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A guide to cancer immunotherapy: from T cell basic science to clinical practice

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Abstract | The T lymphocyte, especially its capacity for antigen-directed cytotoxicity, has become a central focus for engaging the immune system in the fight against cancer. Basic science discoveries elucidating the molecular and cellular biology of the T cell have led to new strategies in this fight, including checkpoint blockade, adoptive cellular therapy and cancer vaccinology. This area of immunological research has been highly active for the past 50 years and is now enjoying unprecedented bench-to-bedside clinical success. Here, we provide a comprehensive historical and biological perspective regarding the advent and clinical implementation of cancer immunotherapeutics, with an emphasis on the fundamental importance of T lymphocyte regulation. We highlight clinical trials that demonstrate therapeutic efficacy and toxicities associated with each class of drug. Finally, we summarize emerging therapies and emphasize the yet to be elucidated questions and future promise within the field of cancer immunotherapy.

Neoantigens
Antigens not expressed by self-tissues under normal conditions that manifest in the context of pathology; in cancer, these could be altered proteins/peptides encoded by mutated genes.

Immune checkpoint
A mechanism of immune cell inhibition that restrains activation.

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The idea to deploy the immune system as a tool to treat neoplastic disease originated in the nineteenth century[1]. Wilhelm Busch and Friedrich Fehleisen were the first to describe an epidemiological association between immune status and cancer. They noticed spontaneous regression of tumours following the development of erysipelas, a superficial skin infection most commonly caused by *Streptococcus pyogenes*[1]. Later, William Coley, often called the 'Father of Cancer Immunotherapy', retrospectively demonstrated that erysipelas was associated with a better outcome in patients with sarcoma[2]. With hopes of prospectively verifying his epidemiological evidence, Coley treated patients with cancer with extracts of heat-inactivated *S. pyogenes* and *Serratia marcescens* to boost immunity[3]. This extract, termed 'Coley's toxins', possessed potent immunostimulatory properties and achieved favourable responses in various cancers[2]. However, lack of scientific rigour and reproducibility, in concert with the discovery of radiotherapy and chemotherapeutic agents, prevented treatment with 'Coley's toxins' from becoming standard practice[1].

The concept of cancer immunotherapy resurfaced in the twentieth century and made significant headway with the advent of new technology. In 1909, Paul Ehrlich hypothesized that the human body constantly generates neoplastic cells that are eradicated by the immune system[3]. Lewis Thomas and Sir Frank Macfarlane Burnet independently conceived the 'cancer immuno-surveillance' hypothesis, stating that tumour-associated neoantigens are recognized and targeted by the immune system to prevent carcinogenesis in a manner similar to graft rejection[1]. Productive immune responses following tumoural adoptive transfer in mice[4] and clinical reports of spontaneous regression of melanoma in patients with concomitant autoimmune disease[5] provided additional evidence supporting this hypothesis, although a unifying mechanism was elusive. The advent of knockout mouse models provided the necessary technology to experimentally demonstrate a link between immunodeficiency and cancer[6]. Additional molecular and biochemical advances led to the identification of tumour-specific immune responses[7]. This provided unequivocal evidence that the immune system, in particular T cells (see BOX 1 and FIG. 1), was capable of waging war on cancer tissue[7]. Cancer immunotherapy has now revolutionized the field of oncology by prolonging survival of patients with rapidly fatal cancers. The number of patients eligible for immune-based cancer treatments continues to skyrocket as these therapies position themselves as the first line for many cancer indications. Novel treatment combinations and newly identified druggable targets will only expand the role of immunotherapy in the treatment of cancer in the decades to come.

In this Review, we emphasize the role of T cells in modern cancer immunotherapies and discuss three different categories of immunotherapeutic approaches to treat cancer: immune checkpoint blockade, an approach that is designed to 'unleash' powerful T cell responses; adoptive cellular therapies, which are based on the infusion of tumour-fighting immune cells into the body; and

