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Review Article

T-cell response to checkpoint blockade immunotherapies: from fundamental mechanisms to treatment signatures

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Immune checkpoint immunotherapies act to block inhibitory receptors on the surface of T cells and other cells of the immune system. This can increase activation of immune cells and promote tumour clearance. Whilst this is very effective in some types of cancer, significant proportions of patients do not respond to single-agent immunotherapy. To improve patient outcomes, we must first mechanistically understand what drives therapy resistance. Many studies have utilised genetic, transcriptional, and histological signatures to find correlates of effective responses to treatment. It is key that we understand pretreatment predictors of response, but also to understand how the immune system becomes treatment resistant during therapy. Here, we review our understanding of the T-cell signatures that are critical for response, how these immune signatures change during treatment, and how this information can be used to rationally design therapeutic strategies. We highlight how chronic antigen recognition drives heterogeneous T-cell exhaustion and the role of T-cell receptor (TCR) signal strength in exhausted T-cell differentiation and molecular response to therapy. We explore how dynamic changes in negative feedback pathways can promote resistance to single-agent therapy. We speculate that this resistance may be circumvented in the future through identifying the most effective combinations of immunotherapies to promote sustained and durable antitumour responses.

Introduction

Activation of T cells is controlled by their T-cell receptor (TCR), which recognises short amino acid sequences (peptides) presented on major histocompatibility complex (MHC) molecules by antigen-presenting cells (APCs). Conventional T cells can be $CD8^+$ 'cytotoxic' or $CD4^+$ 'helper' subsets, defined by their ability to bind peptide-loaded MHC (pMHC) class I or II, respectively. Direct recognition of pMHC on target cells is important for killing [1]; MHC I is constitutively expressed and loaded with self-peptide in almost all cells, whereas MHC II expression is constitutive only in 'professional' APCs such as dendritic cells (DCs). As such, many cancers are MHC II^- [2], making $CD8^+$ T cells the primary cytotoxic population driving the anticancer response. However, in some cases, cells of epithelial lineage can express MHC II in response to interferon signalling [3] and cytotoxic $CD4^+$ T cells can kill MHC II^+ cancer cells [4].

T-cell activation results in production of key cytokines, such as Interferon-gamma (IFNγ) and tumour necrosis factor-alpha (TNF-α), and cytolytic granules such as Perforin and Granzymes. Activated T cells proliferate and can differentiate into memory cells, persisting for long periods of time primarily in the blood and lymphoid tissues, primed to respond quickly to subsequent infection or malignancy. Alternatively, activated T cells can differentiate into an effector phenotype – effectors are more cytotoxic and can reside in tissues to exert their effector function, but retain less proliferative capacity.

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Strength of TCR signalling has important roles in T-cell differentiation and function. T cells need to distinguish between weak tonic signals that occur in the lymphoid environment to promote survival [5,6], and strong, foreign antigen signals that require an immune response. In mice, comparatively stronger TCR signalling promotes effector function over memory in both $CD4^+$ [7] and $CD8^+$ T cells [8–10] during acute stimulation. Also, whilst cytotoxic fate of $CD8^+$ T cells is largely independent of initial signal strength, strength of TCR recognition on target cells dictates directional polarisation of cytolytic granules and effective killing [11,12].

Effective antitumour immunity requires T-cell responses to tumour neoantigens

Tumours are derived from self, making their recognition challenging for T cells that are self-tolerised during thymic development [13]; nevertheless, T cells can recognise a variety of cancer associated patterns through their TCR [14], the most potent of which are neoantigens. Neoantigens are novel amino acid sequences arising from nonsynonymous mutations that can deliver a signal to the TCR when presented on MHC molecules [15]. High numbers of nonsynonymous mutations tend to be associated with exogenous mutagens or certain types of genetic instability [16]. Accordingly, experimental induction of high neoantigen burden via loss of DNA mismatch repair machinery results in tumour rejection by mice [17].

