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THE ROLE AND THERAPEUTIC STRATEGIES OF PRECURSOR EXHAUSTED CD8-positive T CELLS IN TUMOR IMMUNITY

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Abstract: The clinical use of immune checkpoint inhibitors (ICIs) has markedly improved outcomes in a range of malignancies. Even so, overall response rates remain unsatisfactory, and acquired resistance often erodes durable clinical benefit. Accumulating evidence suggests that terminally exhausted CD8-positive T cells are not the principal population that expands after checkpoint blockade. Rather, precursor exhausted CD8-positive T cells (Tpex), which retain self-renewal capacity and the potential to redifferentiate, provide the main cellular foundation for sustained antitumor immunity. In most settings, Tpex are characterized by high TCF1 expression, relatively low levels of terminal exhaustion markers, and responsiveness to PD-1 blockade. Their generation and persistence are shaped by several interconnected factors, including tumor-draining lymph nodes, intratumoral antigen-presenting cell niches, transcription factor circuits, metabolic adaptation, and epigenetic remodeling. Research on Tpex is now reshaping the conceptual basis of cancer immunotherapy. The emphasis is shifting away from simply amplifying immune activation and toward expanding the precursor pool, preserving cellular plasticity, and modulating the timing of differentiation. This review outlines the conceptual definition, biological basis, fate-regulatory mechanisms, and translational significance of Tpex in tumor immunotherapy. It also discusses current challenges, such as the absence of a unified phenotypic definition, the shortage of longitudinal causal evidence, and the still-limited body of clinical translational data. Overall, this review aims to provide a useful framework for refining precision immunotherapeutic strategies.
Keywords: CD8-positive T cells; Precursor exhausted T cells; Tumor immunity; Immune checkpoint inhibitors

1 INTRODUCTION

Immune checkpoint inhibitors (ICIs), especially those directed against PD-1/PD-L1 and CTLA-4, have profoundly changed the treatment landscape for many malignancies. Still, objective response rates remain modest, and acquired resistance occurs frequently. Taken together, these limitations continue to constrain further gains in clinical benefit [1]. In recent years, the key question in the field has gradually shifted. Rather than asking only whether antitumor immunity can be activated, attention has turned to which T-cell subsets can actually be reinvigorated by ICIs and converted into durable tumor control [2]. In this context, identifying the $CD8^+$ T-cell populations that sustain long-term immunotherapeutic responses, and clarifying the basis of their plasticity, has become essential for understanding variation in treatment outcomes and for designing rational combination strategies.

T-cell exhaustion describes a state in which persistent antigen exposure progressively undermines $CD8^+$ T-cell effector functions, including production of IFN-γ, TNF-α, and granzymes, while simultaneously increasing the expression of inhibitory receptors such as PD-1, LAG-3, TIM-3, and TIGIT and diminishing proliferative capacity [3]. Importantly, exhaustion should not be reduced to a simple picture of passive suppression. It is better viewed as a dynamic adaptive program that balances effector activity, survival, and tissue fitness under chronic stimulation. From this perspective, the concept of exhaustion laid the groundwork for the later differentiation hierarchy model.

Work in chronic LCMV infection has shown that exhausted $CD8^+$ T cells do not represent a uniform terminal population. Instead, they include a precursor or stem-like subset marked by high expression of TCF1, encoded by TCF7. This subset retains self-renewal capacity, undergoes vigorous proliferative expansion after PD-1 blockade, and continuously replenishes both effector-like and terminally exhausted populations [4,5]. Exhaustion, then, is more accurately understood as a hierarchically organized differentiation lineage. Terminally exhausted cells (Tex) are not the major reversible targets of ICIs. By contrast, Tpex combine features of exhaustion with preserved redifferentiation potential and therefore act as the upstream reservoir that more directly supports therapeutic responses. Landmark studies further suggest that the proliferative burst observed after PD-1 blockade derives predominantly from Tpex rather than terminal Tex [6]. Their maintenance and expansion depend on coordinated support from TCF1, costimulatory signaling, and specialized tissue niches. In tumor immunotherapy, the Tpex framework offers a more convincing mechanistic explanation: ICIs appear to act mainly on a precursor pool that still retains plasticity, rather than on deeply terminally exhausted cells that have already become epigenetically fixed. Accordingly, therapeutic durability depends to a large extent on the abundance, quality, and niche support of the Tpex pool [4,7,8]. On this basis, the present review systematically discusses the conceptual definition and immunological significance of Tpex, the niche architecture underlying their formation and maintenance, the multilayered networks governing their fate, and the translational

strategies and major bottlenecks involved in targeting Tpex. The broader aim is to provide a systematic reference for designing combination immunotherapies centered on sustainable immune resources.