Box 1 | T cell function, development, activation and fate

The 1960s represented a period of enlightenment within the field of immunology because two major subtypes of lymphocytes, B lymphocytes and T lymphocytes, were characterized[264,265]. This was recognized by the 2019 Lasker Award for Basic Science, awarded for the pioneering work by Jacques A. F. P. Miller and Max Dale Cooper that defined the key roles of T cells and B cells in adaptive immunity. B cells recognize circulating antigen in its native form and respond by secreting protective antibodies[266]. By contrast, T cells recognize peptide antigens, derived from proteins degraded intracellularly, that are loaded onto cell surface MHC molecules, a process called antigen presentation. Two broad classes of T cells that have distinct effector mechanisms are delineated by the expression of either the CD4 or CD8 co-receptor: CD4+ T cells detect antigen in the context of MHC class II molecules and orchestrate the adaptive arm of the immune system by producing cytokines with chemotactic, pro-inflammatory and immunoprotective properties[267]. At least one CD4+ T cell subclass, CD4+CD25+ regulatory T cells, dampens the immune response following challenge[268]. CD8+ T cells detect antigen in the context of MHC class I molecules and carry out direct cytotoxic reactions that kill infected or neoplastic cells[269].

A unique clone-specific cell surface protein complex, the T cell receptor (TCR), specifically recognizes antigens and participates in the developmental selection of T cells that can recognize pathogens but are self-tolerant[270]. The TCR complex comprises highly polymorphic single α- and β-glycoprotein chains (a small T cell population harbours γ- and δ-chains instead) that contain variable and constant regions, akin to immunoglobulins, and a group of non-polymorphic signalling chains, called CD3 γ, δ, ε and ζ. A vast repertoire of T cell clonotypes with unique specificities is generated through rearrangement of α- and β-chain gene segments within the genome of each T cell[271]. Following clonotype production, positive and negative thymic selection functions to entrain a 'tolerant' immune system, one that efficiently responds to pathogens or cancer cells but generally ignores or 'tolerates' self-tissues as non-immunogenic[269,270].

Antigen stimulation of the TCR is necessary for T cell activation and proliferation, but an additional signal, termed co-stimulation, is required for phosphorylation events crucial for early signal transduction[272]. The non-polymorphic surface protein CD28 and its family members are the most potent co-stimulatory receptors on T cells, as elegantly demonstrated by the synergism of anti-CD28 stimulatory antibodies and TCR engagement on T cell activation and proliferation[273,274]. Additional evidence was provided by studies demonstrating the efficient inhibition of T cell activation and proliferation by inhibitory anti-CD28 antibodies[275–278]. The ligands for CD28, B7-1 and B7-2, are expressed on antigen-presenting cells and are upregulated when these cells encounter microorganisms that activate Toll-like receptors or other pathogen sensors[279,280]. Inhibitory molecules, including cytotoxic T lymphocyte-associated protein 4 (CTLA4) and programmed cell death 1 (PD1), are induced during immune responses and represent a 'checkpoint' to dampen T cell hyperactivation[281] (see FIG. 2). The polymorphic TCR signals through a complex of three sets of dimeric CD3 chains, ε–δ, γ–δ and ζ– ζ[282]. The intracellular portions of the CD3 chains contain immunoreceptor tyrosine-based activation motifs that are phosphorylated by lymphocyte-specific protein kinase (LCK), a SRC family kinase[283]. At rest, the surface signalling protein CD45 exhibits phosphatase activity that blocks LCK function[284]. Following activation, CD45 removes an inhibitory phosphate on LCK, permitting phosphorylation of ζ chain-associated protein kinase 70 (ZAP70), a SYK kinase family member that binds to immunoreceptor tyrosine-based activation motifs in the CD3 ζ-chain and recruits the linker for activation of T cells (LAT) and phospholipase Cγ1 (PLCγ)[285]. With ample co-stimulation, downstream signalling affects calcium release, the activation of the GTPase RAS and transcriptional reprogramming essential for activated T cell function[286].

Following activation, circulating naive T cells have three major fates in the periphery (FIG. 1). First, the effector T cell population can contract through apoptosis as the immune response resolves (cytokine withdrawal) or following repeated high-dose stimulation (restimulation-induced cell death)[287–289]. T cells can also exhibit an exhausted phenotype induced by repeated low-dose and low-affinity stimulation, as seen in chronic infections and neoplastic processes[88]. Lastly, a subset of these effector cells are involved in long-term immunological memory. Memory T cells are primed to react more vigorously to the same antigen during a subsequent encounter, making them critical mediators of immune recall responses to pathogens and tumours[290]. Leveraging the power of technological advances in molecular biology, recent single-cell RNA sequencing and epigenomic studies have provided additional molecular insight into T cell fates and the corresponding features of immunotherapy-responsive T cells. These studies collectively implicate that complex transcriptomic, epigenomic and clonotypic changes of tumour-infiltrating T cells determine the success of immunotherapy[291–294].

cancer vaccines, which can be designed to have either prophylactic or therapeutic activity. Finally, we introduce some of the emerging targets and approaches in cancer immunotherapy.

