 4-1BB costimulation reduced exhaustion associated with persistent CAR signaling compared with CD28-based signaling, helping explain differences in persistence between CAR designs.

The intracellular architecture of the CAR therefore has consequences beyond simple activation strength. CD28-containing CARs can produce strong early activation and expansion, whereas 4-1BB signaling may promote metabolic and transcriptional programs more compatible with long-term persistence [25]. The optimal configuration may vary according to antigen density, tumor type, manufacturing platform, and clinical objective.

Chronic antigen exposure after infusion represents another major driver of CAR-T dysfunction. In solid tumors, CAR-T cells may encounter antigen continuously, while simultaneously being exposed to hypoxia, nutrient deprivation, suppressive myeloid cells, regulatory T cells, and inhibitory cytokines. This environment can accelerate progression toward dysfunctional states.

Transcriptional regulators implicated in endogenous T-cell exhaustion can also affect CAR-T cells. NR4A transcription factors are induced following chronic stimulation and contribute to a hyporesponsive state. In experimental models, deletion of NR4A1, NR4A2, and NR4A3 improved CAR-T antitumor activity and promoted effector-associated transcriptional programs [21]. These observations support the concept that exhaustion is partly cell intrinsic and can potentially be modified through genetic engineering.

TOX-related pathways are also relevant to CAR-T biology. Because TOX helps establish and stabilize exhaustion-associated programs, modifying TOX activity may theoretically improve CAR-T function. However, the adaptive role of TOX in supporting persistence under chronic stimulation means that complete elimination may not necessarily be beneficial. More refined approaches that adjust the intensity or timing of TOX-associated signaling may be preferable to permanent deletion.

CAR-T cells can also be engineered to resist inhibitory signaling. Strategies include deletion of inhibitory receptors, expression of dominant-negative receptors, incorporation of switch receptors, manipulation of transcription factors, and remodeling of intracellular signaling pathways [23,24]. These approaches aim to preserve functional capacity despite continuous exposure to tumor-associated inhibitory signals.

Metabolic engineering represents another emerging strategy. Exhausted CAR-T cells often exhibit impaired mitochondrial function and metabolic flexibility. Enhancing mitochondrial fitness, promoting oxidative metabolism, or altering nutrient utilization may improve persistence. Nevertheless, metabolic interventions must be carefully balanced because excessive activation can increase differentiation and reduce long-term persistence.

The relationship between CAR-T exhaustion and Tpex biology is particularly interesting. Conventional CAR-T cells are generated ex vivo and therefore do not necessarily follow exactly the same developmental trajectory as endogenous tumor-specific T cells. Nevertheless, the concept of a stem-like reservoir remains relevant. CAR-T products enriched for less differentiated populations may persist longer and generate differentiated effector cells after infusion. This suggests that preserving developmental potential during manufacturing may be as important as maximizing immediate cytotoxicity.

Recent clinical and experimental evidence also supports the value of stem-like tumor-reactive T cells in adoptive immunotherapy. In patients receiving TIL therapy, stem-like CD8+ T-cell populations were associated with persistence and complete tumor regression, whereas highly differentiated populations were associated with poorer persistence [26]. These observations reinforce a general principle across cellular immunotherapies: durable antitumor responses require a population capable of renewing itself.

The therapeutic objective should therefore shift from eliminating exhaustion altogether toward controlling the trajectory of T-cell differentiation. A successful CAR-T product may not be one that is completely resistant to all exhaustion-associated pathways. Instead, it may be one that maintains an appropriate balance between persistence, controlled differentiation, cytotoxicity, and resistance to terminal dysfunction.

Therapeutic Opportunities and Future Perspectives

The Tpex–Texint–Texterm framework creates several potential therapeutic opportunities. First, therapies could be designed to expand or preserve the Tpex population. Because Tpex cells represent a renewable source of tumor-reactive cells, protecting this compartment may increase the magnitude and durability of responses to checkpoint

blockade. Vaccination, improved antigen presentation, modulation of lymphoid niches, and selected cytokine-based approaches may contribute to this objective.

Second, interventions could target the transition from progenitor to terminal exhaustion. This does not necessarily require preventing differentiation entirely. Effector differentiation is essential for tumor killing. The therapeutic goal may instead be to delay or regulate terminal differentiation sufficiently to maintain a renewable pool while allowing effective effector production.

Third, epigenetic and transcriptional modulation could be combined with checkpoint blockade. Because exhausted cells retain a distinct chromatin landscape even after PD-1 inhibition [20], interventions that alter chromatin accessibility may enhance the depth or durability of reinvigoration. However, this strategy requires precise targeting because exhaustion-associated epigenetic programs also support persistence.

Fourth, the tumor microenvironment must be considered. Even highly functional T cells may fail if they cannot access the tumor, receive adequate antigen-presenting signals, or obtain metabolic resources. Combining immunotherapy with approaches that normalize tumor vasculature, modify myeloid populations, improve antigen presentation, or reduce suppressive cytokines may therefore be essential.

Fifth, cellular therapies can increasingly be engineered at multiple levels. CAR design, costimulatory domains, gene editing, transcriptional regulation, metabolic programming, and resistance to inhibitory signals can all be modified. The goal is to produce T cells that maintain stem-like properties while acquiring sufficient effector capacity to eliminate tumor cells.