2 CONCEPTUAL DEFINITION OF TPEX AND THEIR SIGNIFICANCE IN TUMOR IMMUNOLOGY

The nomenclature surrounding Tpex remains somewhat inconsistent across studies. Terms such as Tpex, Tex prog, stem-like exhausted, and memory-like exhausted are all used in the literature [9]. Despite this variation, the core biological properties described are largely aligned. In operational terms, Tpex can be defined from four complementary dimensions: phenotype, function, transcriptomic and epigenetic features, and therapeutic responsiveness.

2.1 Phenotypic Markers

Tpex generally display intermediate PD-1 expression together with high TCF1 expression. As a major downstream transcription factor in the WNT signaling pathway, TCF1 is currently the most widely accepted core marker. By contrast, high expression of molecules associated with terminal exhaustion, including TIM-3 (HAVCR2), CD39 (ENTPD1), and TOX, is more typical of Tex [10]. That said, these markers are strongly influenced by species, tissue location—including tumor-draining lymph nodes (TDLNs), tumor tissue, and peripheral blood—and tumor type. For that reason, cross-study comparisons must be interpreted cautiously and always with close attention to sample origin.

2.2 Functions of Tpex

The importance of Tpex does not lie in strong immediate cytotoxicity under steady-state conditions. Rather, it rests on their sustained supply capacity and mobilizable functional potential. Their defining characteristics include self-renewal, which allows rapid proliferation and preservation of population size, as shown by adoptive transfer and lineage-tracing studies; multidirectional differentiation potential, enabling continuous generation of effector-like subsets and Tex under persistent antigenic stimulation; and rapid mobilization after restimulation or immune checkpoint blockade, accompanied by increased expression of effector molecules such as IFN-γ, TNF-α, and granzyme B, which reflects preserved functional reserve [2,9,11]. Tpex, therefore, should not be judged solely by short-term cytotoxic output. Their proliferative burst, lineage-replenishing capacity, and responsiveness to therapeutic remodeling are equally important, if not more so.

2.3 Transcriptomic and Epigenetic Features

Single-cell RNA sequencing and ATAC-seq analyses have demonstrated that Tpex possess a distinct molecular program that sets them apart from effector T cells (Teff), memory T cells (Tmem), and Tex. At the transcriptional level, Tpex show high expression of stemness-associated genes, including TCF7, LEF1, MYB, FOXO1, ID3, BACH2, and BCL6, while relatively suppressing effector and terminal exhaustion drivers such as PRDM1, ID2, TIM-3, and CD39 [2,9,11]. At the chromatin level, accessible regions in Tpex are enriched for TCF1, LEF1, and FOXO1 binding motifs, whereas Tex display greater accessibility at T-bet, BATF, and IRF4 binding sites [12,13]. These observations suggest that Tpex and Tex differ not merely in degree of dysfunction, but in regulatory architecture and in the boundaries of cellular plasticity. This distinction provides a critical molecular basis for understanding therapeutic limits and for designing interventions that modulate the tempo of differentiation.

2.4 Therapeutic Response Characteristics

In chronic LCMV infection as well as in multiple transplantable and spontaneous tumor models, the proliferative expansion of $CD8^+$ T cells following PD-1/PD-L1 blockade arises almost entirely from the $TCF1^+$ Tpex subset, whereas $TCF1^-$ terminally exhausted cells show poor responsiveness to checkpoint blockade [5]. A similar pattern has been reported in human tumors. In melanoma, non-small cell lung cancer, and other malignancies, the abundance of $TCF1^+$ $CD8^+$ T cells in baseline tumor tissue or TDLNs correlates positively with objective response rate and overall survival after ICI treatment [14-17]. Thus, Tpex are not only a key mechanistic population in preclinical work, but are also increasingly recognized as a clinically relevant determinant of therapeutic efficacy and a potential component of predictive models.