Immune checkpoint therapy

Several evolutionarily conserved negative regulators of T cell activation act as 'checkpoint molecules' to fine-tune the immune response and regulate hyperactivation. Cytotoxic T lymphocyte antigen 4 (CTLA4) and programmed cell death 1 (PD1) are the most potent examples of T cell immune checkpoint molecules. They exert their biological effect at distinct body sites and times during the T cell lifespan[8]. Therefore, they complement each other functionally and ensure that T cell responses preserve self-tolerance while effectively protecting the body from pathogens and neoplasia. CTLA4 and PD1 have been successfully targeted by several pioneering research groups as treatments for a wide variety of recalcitrant cancers, research that ultimately earned James P. Allison and Tasuku Honjo the 2018 Nobel Prize in Physiology or Medicine.

CTLA4 biological function. After the discovery of T cell co-stimulation mediated by the surface protein CD28 (BOX 1), the search for additional immune regulators led to the identification of CTLA4, a receptor with structural and biochemical similarities to CD28, as a new immunoglobulin superfamily member[9,10]. The *CTLA4* and *CD28* genes are found in the same region of chromosome 2 (2q33.2) and are selectively expressed in the haematopoietic compartment[11]. However, in contrast to the high levels of basal CD28 expression on conventional T cells, CTLA4 is expressed at a low basal level and is strongly induced following antigen activation. Interestingly, CD4+CD25+ regulatory T (T_{reg}) cells, which have an immunosuppressive function, express CTLA4 constitutively. Structurally, both CTLA4 and CD28 form membrane-bound homodimers comprising an extracellular immunoglobulin-like domain, a transmembrane region and a cytoplasmic tail capable of recruiting signalling proteins and controlling surface expression[10,12,13]. The trafficking of CTLA4-containing vesicles to the cell surface after activation is controlled by a physical interaction with the lipopolysaccharide-responsive and

Immunoglobulin superfamily
A group of proteins with genetic and structural similarities to antibodies.

Antigen-presenting cells
(APCs). Immune cells involved in the uptake and processing of antigens to initiate cellular immune responses.

CTLA4Ig
Soluble recombinant human cytotoxic T lymphocyte antigen 4 (CTLA4) fused to the immunoglobulin Fc domain that competes with endogenous CD28 for its ligands.

Immunological synapse
An interface between interacting lymphocytes and antigen-presenting cells that controls antigen-induced signalling.

beige-like anchor protein (LRBA)[13]. The sequence similarity between CTLA4 and CD28 is highest within their extracellular binding domain and they therefore bind to the same ligands, called B7-1 (also known as CD80) and B7-2 (also known as CD86), which are expressed by antigen-presenting cells (APCs; BOX 1). However, CTLA4 has greater affinity and avidity than CD28 for B7 ligands, representing a key difference in their biology[14–16].

With further characterization, it became clear that CD28 and CTLA4 had opposite immunoregulatory functions. For example, soluble CTLA4 was shown to inhibit the proliferation of T cells co-cultured with B7-expressing APCs because it interfered with the CD28–B7 interaction[14]. T cell receptor (TCR) signalling studies unequivocally demonstrated that CTLA4 inhibits T cell activation and proliferation[12,17,18]. The negative tolerogenic role of CTLA4 was also evident in vivo, because *Ctla4*-knockout mice developed a characteristic T cell-mediated lymphoproliferative autoimmune

disease[19]. The absence of *Ctla4* was sufficient to cause this phenotype, as treatment with an engineered soluble version of a CTLA4:Fc fusion protein (CTLA4Ig) and genetic crosses to B7-deficient mice ameliorated disease[20,21]. The autoimmune lymphoproliferative disorder caused by *Ctla4* loss depends on the activity of CD28 because mutation of an LCK-binding carboxy-terminal proline motif in the intracellular tail of CD28 abrogates disease in mouse models[22]. Moreover, human patients with *CTLA4* haploinsufficiency exhibit similar severe multiorgan lymphocytic infiltration and autoimmunity (CHAI disease) that can be treated with abatacept, an FDA-approved CTLA4Ig[23,24].