In addition to key effector functions, TCR ligation drives the expression of multiple costimulatory and coinhibitory receptors that can recruit kinases or phosphatases to positively or negatively regulate TCR signalling [18]. Programmed cell death 1 (PD-1) appears to be the dominant coinhibitory receptor and is expressed by both naïve and antigen experienced T cells upon TCR ligation. Inhibitory signalling via PD-1 targets both the CD28 costimulatory molecule [19,20] and the TCR itself [21]. Other coinhibitory receptors, such as lymphocyte-activation gene 3 (LAG-3), T-cell immunoreceptor with Ig and ITIM domains (TIGIT), and T-cell immunoglobulin and mucin domain 3 (TIM-3), are expressed only in T cells with more prolonged antigen experience due to epigenetic inaccessibility of their loci in naïve populations [22]. Signalling via coinhibitory receptors raises the TCR signalling threshold, such that an antigenic stimulus that would otherwise activate a T cell fails to elicit a response [23]. Blockade of coinhibitory receptors, or their ligands, using therapeutic monoclonal antibodies (immune checkpoint blockade (ICB)) can increase T-cell function and drive clinical benefit in some types of cancer [24].

In the U.K., NICE approval for ICB therapy is based on evidence of improved survival from clinical trials. Response to ICB is highly variable between different types of cancer. Cancers with high mutation rates, such as melanoma and lung squamous-cell carcinoma, tend to respond well [25]. However, even within patient groups that respond well, such as melanoma, there are some patients that are therapy resistant. Therapy resistance can broadly be defined as primary resistance, where patients fail to respond to primary treatment, and acquired resistance, where patients initially respond but then become resistant [26].

Much of what drives resistance is tumour intrinsic [27]. Analysis of genetic signatures in pretreatment tumour biopsies has revealed predictors of response to ICB, such as clonal mutational burden and signatures of immune recruitment such as CXCL9 expression [27]. In patients that do initially respond, ICB exerts immune pressure on the tumour, to which it can often adapt. Tumours can undergo immunoediting by acquisition of mutations in key antigens that are driving the immune response, or the pathways that present these antigens to T cells. However, in some cases, patients may still respond to ICB in the absence of antigen recognition. For example, in β2-Microglobulin-mutated dMMR colorectal cancer where γδ T cells recognise stress ligands on tumour cells via NKG2D [28].

Tumours may also acquire mutations in JAK/STAT signalling pathways that mediate the response to IFNγ signalling [29], an important effector mechanism of the anticancer CD8 T-cell response. Tumours can remodel their microenvironment to promote therapy resistance [30]; factors such as hypoxia [31], immune exclusion by tumour stroma [32], and infiltration of regulatory immune cells such as myeloid-derived suppressor cells [33] can subvert the anticancer immune response.

Distinct from tumour intrinsic factors, there are T-cell intrinsic factors that can also mediate resistance. Understanding the T-cell signatures (or biomarkers) that predict response to therapy, but also how these signatures change during therapy can provide insight into mechanisms of therapy resistance. Experimental work in animal models of chronic antigen exposure can help us understand the fundamental processes that govern T-cell regulation, and therefore T-cell intrinsic therapy resistance. Here, we discuss the fundamental mechanisms that regulate T-cell biology in the face of chronic antigen exposure. We overview how preclinical mouse models have set a platform for translational discovery and highlight key similarities and differences that have been observed in human clinical studies of patients on ICB.

Table 1 Phenotypic and functional differences between naive and exhausted subsets of CD8⁺ T cells during chronic LCMV infection in mice

	Naive	T_{PEX}	T_{EX} intermediate	T_{EX} terminal
TCF1	++	+	-	-
TOX	-	+	++	++
TBET	-	+	-	-
EOMES	-	-	+	+
CD127	+	+/-	-	-
CD62L	++	+/-	-	-
CX3CR1	-	-	+	-
CXCR6	-	-	-	+
PD-1	-	+	++	++
TIM-3	-	-	+	+
KLRG1	-	-	+	-
Proliferative capacity	+	+	-	-
Cytotoxicity	-	-	+	+/-
Cytokine production	+	+	+	-

Negative feedback control of T cells in mouse models of infection and tolerance

Experimental models of chronic antigen exposure, such as lymphocytic choriomeningitis virus (LCMV) infection, and techniques to identify antigen-specific T cells such as TCR transgenics and pMHC tetramers, have informed our fundamental understanding of negative feedback control of T cells.