3 FOUNDATIONS OF TPEX FORMATION AND FATE REGULATION

3.1 The Tumor Microenvironment Shapes the Initiation and Maintenance of Tpex

Current evidence supports a two-stage model of Tpex development. The first stage occurs mainly in TDLNs, where organized antigen presentation, abundant costimulatory signals, and stromal networks that support T-cell expansion and stem-like programming favor the establishment and maintenance of precursor features in newly activated $CD8^+$ T cells. The second stage takes place within the tumor itself, where activated $CD8^+$ T cells migrate into the lesion and gradually initiate effector programs while entering the exhaustion trajectory under the combined influence of persistent antigen exposure, local APC-mediated costimulation, and immunosuppressive microenvironmental signals [18,19]. Integrated analyses of paired human TDLN and tumor samples have shown that activated $CD8^+$ T cells in TDLNs closely

resemble intratumoral TCF1$^+$ Tpex at both the transcriptomic and epigenetic levels. Substantial TCR clonal overlap between these compartments further supports the view that TDLNs are a major source of intratumoral Tpex [15].

In vivo lineage-tracing studies further indicate that Tpex are not confined to a single tissue compartment. Rather, they continuously circulate between tumors and lymphoid organs. TCF1$^+$ non-effector-like CD8$^+$ T cells can migrate from peripheral lymphoid tissues into tumors, and some may also return to TDLNs for functional renewal. This pattern implies that although local tumor environments rapidly impose exhaustion pressure, the maintenance of intratumoral Tpex depends on sustained systemic replenishment and cyclic renewal. Therapeutic evidence supports this idea: blocking the migration of Tpex from TDLNs into tumors significantly compromises the efficacy of anti-PD-1 therapy, indicating that circulatory supply is indispensable for durable tumor control [18,20]. In addition, a circulating CD8$^+$ T-cell subset in peripheral blood that shares TCR clonotypes with intratumoral Tpex can be detected. These circulating Tpex-like cells are usually PD-1$^+$TCF1$^+$ and transiently increase after anti-PD-1 therapy, corresponding to the so-called proliferative burst [21].

Within tumors, immune niches composed of dendritic cells (DCs) and CD4$^+$ helper T cells critically influence Tpex fate. In these niches, DCs maintain antigen recognition through cross-presentation and support Tpex survival and controlled effector differentiation via the CD80/CD86-CD28 costimulatory axis and cytokines such as IL-12. CD4$^+$ T cells further license DCs through CD40L-CD40 interactions, thereby enhancing cross-presentation, and they directly sustain Tpex maintenance and expansion through factors such as IL-21 [22,23]. PD-1 blockade relieves inhibition within these niches, allowing Tpex to expand and generate effector progeny. In this way, spatial niche organization is directly linked to therapeutic responsiveness. Enhancing the abundance and function of DCs may therefore improve antigen cross-presentation and costimulatory support, thereby modulating the balance among precursor maintenance, effector differentiation, and terminal exhaustion. The key point, perhaps, is not merely to intensify stimulation, but to provide a sustained and appropriately calibrated antigen-presenting environment [8,11].

3.2 Transcription Factor Networks Determine Tpex Fate Decisions

The formation, maintenance, and fate bifurcation of Tpex are governed by a hierarchical transcription factor network. At its core lies a dynamic antagonism between stemness-maintaining factors and effector- or exhaustion-driving factors, and this antagonism determines the balance between precursor preservation and terminal differentiation. This balance is not fixed. Instead, it shifts in response to antigen strength, costimulatory thresholds, cytokine input, and metabolic stress. Tpex are therefore highly plastic populations whose stability depends on environmental support. This may also explain why therapeutic interventions that ignore differentiation stage and stimulation timing sometimes produce transient enhancement at the cost of long-term exhaustion.

TCF1 is one of the central transcription factors controlling Tpex formation and maintenance and serves as a major molecular hub linking stem-like properties, self-renewal, and therapeutic responsiveness. As a downstream effector of the Wnt signaling pathway, TCF1 regulates a broad transcriptional program through its HMG-box DNA-binding domain. It promotes stemness- and memory-associated genes such as EOMES and BCL6 while suppressing effector and exhaustion-driving genes including BLIMP1 and ID2 [14]. Functionally, TCF1 is best viewed not as a single switch that reverses deep exhaustion, but as a regulator that preserves precursor identity and delays terminal differentiation. Accordingly, translational strategies centered on TCF1 should focus on maintaining Tpex that still reside within a plasticity window. Conditional deletion of TCF7 nearly abolishes the Tpex population, leading to rapid collapse of the CD8$^+$ T-cell response in chronic infection and tumor settings and markedly weakening anti-PD-1 efficacy [24]. Conversely, sustained upregulation of TCF1 can expand the Tpex pool and prolong response durability, but this effect is stage-dependent. Once cells enter the CX3CR1$^+$ intermediate exhausted state and pass the precursor-to-effector-like transition point, reinduction of TCF1 is insufficient to restore a precursor-like phenotype [25]. This observation suggests that controlling the pace of differentiation may be more feasible than trying to reverse terminal states outright. Interventions aimed at expanding and stabilizing the precursor pool are therefore likely to work best before Tpex cross crucial transitional checkpoints. TCF1 also contributes to epigenetic stabilization through interactions with histone deacetylase complexes and through participation in chromatin remodeling at target loci, thereby consolidating the precursor program at the epigenetic level [14,26]. Thus, the impact of TCF1 depends not only on how much of it is expressed, but also on chromatin accessibility and cofactor availability, which provides a mechanistic rationale for epigenetic priming strategies.