CTLA4 restrains T cell activation through multiple mechanisms: by directly antagonizing CD28, by competing for co-stimulatory ligands, by preventing immune conjugate formation and by recruiting inhibitory effectors[25] (FIG. 2). To directly oppose CD28 activity, intracellular vesicles release CTLA4 at the immunological synapse
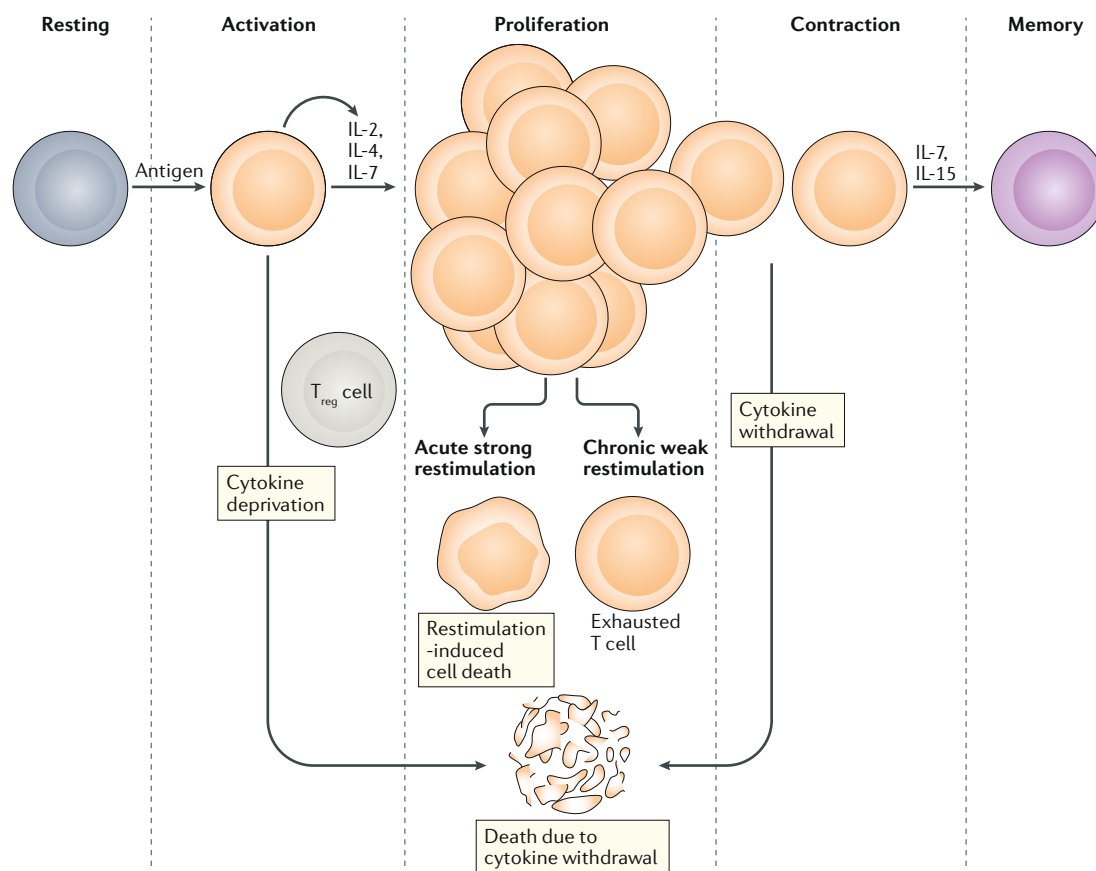
Fig. 1 | **Peripheral T cell fates after antigenic activation.** Resting T cells become activated after stimulation by cognate antigen in the context of an antigen-presenting cell and co-stimulatory signals. Activated T cells produce and consume proliferative/survival cytokines, for example, IL-2, IL-4 and IL-7, and begin to expand in number. If CD4+CD25+ regulatory T (T_{reg}) cells are present, they can deprive the cycling T cells of proliferative/survival cytokines, especially IL-2, causing them to undergo apoptosis. Once cells are proliferating rapidly, they have different fates depending on their environment. If they receive acute strong antigenic stimulation, especially if it is encountered repeatedly, the cells will undergo restimulation-induced cell death. By contrast, if they receive chronic weak antigenic stimulation, the cells will survive but become reprogrammed into a specific unresponsive transcriptional state known as 'T cell exhaustion'. Finally, as the antigen and cytokine stimulation diminishes as the immune response wanes, usually once the pathogen has been cleared, cytokine withdrawal can occur passively to contract the expanded population of antigen-specific T cells. A small fraction of cells will be reprogrammed to enter a 'memory' phenotype, and this differentiation step is facilitated by IL-7 and IL-15. Memory T cells will continue to persist in the immune system and form the basis of anamnestic responses. In these regulatory processes, T cell death usually takes the form of apoptosis.