Negative feedback mechanisms, including coinhibitory receptors, are an intrinsic and indispensable feature of T-cell function; their absence can cause activation-induced T-cell death [34] and lethal immunopathology during infection [35]. Chronic antigen exposure in cancer or infection can drive 'exhaustion' – a state of T-cell differentiation distinct from effector/memory phenotypes, characterised by expression of multiple coinhibitory receptors and progressive loss of function [36,37]. Exhausted T cells lose proliferative potential, long-term survival, ability to produce cytokines, and ability to kill target cells. Although there are many similarities between exhausted $CD8^+$ T cells and effectors arising from acute antigen stimulus, there are transcriptional signatures and characteristics specific to T-cell exhaustion, uncoupled from effector function [38]. In mice, blockade of the ligand for PD-1 (PD-L1), can induce proliferation in LCMV specific exhausted $CD8^+$ T cells [35].

LCMV studies have highlighted the significant heterogeneity in T-cell exhaustion (Table 1). Many have drawn a distinction between T cells that are exhausted (T_{EX}, usually indicated by coexpression of multiple coinhibitory receptors including PD-1 and Tim-3 or CD39 [36]), and the precursor to these cells (T_{PEX}, usually indicated by coexpression of PD-1 and transcription factor T-cell factor 1 (TCF1) [39] or CXCR5 [40,41]). Loss of T-cell functionality in response to chronic antigen is graded and T_{PEX} cells are at an early point in the trajectory. T_{PEX} cells can undergo a TCR signal-dependent conversion to T_{EX} cells [42], a process that is augmented by T-cell intrinsic IFNγ signalling [43]. In contrast, T_{EX} cells are a stable population that maintain phenotypic and functional features in transfer experiments [44]. In many ways, T_{PEX} and T_{EX} demonstrate a functional polarisation like memory and effector $CD8^+$ T cells during acute stimulation. T_{PEX}, unlike T_{EX}, show a selective ability to undergo proliferative burst following ICB [39,40].

Transcription factors such as TOX [34,45,46], BLIMP-1 [47], MYB [48], NR4A1 [49,50], and NFAT [51] mediate the exhaustion programme by controlling the expression of negative regulators of TCR signalling. Exhausted T cells also undergo significant epigenetic rewiring following as little as 5 days of chronic antigen exposure [52,53]. In mouse models, it is largely thought that terminally exhausted T cells are incapable of response to ICB due to the epigenetic repression of genes involved in important T-cell functions [54] and *TCF7* (encoding TCF1) itself [55].

Failure of T_{EX} cells to respond to ICB monotherapy may be a result of functional redundancy between numerous coinhibitory receptors [56]. In contrast, negative signalling via PD-1 may be the limiting factor for T_{PEX} cells to signal via their TCR, explaining their selective response to monotherapy. This may then suggest that blockade of multiple coinhibitory receptors is necessary to restore signalling in T_{EX}. For example, it has been shown that dual blockade of coinhibitory receptors PD-1 and TIGIT can restore cytokine production in TOX^+ $CD8^+$ tumour-infiltrating lymphocytes (TILs) *in vitro* [57].

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Some studies have challenged this idea. For example, combining blockade of PD-L1 with a vaccination approach has been shown to expand PD-1hi antigen-specific $CD8^+$ T cells in an infection model [58]. Additionally, TIM-3^+ exhausted T cells can proliferate in response to engineered Interleukin-10 [59]. These data show it may be possible to reinvigorate terminally exhausted T cells. Discrepancies between studies may be due to functional heterogeneity within $TCF1^-$-exhausted subsets. Some $TCF1^-$ subsets are at an intermediate stage of exhaustion, they express CX3CR1 and KLRG1 and make IFNγ. Other more terminally exhausted cells express CXCR6 and produce very low levels of IFNγ [60].