FOXO1 is another important regulator of Tpex maintenance. It is highly expressed in Tpex and preserves the stem-like phenotype by promoting transcription of genes such as TCF7, SELL, and IL7R. Its nuclear localization is negatively regulated by the PI3K-AKT axis. When TCR signaling becomes excessive or growth factor signaling is amplified, AKT-mediated phosphorylation retains FOXO1 in the cytoplasm and suppresses its transcriptional activity. This, in turn, downregulates stemness genes and activates effector programs. Such a mechanism helps explain why chronic strong antigenic stimulation drives Tpex more rapidly toward terminal exhaustion. Functionally, conditional deletion of FOXO1 accelerates terminal differentiation and weakens Tpex maintenance, whereas enhancing FOXO1 activity through PI3K inhibition promotes expansion of the Tpex pool [27].

The antagonistic BCL6-BLIMP1 axis also represents a key transcriptional module that controls the balance between Tpex maintenance and terminal differentiation. BCL6 supports stemness programs and represses genes associated with terminal exhaustion, including multiple inhibitory receptors and effector molecules. BLIMP1, by contrast, drives effector output and terminal differentiation. This axis is finely tuned by the cytokine milieu. TGF-β-SMAD2 signaling

can upregulate BCL6 and thereby preserve the Tpex state, whereas IL-2-STAT5 signaling suppresses BCL6 and promotes BLIMP1 expression, pushing cells toward effector and terminally exhausted states [28].

TOX is a major driver of the exhaustion program during chronic antigen stimulation, yet its role is not entirely detrimental. Persistent TCR signaling induces TOX through an NFAT-dependent pathway, after which TOX reshapes chromatin accessibility through interactions with histone acetyltransferase and deacetylase complexes, thereby establishing an exhaustion-associated epigenetic landscape. Still, TOX cannot be regarded as simply harmful. Although TOX deficiency can reduce inhibitory receptor expression and partially attenuate exhaustion-associated remodeling, it also biases cells toward short-lived effector differentiation and ultimately reduces the overall number of antigen-specific $CD8^+$ T cells. This suggests that the exhaustion program itself has adaptive value, sacrificing part of immediate effector function in order to preserve long-term survival and immune persistence. Complete de-exhaustion, therefore, may not always be desirable, especially if it undermines durability [29].

A MYB-defined $CD62L^+$ Tpex subset further reveals hierarchical organization within the Tpex compartment. Preclinical studies have shown that MYB is highly expressed in $CD62L^+$ Tpex, a subset located at the apex of the differentiation hierarchy and characterized by stronger self-renewal, lower differentiation commitment, and greater regenerative potential. After PD-1 blockade, $CD62L^+$ Tpex exhibit robust proliferative capacity and can sequentially generate $CD62L^-$ Tpex, $CX3CR1^+$ effector-like transitional cells, and terminally exhausted cells. MYB supports their survival and stem-like architecture by promoting TCF7 and BCL2 expression [5]. This finding suggests that clinical assessment based only on total $TCF1^+$ Tpex abundance may not fully capture expansion potential. More refined stratification of upstream Tpex subsets, such as $CD62L^+$ cells, may improve prediction of therapeutic response and help guide strategy selection.