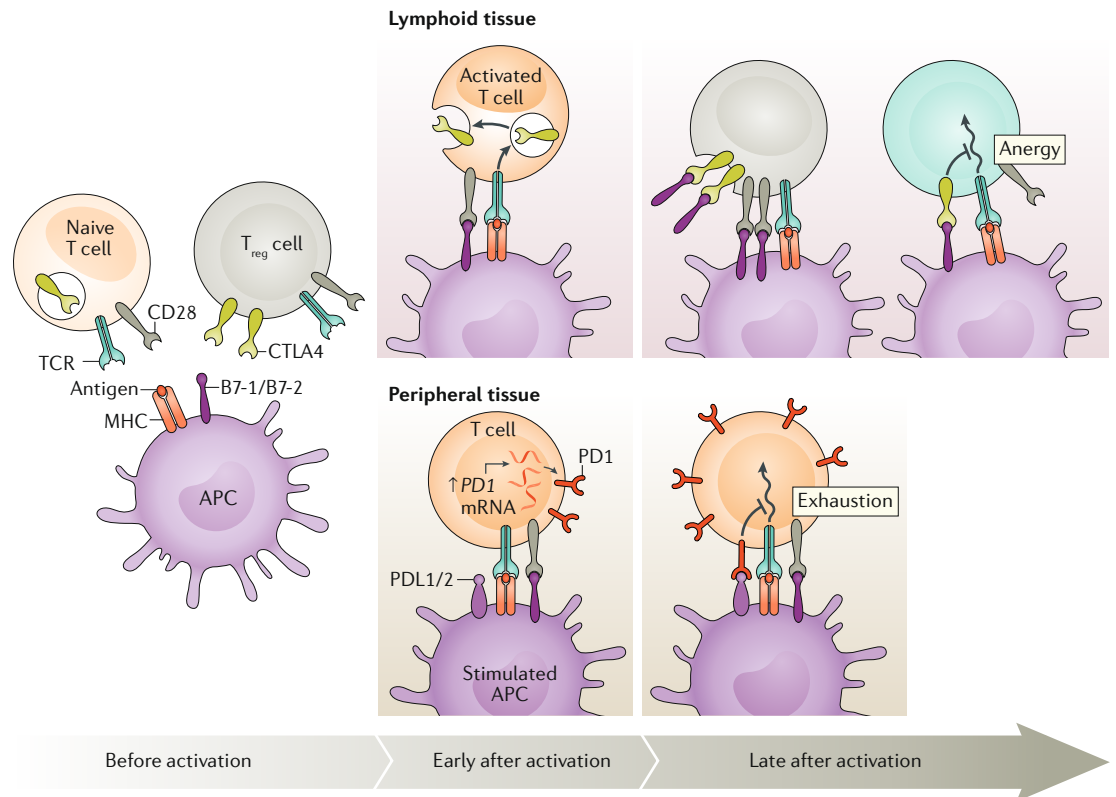
Fig. 2 | **Mechanisms of T cell activation and regulation.** Before activation, antigen-presenting cells (APCs) load antigen onto MHC molecules to prepare for contact with a T cell that displays a cognate T cell receptor (TCR) while also providing necessary co-stimulatory ligands B7-1 and B7-2. The inhibitory molecule cytotoxic T lymphocyte antigen 4 (CTLA4) is contained within intracellular vesicles in naive T cells, whereas it is constitutively expressed on the cell surface of CD4^{+}CD25^{+} regulatory T (T_{reg}) cells. Both classes of T cells express the co-stimulatory receptor CD28. Early after activation, generally in the lymphoid tissue, T cells are activated when their TCRs bind to their cognate antigen presented by APCs in conjunction with CD28 binding to B7-1/B7-2. Also, the activated T cells begin the process of displaying CTLA4 on the cell surface. T cells within peripheral tissues upregulate *PD1* at the mRNA level early after activation. Late after activation, in lymphoid tissue, CTLA4 expressed by activated T cells binds to the B7-1 and B7-2 molecules on APCs, thereby preventing their binding to CD28 and promoting anergy by decreasing the T cell activation state. At the same time, constitutive expression of CTLA4 on T_{reg} cells leads to trans-endocytosis of B7 ligands and interferes with the CD28 co-stimulatory ability of APCs. Late after activation in peripheral tissues, *PD1* is further upregulated transcriptionally, leading to greater surface expression of programmed cell death 1 (PD1), which binds to its ligands PDL1 and PDL2, thereby promoting T cell exhaustion at sites of infection or when confronted with neoplasms. Image courtesy of the National Institute of Allergy and Infectious Diseases.