Not just duration, but TCR signal strength appears to be a key determinant of fate decisions among exhausted CD8 T-cell subsets. T cells with higher avidity for antigen, as determined by tetramer staining, showed a biased for terminal exhaustion differentiation [60]. Additionally, utilising the *Nr4a3*-Tocky reporter of TCR signalling and a model of adaptive tolerance, we have highlighted $CD4^+$ T-cell transcriptional programmes that are related to defined TCR signal strengths [23]. Crucially, we found that strong TCR signalling increased the expression of many coinhibitory receptors, in agreement with work on $CD8^+$ T cells in tumour models [61]. Additionally, we have found significant overlap in genes associated with strong TCR signalling, and genes up-regulated in antigen experienced T cells reactivating in response to PD-1 blockade. This evidence suggests that blockade of inhibitory signalling does not only increase the probability that a given antigen-experienced T cell will reactivate in response to subsequent TCR signals, but that the qualitative strength of the signal is higher. This agrees with *in vitro* studies that have shown selective sensitivity to PD-1-mediated inhibition in genes that require strong TCR signalling [62].

Negative feedback control of T cells in mouse models of cancer

Chronic infection models have some key limitations in understanding T-cell negative feedback in cancer. Tumour-reactive T cells circulate between the tumour, blood, and lymphoid tissue and a systemic immune response is key for effective anticancer immunity [63]. In contrast with systemic LCMV infections, tumour antigens are largely restricted to the tumour site. This is an important consideration, as cellular therapy studies have found withdrawal from chronic antigen stimulation can restore function to exhausted T cells [64].

Many paradigms from LCMV studies have been replicated in mouse models of cancer. Subcutaneous implantation of tumour cells leads to generation of T_{EX} and T_{PEX} cells that are transcriptionally analogous to those generated during LCMV infection [42]. T_{PEX} are essential for the maintenance of a T-cell response in mouse models of cancer, they can traffic between lymph nodes and tumours [65,66], acting as a reservoir [67] to supply tumours with cells that gain effector functions upon arrival [68]. Despite representing a small proportion of tumour-specific $CD8^+$ T cells, selective deletion of tumour-specific *Tcf7*$^+$ $CD8^+$ T cells accelerates tumour growth and diminishes response to ICB in mouse models [69]. These data in mouse models suggest that signatures relating to T_{PEX} may indicate an effective ongoing anticancer immune response, and the presence of cells that can respond well to ICB therapy.

T-cell exhaustion is not limited to $CD8^+$ T cells. Cancer neoantigens are frequently restricted to MHC II and recognised by $CD4^+$ T cells in both mice [70] and humans [71]. In a poorly immunogenic mouse model of cancer, experimental induction of an MHC I restricted neoantigen only conferred sensitivity to ICB when an MHC II restricted neoantigen was also induced [72]. $CD4^+$ T-cell recognition of tumour antigen led to increased numbers of $CD8^+$ T cells through the well-established principle of $CD4^+$ T-cell help [73], whereby $CD4^+$ T cells provide support to $CD8^+$ T cells in the form of costimulation and cytokine production, either directly or via DCs (Figure 1). This happened despite lack of MHC II expression on tumour cells, indicating that $CD4^+$ T cells recognised tumour antigens that were phagocytosed and presented by professional APCs. Therefore, whilst direct recognition of MHC II^+ tumour cells can mediate tumour rejection [74], it is not prerequisite for $CD4^+$ T-cell involvement in the anticancer immune response.

Like work in models of chronic infection and adaptive tolerance, strength of TCR signalling can dictate fate of antitumour $CD8^+$ T cells. High-affinity interactions between the TCR and pMHC drive higher levels of negative feedback, whilst particularly low affinity interactions lead to ineffective killing [61]. TCR signal strength appears to be a key determinant of T_{EX} versus T_{PEX} fate; whilst both subsets can share clonotypes [68], it appears that lower-affinity TCR-pMHC interactions favour T_{PEX} differentiation [75].