Beyond TCF1, FOXO1, BCL6, TOX, and MYB, several additional transcription factors participate in Tpex formation and fate regulation in stage-specific and context-dependent ways. BACH2 promotes long-term stem-like transcriptional programs by suppressing genomic binding of the AP-1 family member JUNB, thereby enhancing in vivo expansion of CAR T cells and increasing resistance to exhaustion [30]. Within the ID3-ID2 axis, ID3 is highly expressed in Tpex and supports stem-like features, whereas ID2 is more commonly upregulated in effector and terminally exhausted subsets [31,32]. BATF can promote survival and expansion of tumor-infiltrating CAR T cells, increase effector cytokine production, reduce inhibitory receptor expression and TOX levels, and support the generation of long-lived memory T cells capable of controlling tumor relapse [33]. Taken together, these findings underscore that Tpex fate is controlled by a multicomponent regulatory network, and translational conclusions based on single factors should therefore be interpreted with care.

3.3 Metabolic Adaptation Supports the Stem-like Properties and Persistence of Tpex

3.3.1 Mitochondrial homeostasis

The maintenance of Tpex depends not only on transcriptional regulation but also on metabolic adaptation. Compared with terminally exhausted T cells or short-lived effector cells, Tpex must survive over prolonged periods and retain proliferative capacity within the tumor microenvironment, where nutrients are limited, metabolic competition is intense, and hypoxia and oxidative stress are common. This demands stronger mitochondrial homeostasis, greater metabolic flexibility, and improved stress tolerance. Importantly, metabolic state and epigenetic regulation are tightly interconnected, because metabolic substrates and redox balance influence the activity of chromatin-modifying enzymes and thereby shape both the breadth and duration of the plasticity window.

Unlike effector T cells, which depend heavily on glycolysis, Tpex preferentially use mitochondrial oxidative phosphorylation (OXPHOS) and fatty acid oxidation (FAO), resembling the metabolic profile of memory T cells. Functionally intact mitochondria are therefore essential for preserving the resilience and self-renewal capacity of Tpex. Within the tumor microenvironment, however, persistent oxidative stress, hypoxia, and nutrient competition can progressively damage mitochondria and ultimately compromise Tpex survival and expansion. Long-term mitochondrial fitness, in other words, is a major determinant of whether Tpex can maintain immune supply and therapeutic efficacy [34]. Preclinical studies have shown that PTMA (prothymosin alpha) is highly expressed in Tpex and preserves OXPHOS capacity and resistance to metabolic stress by maintaining TFAM-dependent mitochondrial DNA integrity. Disruption of this axis significantly reduces the persistence of intratumoral $CD8^+$ T cells and weakens the efficacy of PD-1 blockade [35]. These findings imply that sustaining Tpex requires more than limiting terminal exhaustion marker expression; it also requires preservation of mitochondrial genomic stability and metabolic resilience.

Proteostasis and mitochondrial dynamics further shape the rate at which Tpex progress toward terminal exhaustion. KLHL6 has emerged as a key node linking exhaustion control with mitochondrial adaptation and is highly expressed in Tpex. As a substrate adaptor for the Cullin3 E3 ubiquitin ligase complex, KLHL6 promotes ubiquitination and degradation of TOX, thereby slowing the transition from Tpex to Tex. In parallel, it restricts excessive mitochondrial fission, possibly by regulating the stability of the fission protein DRP1, thus preserving mitochondrial function and cell survival during chronic stimulation [36]. Loss of KLHL6 accelerates TOX accumulation and mitochondrial dysfunction, leading to depletion of the Tpex pool and reduced anti-PD-1 efficacy. This mechanism suggests that protein quality control and mitochondrial dynamics are not merely parallel processes; rather, they may jointly form a coupled rate-limiting step that determines Tpex lifespan and fate.

3.3.2 Metabolic factors in the tumor microenvironment

Multiple metabolites that accumulate within the tumor microenvironment exert complex and context-dependent effects on Tpex fate. Traditional views have emphasized the suppressive roles of metabolites such as lactate, adenosine, and kynurenine in T-cell function. More recent studies, however, suggest that metabolic signals can produce marked dose-, duration-, and differentiation stage-dependent effects on Tpex. The same metabolite may dampen immediate effector function under one set of conditions, yet under another may help preserve the precursor pool by improving mitochondrial quality control or stress adaptation. Thus, in the context of Tpex biology, metabolic suppression should not be evaluated only in terms of short-term effector inhibition, but also in terms of long-term effects on precursor pool stability [37–39].