where it associates with the TCR[26]. In the context of the immunological synapse, CTLA4 can also reorganize the cytoskeleton and disturb T cell–APC immune conjugate formation[27]. CTLA4 also mediates the internalization of its ligands, thereby preventing their binding to CD28, which, in turn, reduces IL-2 secretion and T cell proliferation[17,28,29]. Lastly, phosphatases, including SH2 domain-containing tyrosine phosphatase 2 (SHP2) and protein phosphatase 2A (PP2A), are recruited and interact with the cytoplasmic tail of CTLA4, thereby contributing to its negative effect on T cell activation. SHP2 is an inhibitor of phosphorylation of the CD3 ζ-subunit of the TCR and also inhibits phosphorylation of the adaptor protein linker of activated T cells (LAT)[30,31]. PP2A is hypothesized to inhibit extracellular signal-regulated kinase (ERK), a kinase that acts as a signalling protein downstream of the TCR[32]. However, there is significant debate about which of the molecules that associate with the cytoplasmic tail of CTLA4 are most important for inhibiting T cell activity. Nevertheless, these inhibitory

signals reduce the activation of transcription factors, such as activator protein 1 (AP-1), nuclear factor-κB (NF-κB) and nuclear factor of activated T cells (NFAT), which reprogrammes T cells towards an anergic fate[29,33].

Beyond its function in activated conventional T cells, CTLA4 expression on T_{reg} cells is essential for the direct and indirect immunosuppressive activity of these cells[34,35]. In vitro studies showed that CTLA4 was necessary for anti-inflammatory cytokine release by T_{reg} cells, which reduces polyclonal activation and proliferation of conventional T cells nearby[36,37]. This result was confirmed in vivo by adoptive transfer of CTLA4-bearing T_{reg} cells to prevent autoimmunity induced by CTLA4-deficient T cells that had been transferred to T cell- and B cell-deficient mice (*Rag*^{−/−} mice)[38,39]. This treatment effect was nullified by antibody-mediated neutralization of CTLA4 (REFS[38,40,41]). Thus, T_{reg} cell-expressed CTLA4 can compensate for lack of CTLA4 expression by conventional T cells[42,43]. Beyond direct immunosuppression, T_{reg} cells also prime

Immune conjugate
A biological unit that comprises interacting lymphocytes and antigen-presenting cells.

IL-2
A cytokine essential for lymphocyte activation, proliferation and tolerance.

Adaptor protein
An intracytoplasmic protein that facilitates molecular interactions and signal transduction.
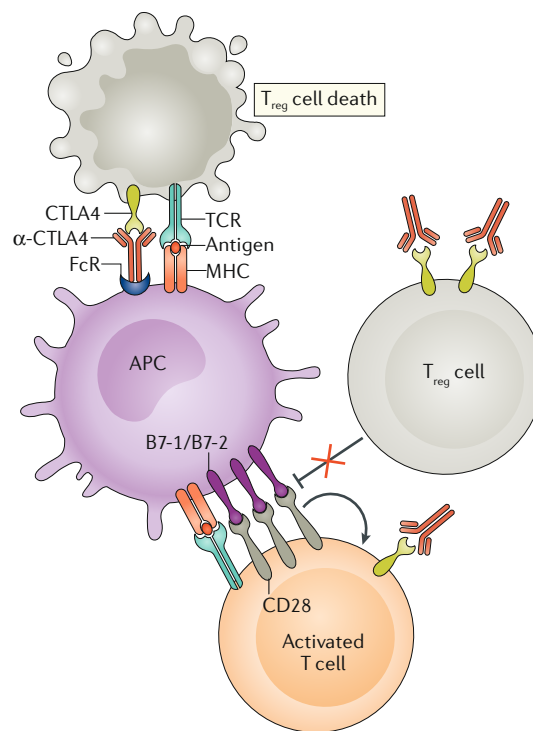
Antibody-dependent cell-mediated cytotoxicity
The process by which antibody-based opsonization of target cells promotes their lysis by immune cytotoxic cells.

dendritic cells to induce anergy of conventional T cells in a CTLA4-dependent fashion by binding to B7 ligands on APCs, followed by internalizing and degrading them, a process termed trans-endocytosis[28,44].