Expression levels of coinhibitory receptors are dependent on TCR signal strength and PD-1 blockade increases TCR signal strength [23,60]. We have observed elevated expression of genes encoding multiple coinhibitory receptors in antigen-adapted T cells that reactivated their TCR signalling in response to PD-1 blockade [23]. In mouse models of cancer, it has been observed that T cells bound by therapeutic PD-1 antibodies have increased expression of TIM-3 [76]. Consequently, combination of PD-1 and TIM-3 blockade showed better clinical response than PD-1 blockade alone [76,77]. Additionally, PD-L1 and LAG-3 combination therapy has shown synergistic effects on $CD4^+$ and $CD8^+$

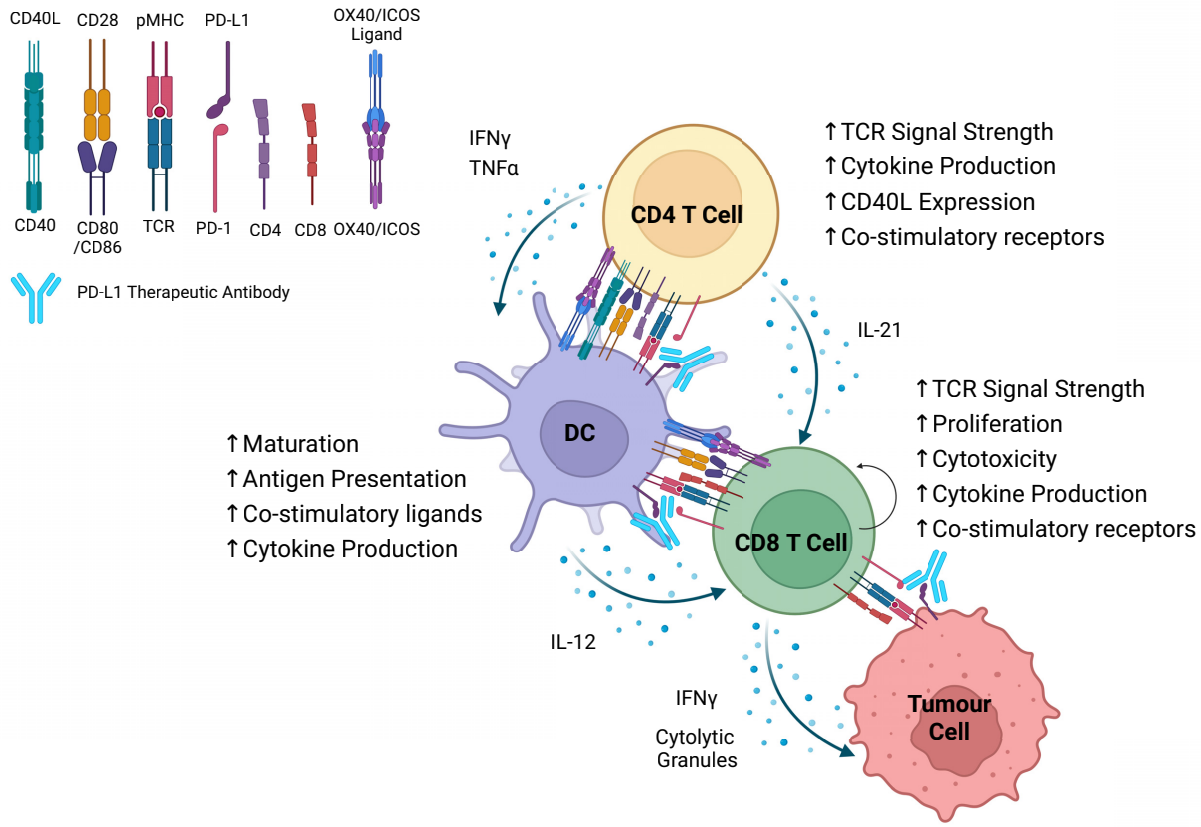

Figure 1. Graphical representation of reported changes to function of CD4⁺ T cells, CD8⁺ T cells, and DCs in response to αPD-L1 immunotherapy

Distinct from direct effect on CD8⁺ T cells, immunotherapy can act via CD4⁺ T cells and DCs to support CD8⁺ T-cell function via cytokine production and costimulation.

T-cell cytokine production and tumour control [78]. Therefore, combination blockade is an attractive strategy to not only target T cells with very high levels of inhibitory signalling but also to combat therapy induced dynamic feedback and acquired therapy resistance [79]. Signatures that can identify dynamic changes in negative feedback may therefore be valuable in the design of combinatorial ICB therapies and on-therapy monitoring of efficacy.