Preclinical studies have shown that succinate, a tricarboxylic acid cycle intermediate, may accumulate in the tumor microenvironment because of reduced succinate dehydrogenase activity. Rather than acting solely as an immunosuppressive metabolite, succinate may support the survival and maintenance of tumor-reactive $CD8^+$ T cells, particularly Tpex, by enhancing BNIP3-mediated mitophagy and metabolic readaptation. BNIP3-mediated mitophagy removes damaged mitochondria and reduces reactive oxygen species accumulation, thereby improving metabolic fitness under chronic stimulation. This finding suggests that strengthening mitochondrial quality control may be a more sustainable means of stabilizing Tpex than simply boosting effector function [40].

By contrast, the immunosuppressive role of the tryptophan-kynurenine pathway is supported by more consistent evidence. Tumor cells and tumor-associated myeloid cells frequently express high levels of IDO1 and TDO2, which convert tryptophan into kynurenine. As an endogenous ligand of the aryl hydrocarbon receptor (AHR), kynurenine activates AHR and drives the exhaustion program in $CD8^+$ T cells through AHR-FANCD2-mediated chromatin remodeling, including upregulation of inhibitory receptors and downregulation of effector genes. At the same time, tryptophan depletion activates the GCN2 stress pathway, suppressing proliferation and function [39]. This example illustrates how metabolic suppression and epigenetic dysregulation can cooperate to push Tpex beyond the plasticity window and into irreversible terminal differentiation.

Other metabolic features of the tumor microenvironment may also affect Tpex fate. Hypoxia promotes glycolysis-dependent reprogramming through HIF-1α, which is unfavorable for maintaining OXPHOS-dependent Tpex [41]. Abnormal cholesterol metabolism and oxysterols may compromise adaptation through endoplasmic reticulum stress and lipid peroxidation [42]. Elevated extracellular potassium released from necrotic regions may suppress TCR signaling, yet under certain conditions may indirectly favor maintenance of stem-like programs associated with lower activation intensity [43]. Taken together, these observations suggest that the metabolic environment shapes Tpex fate in a systemic way by simultaneously altering signaling intensity, mitochondrial stress, and the epigenetic baseline. Its effects, therefore, cannot be neatly classified as either inhibitory or supportive.

3.4 Epigenetic Remodeling Determines Tpex Stability and Plasticity

Epigenetic studies have offered deeper insight into the mechanisms governing Tpex fate. Systematic ATAC-seq analyses of $CD8^+$ T cells across different differentiation stages have shown that Tpex, effector-like intermediate states, and terminally exhausted T cells possess clearly distinct chromatin accessibility landscapes. Accessible regions in Tpex are enriched for motifs recognized by the TCF/LEF, FOXO, and ETS families, whereas terminally exhausted cells show greater accessibility at NR4A, NFAT, BATF/IRF, and AP-1 family sites [2,12,13]. These findings indicate that exhaustion is not merely a reduction in transcriptional output, but a stable state transition accompanied by extensive chromatin remodeling. Consequently, therapies that enhance only short-term transcriptional activity may fail to overcome established chromatin barriers. Notably, exhaustion-associated epigenetic remodeling is not fully reversible. Although PD-1 blockade can transiently improve certain effector functions, chromatin accessibility often remains characteristic of exhaustion, and cells rapidly return to the exhausted state once treatment is withdrawn. This helps explain why ICIs primarily act by expanding Tpex that have not yet undergone irreversible epigenetic locking, rather than by fully reprogramming terminal Tex [11]. A more realistic strategy for improving durable responses may therefore be to expand the Tpex pool and delay epigenetic fixation, rather than expecting complete de-exhaustion of terminal Tex.

DNMT3A, TET2, and ASXL1 are common epigenetic regulators associated with clonal hematopoiesis. In $CD8^+$ T cells, knockout of any of these genes can preserve the Tpex subset over the long term under chronic antigen exposure, including both chronic infection and tumor conditions. DNMT3A and TET2 regulate DNA methylation and demethylation, respectively, whereas ASXL1 controls the PR-DUB complex and thereby influences H2AK119 monoubiquitination, regulating a checkpoint in the differentiation of Tpex into terminally exhausted cells [13].

From a translational standpoint, epigenetic priming may represent a practical strategy for expanding the Tpex pool [44]. Low-dose decitabine, a DNA methylation inhibitor, can enhance Tpex proliferation and effector potential while preserving expression of stemness genes such as TCF1 by modulating methylation at effector- and exhaustion-related loci. When combined with anti-PD-1 therapy, decitabine promotes intratumoral Tpex expansion and restricts excessive terminal differentiation [45]. These findings suggest that even without direct genetic modification of T cells, remodeling the epigenetic landscape can improve Tpex expandability and provide a clinically accessible foundation for combination therapy.