CTLA4 blockade in cancer. The recognition of CTLA4 as a negative regulator of T cell activation gave rise to the idea that blocking its actions could unleash a therapeutic response of T cells against cancer[45] (FIG. 3). James Allison and colleagues first tested this idea and demonstrated that neutralizing anti-CTLA4 antibodies enhanced antitumoural immunity in mice against transplanted and established colon carcinoma and fibrosarcoma[46]. In addition, during rechallenge, animals treated with anti-CTLA4 were able to rapidly eliminate tumour cells through immune mechanisms, providing evidence that blocking of CTLA4 induces long-lasting immunological memory[46,47]. Although CTLA4-targeted monotherapy was shown to confer benefit in animal models of brain[48], ovarian[49], bladder[50], colon[46], prostate[47] and soft tissue[46] cancers, less immunogenic cancers, including SM1 mammary carcinoma[51] and B16 melanoma[52], did not respond as favourably. Furthermore, heterogeneity between cancer models yielded discordant tissue-specific results[45,53]. In addition, a greater tumour burden correlated with reduced tumour responses to anti-CTLA4 treatment because larger tumours foster a more robust anti-inflammatory tumour microenvironment[45,49].

Despite the mixed success in preclinical studies, mAbs targeting CTLA4 proved effective in clinical trials of melanoma[45]. Ipilimumab, a human IgG1κ anti-CTLA4 mAb, gained FDA approval in 2011 for non-resectable stage III/IV melanoma following evidence that it elicited potent tumour necrosis[54] and conferred a 3.6-month short-term survival benefit[55]. Long-term survival data demonstrated that 22% of patients with advanced melanoma treated with ipilimumab benefited from an additional 3 years or more of life[56]. Additional long-term studies have demonstrated the durability of this survival benefit, indicating the persistence of antitumoural immunity following CTLA4 blockade[56,57]. Unfortunately, trial results in renal cell carcinoma[58], non-small-cell lung cancer[59], small-cell lung cancer[60] and prostate cancer[61] have yielded less impressive effects than those seen in patients with melanoma. Tremelimumab, an IgG2 isotype form of a CTLA4-blocking antibody, has yet to receive FDA approval as it did not increase survival in advanced melanoma[62]. It is hypothesized that effectiveness varies between ipilimumab and tremelimumab owing to differences in binding kinetics and the capacity to mediate antibody-dependent cell-mediated cytotoxicity[63,64].

The mechanisms of CTLA4-mediated tumour regression are pleiotropic but unified by the action of one cell type, the T lymphocyte (FIG. 3). T cell responses are necessary for the therapeutic effects of CTLA4-targeted agents because T cell depletion in animal models abolishes tumoricidal activity[65]. Inhibition of CTLA4 enhances T cell clonal responses to tumour-associated neoantigens and a high neoantigen burden portends a favourable response to anti-CTLA4 therapy[66,67]. Apart from boosting effector T cell responses, anti-CTLA4

Fig. 3 | **Effects of CTLA4-blocking antibodies.** Cytotoxic T lymphocyte antigen 4 (CTLA4)-blocking antibodies (α-CTLA4), especially when bound to an Fc receptor (FcR) on an antigen-presenting cell (APC), can promote antibody-dependent cellular cytotoxicity (ADCC). CD4⁺CD25⁺ regulatory T (T$_{reg}$) cells express higher amounts of CTLA4 than conventional T cells and are therefore more prone to α-CTLA4-induced ADCC than conventional T cells. In addition, α-CTLA4 can bind to CTLA4 on the surface of the T$_{reg}$ cell and prevent it from counter-regulating the CD28-mediated co-stimulatory pathways that are playing a role in T cell activation. At the same time, α-CTLA4 can also promote T cell responses by blocking CTLA4 on the surface of conventional T cells as they undergo activation. TCR, T cell receptor. Adapted from ©2019 Fritz, J. M. & Lenardo, M. J. Originally published in *J. Exp. Med.* https://doi.org/10.1084/jem.20182395 (REF.[135]).