T-cell signatures in response to ICB in human cancer

Mouse models are a valuable tool for studying the processes that underlie ICB therapeutic success. However, the time periods for disease development differ significantly, and many mouse models are unable to account for the significant interpatient heterogeneity in human cancer.

Systemic response to ICB

Whilst many studies in mouse models of cancer focus on intratumoural T cells, it is now recognised that an effective response to ICB is in fact systemic [63]. Interaction between T cell PD-1 and DC PD-L1 in tumour-draining lymph nodes is an important facet of ICB activity in mice [80]. In melanoma patients, ICB can lead to clonal replacement of intratumoural CD8⁺ T cells with those from peripheral sites [81]. In addition, sequencing of peripheral blood T cells before ICB therapy has found that expanded cytotoxic CD8 T-cell clones are associated with beneficial response [82]. The peripheral blood of melanoma patients exhibits an increased number of highly expanded clones as compared with healthy controls, reflective of T-cell proliferation in response to recognition of tumour antigens [82]. An increase in the number of highly expanded clones in response to therapy is associated with good clinical outcomes [83]. Clones possessing a central memory signature can convert to a cytotoxic effector signature in the peripheral blood of patients responding to ICB [82]. This demonstrates how ICB can increase TCR signal strength to drive differentiation of

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cytotoxic effector cells to mediate tumour cell killing. These findings are corroborated by analysis of tumour reactive T cells from peripheral blood of lung cancer patients [84]: successful ICB responses were associated with an increase in expression of effector/exhaustion markers and a decrease in memory phenotypes during treatment.

In the periphery, highly expanded cytotoxic CD8 T-cell clones show the greatest transcriptional response to ICB, without increasing their clonal size [82]. These cells express many genes typically associated with CD8 T-cell exhaustion such as *TOX*, *HAVCR2*, and *TIGIT*. Similar studies in urothelial cancer have shown expanded tumour-specific CD8 clones with a cytotoxic phenotype in the periphery are predictive of effective ICB response [85]. This marks a clear difference between mouse LCMV models and human disease-exhausted $CD8^+$ T cells in human cancer are likely not 'nonresponders' but respond differently to $CD8^+$ T cells at an earlier stage of differentiation. This highlights a mechanism distinct from proliferation as a key determinant of response to ICB and agrees with mouse *in-vitro* studies that have found exhausted $CD8^+$ T cells to be effective killers [61].

Intratumoural responses to ICB

Early studies in humans receiving ICB identified TIL intrinsic signatures of response, including increased CD8 T-cell infiltration, T-cell proliferation, IFNγ signalling, and granzyme B production [86]. Tumour antigen-specific $CD4^+$ T cells can accumulate within human cancers [87], exhibiting hallmarks of exhaustion such as coexpression of multiple inhibitory receptors (including CD39), loss of cytokine production (a key mechanism for helping $CD8^+$ T cells), and transcriptional similarities to TOX^+ $CD8^+$ T cells [88]. Exhausted $CD4^+$ T cells can respond to immunotherapy: blockade of PD-1 during *in-vitro* stimulation of patient-derived $PD\text{-}1^+$ $CD39^+$ $CD4^+$ TILs led to increased cytokine production and expression of the costimulatory ligand CD40L. Following pretreatment with anti-PD-1, coculture of exhausted $CD4^+$ TILs with autologous DCs and $CD8^+$ T cells led to DC priming and $CD8^+$ T-cell proliferation [88]. These studies highlight the fact that both $CD4^+$ and $CD8^+$ T cells can lose critical antitumour functions, following chronic antigen stimulation and are both targets of ICB (Figure 1). Indeed, we recently cross-referenced transcriptional signatures of ICB in melanoma patients with those found in mouse CD4 T cells receiving high-strength TCR signals. We identified a five gene signature associated with clinical response, highlighting increased TCR signal strength as a key factor in driving patient survival. We termed this gene signature TCR.strong (*ICOS, TNFRS4* (OX40), *STAT4, TNIP3, IRF8*) [23]. Elevated expression of ICOS and OX40 suggests that the ICB-mediated increase in TCR signal strength acts to increase costimulation via inducible receptors (Figure 1).