One promising direction is the use of multi-omic profiling to define exhaustion states at single-cell resolution. Integration of transcriptomic, epigenomic, proteomic, metabolic, and TCR-clonotype data may allow investigators to identify which cells are true therapeutic reservoirs and which represent terminal states. Spatial transcriptomics may further clarify how specific niches maintain Tpex cells and regulate their differentiation.

Artificial intelligence and computational modeling may also facilitate the identification of clinically useful exhaustion signatures. Rather than relying on individual markers such as PD-1, future models may integrate TCF1, TOX, inhibitory receptors, cytotoxic genes, metabolic pathways, clonotype information, and spatial features. Such composite biomarkers could improve patient selection for checkpoint blockade and cellular therapies.

Another important issue is tumor type. The Tpex–Texint–Texterm framework is supported by substantial evidence, but the relative abundance, phenotype, and function of these populations can vary between tumors. Differences in antigenicity, tissue architecture, immune infiltration, oncogenic pathways, and treatment history can influence T-cell differentiation. Therefore, universal classification systems should be applied cautiously.

Similarly, the terminology of "exhaustion" requires careful interpretation. Exhausted T cells are not simply defective cells. The exhaustion program represents an adaptation to persistent antigen that prevents uncontrolled activation and may preserve cellular survival under chronic stimulation [2,13]. The objective of immunotherapy is therefore not necessarily to abolish exhaustion-associated biology but to selectively remove its most restrictive components while preserving beneficial features such as persistence and self-renewal.

For CAR-T therapy, future development may increasingly focus on "programmed persistence." Instead of creating cells that are maximally activated at infusion, manufacturing protocols may intentionally generate products enriched for memory-like and stem-like characteristics, followed by controlled differentiation in vivo. This approach could improve persistence while reducing premature terminal differentiation.

Combination strategies are likely to be particularly important. A future therapeutic regimen might simultaneously preserve Tpex cells, inhibit suppressive signaling, improve antigen presentation, remodel the metabolic environment, and enhance CAR-T persistence. However, such combinations must be rationally designed because excessive immune stimulation can increase toxicity without necessarily improving tumor control.

An important unresolved question concerns the precise relationship between Tpex and conventional memory T cells. Although Tpex cells share several features with memory populations, they are shaped by persistent antigen and display a distinct exhaustion-associated epigenetic program [8,20]. Better understanding of this distinction could identify mechanisms through which T cells can be maintained in a highly persistent state without sacrificing tumor recognition.

Another challenge is the identification of the optimal balance between TCF1 and effector differentiation. High TCF1 activity may preserve stemness but limit immediate cytotoxic function, whereas excessive differentiation can produce potent effector cells that rapidly lose persistence. Effective immunotherapy may therefore depend on maintaining a dynamic equilibrium rather than maximizing either state.

The future of cancer immunotherapy will likely involve moving beyond the simplistic goal of "reversing exhaustion." Instead, therapies may be designed to manipulate the developmental trajectory of T cells. In this framework, Tpex cells represent a valuable renewable reservoir, Texint cells represent a therapeutically exploitable transition state, and Texterm cells represent a more constrained endpoint. The ability to control movement between these states could become a central objective of next-generation immunotherapy.

Conclusion

T-cell exhaustion is a complex and dynamic differentiation process that plays a central role in the failure of antitumor immunity. Current evidence supports a heterogeneous model in which tumor-reactive CD8+ T cells occupy multiple developmental states ranging from progenitor exhausted Tpex cells to intermediate and terminally exhausted populations. Tpex cells are particularly important because they retain self-renewal and proliferative potential and provide a renewable source of differentiated progeny. Terminally exhausted cells, although capable of residual cytotoxic activity, have substantially reduced capacity for durable expansion and therapeutic reinvigoration.

TCF1 and TOX are central components of this biology. TCF1 supports the stem-like properties and persistence of progenitor exhausted cells, whereas TOX contributes to the establishment and stabilization of the exhaustion-associated transcriptional and epigenetic program. Their functions should not be interpreted as simple opposites. Instead, they operate within a broader regulatory network that allows T cells to persist during chronic antigen exposure while limiting conventional effector differentiation.

The implications for immunotherapy are substantial. PD-1/PD-L1 blockade appears to act predominantly on responsive progenitor-like populations rather than completely restoring terminally exhausted cells. Consequently, the presence and preservation of a Tpex reservoir may be a major determinant of treatment durability. Similarly, CAR-T therapy is strongly influenced by the differentiation state, signaling architecture, metabolic fitness, and susceptibility to exhaustion of the therapeutic cells.

Future strategies should therefore focus on controlling the trajectory of T-cell differentiation rather than attempting to eliminate exhaustion as a whole. Preserving stem-like populations, selectively modulating TOX- and TCF1-associated pathways, improving the tumor microenvironment, optimizing CAR signaling, and integrating epigenetic and metabolic interventions may collectively produce more durable antitumor responses. A deeper understanding of T-cell developmental states will be essential for designing the next generation of checkpoint inhibitors and adoptive cellular therapies.

Declarations

Competing Interests

The authors declare that they have no known financial conflicts of interest or personal relationships that could have influenced the work presented in this manuscript.

Ethical approval

This review article does not include any new studies involving human participants or animals conducted by the authors.

Funding

The authors received no specific financial support for the research, authorship, or publication of this review.

Availability of data and materials

Data sharing is not applicable because no new datasets were generated or analyzed for this review.

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