4 TUMOR IMMUNOTHERAPEUTIC STRATEGIES TARGETING TPEX AND TRANSLATIONAL PROGRESS

Therapeutic development centered on Tpex is redirecting tumor immunotherapy away from an exclusive focus on effector enhancement and toward expansion of the precursor pool, preservation of differentiation plasticity, and optimization of effector timing. Current evidence indicates that durable benefit from immune checkpoint blockade depends heavily on both the quantity and quality of Tpex, as well as on the integrity of the niches that support them. Compared with attempts to reverse deeply terminal Tex, protecting and mobilizing Tpex—which still retain self-renewal and redifferentiation potential—may offer a more realistic path toward improving long-term therapeutic efficacy.

4.1 Expanding and Stabilizing the Tpex Pool: From Immune Enhancement to Differentiation Control

A major strategy for targeting Tpex is to expand the precursor pool and delay terminal differentiation through epigenetic or cytokine-based modulation. Representative studies have shown that decitabine combined with anti-PD-1 therapy promotes Tpex expansion while restricting terminal differentiation, suggesting that a sequential strategy of priming followed by checkpoint blockade may be more effective than simply intensifying immune activation. Compared with direct intervention on individual epigenetic regulators, short-course, reversible, and dose-controllable pharmacologic approaches currently appear more feasible for clinical translation [46].

IL-2 is a key cytokine in T-cell proliferation and differentiation, but conventional high-dose IL-2 therapy is limited by severe toxicity and by preferential stimulation of regulatory T cells, which express high levels of IL-2Rα/CD25. Of note, engineered IL-2 variants developed in recent years can selectively bind the IL-2Rβγ complex rather than IL-2Rα, thereby preferentially stimulating $CD8^+$ T cells or adoptively transferred CAR T cells [47-49]. Excessively strong IL-2 signaling, however, may accelerate Tpex differentiation toward terminal effector and exhausted states through the STAT5-BLIMP1 axis, thereby shrinking the Tpex pool. The intensity, timing, and combination of IL-2 signaling with ICIs must therefore be carefully optimized so that Tpex can expand without being prematurely driven into differentiation.

4.2 Reconstructing the Tpex Niche: TDLNs and APC Niches as Therapeutic Targets

In addition to acting directly on T cells, reconstructing the niches required for Tpex generation and maintenance represents another important translational direction. Existing studies suggest that TDLNs are not merely sites of antigen drainage, but also critical locations for Tpex formation, storage, and sustained replenishment. Similarly, intratumoral APC-rich regions are closely associated with Tpex reactivation and differentiation. Accordingly, the target organs of Tpex-directed therapy should not be restricted to the tumor alone, but should also include TDLNs and related immune tissues [50]. Clinical and preclinical evidence indicates that preserving intact TDLN structure may help maintain responsiveness to immunotherapy, whereas targeted delivery of immunomodulatory agents to TDLNs may enhance expansion of both Tpex and tissue-resident-like $CD8^+$ T cells [50,51]. These observations suggest that the integration of surgery, local therapy, and immunotherapy should explicitly take immune niche preservation into account. This point may be particularly relevant in the neoadjuvant setting, where TDLN function could shape both the therapeutic window and long-term benefit.

4.3 Combined Blockade of Resistance-Associated Inhibitory Pathways

A defining feature of terminally exhausted T cells is the coexpression of multiple inhibitory receptors. PD-1, LAG-3, TIM-3, TIGIT, and CD39 are often expressed simultaneously, forming a redundant inhibitory network. As a result, single-checkpoint blockade may have limited efficacy because other inhibitory receptors can compensate. Consequently, dual or multiple checkpoint blockade strategies, such as anti-PD-1 plus anti-LAG-3 or anti-PD-1 plus anti-TIGIT, are being actively explored in clinical trials [52,53]. From the perspective of Tpex biology, the principal value of these combinations may lie less in reversing already terminal states and more in slowing the conversion of Tpex into terminally exhausted cells. This view provides a more mechanistically grounded basis for rationally designing combination regimens.

5 CURRENT EVIDENTIARY BOTTLENECKS AND FUTURE RESEARCH DIRECTIONS

5.1 Current Bottlenecks

Although research on Tpex has greatly deepened our understanding of tumor immunotherapy responses, several major limitations remain. First, the phenotypic definition of Tpex has not been standardized. Different studies use markedly different marker combinations, thresholds, and tissue sources to define Tpex, including TCF1, SLAMF6, CXCR5, TIM-3, CD39, CD62L, CX3CR1, and TOX. This inconsistency reduces comparability across studies and hinders translation into robust clinical detection systems. Future work should establish a standardized framework that integrates phenotype, spatial localization, and functional readouts [2,9,11].