A study of human lung cancer TILs utilised sequencing of complimentary determining region 3 (CDR3) loci to determine TCR specificity and cross-referenced to scRNA-seq data [84]. This allowed transcriptional comparisons of TILs reactive for tumour neoantigens and the numerous bystanders [89] reactive for resolved (influenza) or latent (EBV) infections. Tumour antigen-reactive TILs contained features of mouse T_{EX} and were enriched for gene signatures associated with tissue residency (*CD103, HOBIT*), effector function (*GZMB, IFNG*), negative feedback (*HAVCR2, ENDTP1* (CD39)), and transcriptional control of exhaustion (*TOX, BLIMP1*). Accordingly, they also expressed low levels of genes associated with memory. Similar results have been shown in melanoma [90].

Transcriptional comparisons of tumour-reactive TILs between patients found that signatures associated with memory (*IL7R, TCF7*) were correlated with a good clinical response, whereas many genes associated with effector function, exhaustion, and tissue residency were associated with treatment resistance [84]. This highlights that whilst most TILs have an exhausted signature, it is atypical TILs – resembling descriptions of T_{PEX} – that are crucial for effective therapy responses. In support of this notion, T_{PEX} can be sustained within intratumoural niches [91] to promote survival, and the presence of intratumoural $TCF1^+$ $PD\text{-}1^+$ CD8 T cells is predictive of a positive response to ICB in melanoma patients [90,92].

T-cell signatures of exhaustion and therapy resistance

T-cell signatures from human studies suggest that in addition to a weak pre-existing response, homogenous exhaustion to a strong pre-existing response can also drive primary therapy resistance (Figure 2). These data suggest it may be possible to avoid primary resistance by early intervention with ICB, prior to homogenous T-cell exhaustion. Treatment with ICB in the neoadjuvant setting has led to improved survival over adjuvant treatment in melanoma [93] and can drive pathological responses in canonical ICB nonresponders such as pMMR colorectal cancer [94].

ICB-induced dynamic negative feedback may contribute to acquired therapy resistance in humans. In the peripheral blood of melanoma patients responding to ICB, CD8 T cells that proliferate in response to ICB increase their expression levels of PD-1 and CTLA-4 [95]. This is not a peripheral phenomenon, since other studies have highlighted signatures of increased intratumoural negative feedback following ICB including expression of PD-1 and LAG-3 [96], and V-domain immunoglobulin suppressor of T-cell activation (VISTA) [97]. Increases in TCR strong signature following ICB were higher in immunotherapy naive patients as compared with those who progressed following CTLA-4 blockade [23]. This suggests that ICB can lead to enhanced negative feedback, driving acquired resistance to future

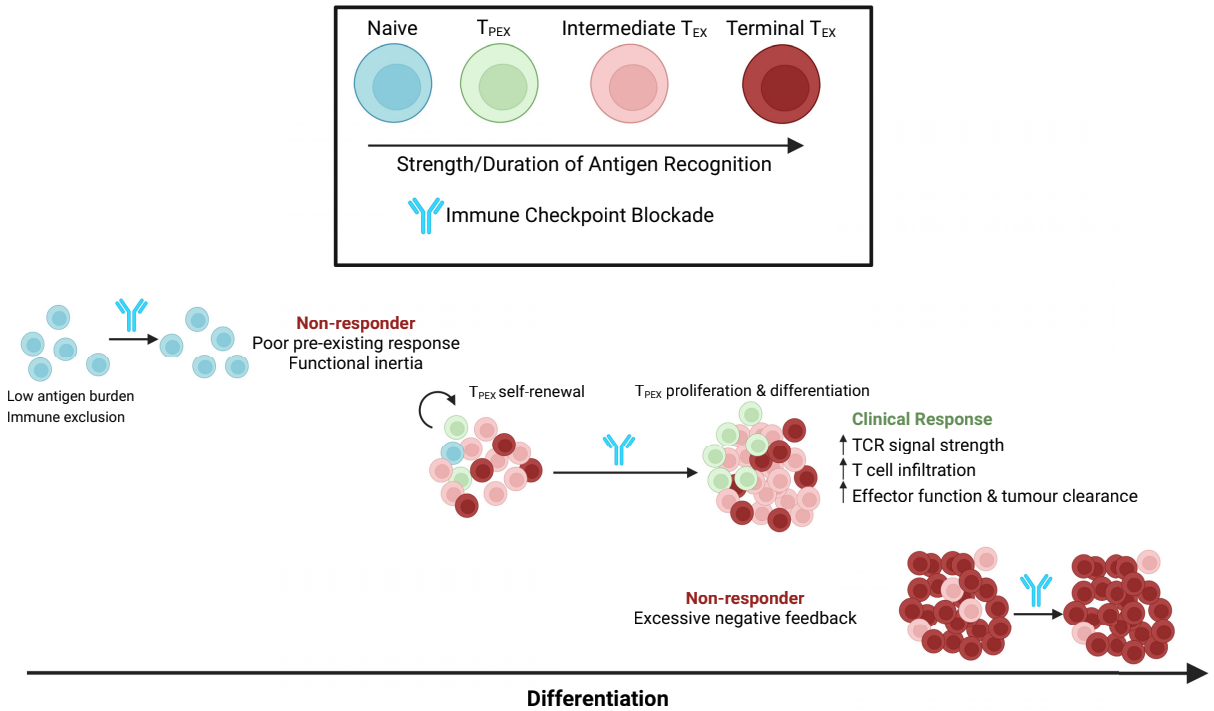

Figure 2. Graphical representation showing how αPD-L1 immunotherapy can modulate intratumoural CD8⁺ T-cell differentiation during treatment

Ineffective response may be a result of poor pre-existing response or previous terminal differentiation. Presence of less-differentiated T_{PEX} cells allows for proliferative burst and differentiation, driving clinical benefit.

ICB therapy. In support of this, new combination approaches that target coinhibitory receptors, such as LAG-3, in addition to PD-1 are showing promising results in trials [98,99]. Future work should focus on rationally identifying optimal combinations of ICB that can promote sustained antitumour immune responses. To achieve this, fundamental insight into the molecular functions of different classes of immune checkpoint is still required to inform treatment strategies in humans.

Future perspectives

To understand which therapeutic strategies are appropriate and for which patients, future research must answer two key questions: (i) what are the signatures of T cells in which functionality is restored during single agent/combination ICB? And (ii): what is the molecular response to ICB that underpins resistance in responding T cells? The former will help target them at the right patients, maximising clinical benefit and minimising unnecessary toxicity and the latter will inform design of rational therapy combinations.

Thorough mechanistic work in experimental models of cancer, tracking dynamic changes in T cells during ICB, will be key to answering the above questions. Translating work in mouse to human is a key challenge in the field: innovative approaches such as tumour-explant models [100] have allowed well-controlled comparisons between ICB approaches, providing valuable information regarding T-cell differentiation during therapy. This will help us understand what therapies synergise with blockade of ICs, such as PD-1, and how they might be used to augment or prolong the clinical benefit of ICB.

Summary

- TCR signal duration and strength drive heterogeneous T-cell exhaustion.
- Stage of T-cell exhaustion dictates molecular response to ICB.
- T-cell proliferation and effector differentiation are signatures of effective ICB therapy.

- Poor pre-existing immune response and homologous terminal exhaustion can both drive primary resistance to ICB.
- ICB may drive acquired resistance via enhanced negative feedback.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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Abbreviations

APC , antigen-presenting cell; CDR3, complimentary determining region 3; DC, dendritic cell; ICB, immune checkpoint blockade; IFNγ, Interferon-gamma; LAG-3, lymphocyte-activation gene 3; LCMV, lymphocytic choriomeningitis virus; MHC, major histocompatibility complex; PD-1, programmed cell death 1; PD-L1, ligand for PD-1; pMHC, peptide-loaded MHC; TCF, T-cell factor 1; TCR, T-cell receptor; TIGIT, T-cell immunoreceptor with Ig and ITIM domains; TIL, tumour-infiltrating lymphocyte; TIM, T-cell immunoglobulin and mucin domain 3; TNF, tumour necrosis factor-alpha.

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