Second, longitudinal causal evidence remains insufficient. Although most studies support an association between Tpex abundance and ICI response or prognosis, truly prospective investigations spanning pre-treatment, on-treatment, and post-treatment phases—and integrating lineage tracing, spatial analysis, and functional validation—are still rare. It

therefore remains difficult to determine conclusively whether Tpex are direct drivers of therapeutic benefit or primarily correlative markers of response [1,10,44].

Third, most current studies focus on single-factor analyses, whereas Tpex formation and maintenance are inherently shaped by the coupled effects of transcriptional regulation, metabolic state, epigenetic remodeling, and niche-derived signals. Intervening in one pathway may improve a local process, but it may also trigger compensatory changes or even accelerate depletion of the precursor pool. Future work should therefore adopt systems biology approaches, multifactor perturbation strategies, and integrative spatial multi-omics to identify combinatorial targets with genuine translational promise [11,53].

In addition, many of the central insights in the Tpex field still come from chronic infection models and mouse tumor models, and their applicability to human tumors requires further validation [4,29]. Humans and mice may differ in marker expression, tissue distribution, niche structure, and dynamic responses to therapy. Strengthening comparative studies using clinical samples, multiregional tissues, and humanized models will therefore be essential for improving the external validity of current conclusions.

5.2 Future Directions

Future research will likely move forward in three main directions. First, spatial resolution and dynamic tracking need to be strengthened. Spatial transcriptomics, spatial proteomics, and lineage-tracing approaches should be integrated to systematically characterize the distribution, neighboring cellular interactions, and differentiation trajectories of Tpex in TDLNs, tertiary lymphoid structures, the invasive margin, and the tumor core. This should clarify the specific roles played by different niches in Tpex generation, maintenance, and output [54,55].

Second, temporally informed therapeutic frameworks should be established for clinical use. Tpex should be managed as dynamic immune resources rather than assessed only as static biomarkers. Future combination strategies ought to place greater emphasis on intervention sequence and therapeutic windows. For example, therapies may be optimized around a sequential framework that first expands the precursor pool, then releases inhibitory constraints, and finally promotes effective effector output.

Third, patient selection systems should be refined. Beyond measuring baseline Tpex abundance, future models should incorporate indicators such as Tpex expandability, TDLN functional status, the presence of tertiary lymphoid structures, cDC1 infiltration, and the spatial proximity between Tpex and APCs. These parameters should be integrated with circulating immune biomarkers and intratumoral multi-omics features into multimodal predictive models. Such an approach may improve response prediction and provide a stronger basis for individualized combination immunotherapy.

6 CONCLUSION AND PERSPECTIVES

As a key subset within the exhaustion differentiation hierarchy, Tpex combine self-renewal capacity with redifferentiation potential and have emerged as a central entry point for understanding durable responses to tumor immunotherapy. Current evidence indicates that the long-term efficacy of immune checkpoint blockade depends less on functional recovery of terminally exhausted cells than on the abundance, differentiation plasticity, and niche support of Tpex. Accordingly, optimization of tumor immunotherapy is shifting from a narrow emphasis on enhancing effector function to a broader strategy aimed at maintaining, expanding, and efficiently mobilizing the precursor pool.

At the same time, the formation and maintenance of Tpex are jointly shaped by transcriptional regulation, metabolic adaptation, epigenetic remodeling, and microenvironmental signals, suggesting that no single universal regulatory switch exists. Future strategies with stronger translational potential should therefore respect differentiation hierarchy and therapeutic timing, and should emphasize systematic combination designs centered on Tpex expansion, niche remodeling, and intervention in resistance-associated pathways, rather than focusing only on reversal of terminal Tex. Overall, research on Tpex is moving tumor immunotherapy beyond short-term effector intensity and toward a broader concern with durability, plasticity, and the tissue ecology of immune responses. Continued advances in spatial multi-omics, lineage tracing, and dynamic clinical monitoring are expected to deepen both mechanistic understanding and translational application of Tpex biology, thereby providing a stronger theoretical foundation for precision immunotherapy.

COMPETING INTERESTS

The authors have no relevant financial or non-financial interests to disclose.

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