therapy depletes local intratumoural T$_{reg}$ cells through antibody-dependent cell-mediated cytotoxicity in mouse models and shifts the balance of the tumour microenvironment away from immunosuppression[68,69]. This phenomenon requires further study in human cancer as current data are inconclusive[70,71]. The relative role of effector T cells and T$_{reg}$ cells in conferring a clinical benefit has been contested, although specific blocking of CTLA4 in both cell populations can lead to synergistic increases in tumour regression[69]. Overall, current data suggest that the most critical factor in predicting outcome is the ratio of effector T cells to T$_{reg}$ cells infiltrating the tumour[45,49].

PD1/PDL1 biological function. PD1 was first identified in 1992 as a putative mediator of apoptosis, although later evidence suggested a role in restraining immune system hyperactivation, analogous to CTLA4 (REF.[72]). As a type 1 transmembrane glycoprotein within the immunoglobulin superfamily, PD1 exhibits a 20% and 15%

BALB/c
An albino inbred mouse strain commonly used in immunology research.

Non-obese diabetic
An inbred mouse stain with enhanced susceptibility to spontaneous development of type 1 diabetes mellitus.

Immunoreceptor tyrosine-based switch motif
A conserved amino acid sequence (TxYxx(V/I)) involved in both activation and inhibition of downstream signalling depending on the cell type and biological context.

Immunoreceptor tyrosine-based inhibitory motif
A conserved amino acid sequence (S/I/V/LxYxxI/V/L) involved in the recruitment of inhibitory phosphatases to dampen downstream signalling.

T cell exhaustion
The progressive loss of effector function due to chronic low-affinity antigen stimulation.

amino acid identity to CTLA4 and CD28, respectively[73]. Human PD1 is expressed on T cells after TCR stimulation and binds the B7 homologues PDL1 (also known as B7-H1) and PDL2 (also known as B7-DC), which are present constitutively on APCs and can be induced in non-haematopoietic tissues by pro-inflammatory cytokines[74–76]. In this review, we refer to PD1 and its ligands as the 'PD1 axis'. The predominant role of the PD1 axis in the negative regulation of T cell activation became clear in 1999 when loss of the mouse *PD1* orthologue, *Pdcd1*, was found to cause autoimmunity in vivo. C57BL/6 mice lacking functional PD1 protein developed splenomegaly[77]. Ageing of these animals led to mild T cell-mediated lupus-like glomerulonephritis and arthritis that was exacerbated by concurrent *lpr* mutations in the *Fas* gene[78]. Characterization of additional mouse strains showed that *Pdcd1*^{−/−} mice of the BALB/c strain exhibited cardiac inflammation leading to dilated cardiomyopathy[79]. By comparison, non-obese diabetic *Pdcd1*^{−/−} mice had accelerated type 1 diabetes mellitus compared with their *Pdcd1*-sufficient counterparts[80]. The heterogeneous and late-onset autoimmune phenotypes of *Pdcd1*^{−/−} mice were distinct from *Ctla4*^{−/−} animals, demonstrating that the PD1 axis regulates T cell biology differently to CTLA4. Spatially, CTLA4 exerts its regulatory effect predominantly within lymphoid organs, whereas PD1 tends towards tempering T cell activation locally within peripheral tissues[8]. Temporally,

PD1 acts later in the course of T cell activation and fate determination. Overall, the PD1 axis plays a unique role in maintaining T cell tolerance to self.

PD1 restrains immune responses primarily through inhibitory intracellular signalling in effector T cells and T_{reg} cells[81]. The immunoreceptor tyrosine-based switch motif and the immunoreceptor tyrosine-based inhibitory motif of PD1 are phosphorylated and recruit the phosphatases SHP1 and SHP2, which dephosphorylate, and thereby inactivate, downstream effectors (that is, the CD3 ζ-subunit and ZAP70) that are important for early T cell activation[76] and CD28 signalling[82]. Both CTLA4 and PD1 inhibit protein kinase B (PKB; also known as AKT) signalling to reduce glucose uptake and utilization, the former through PP2A and the latter by reducing phosphoinositide 3-kinase (PI3K) activity[83]. In contrast to CTLA4, the PD1 axis is essential for controlling the continued activation and proliferation of differentiated effectors; when PD1 engages its ligands, it can induce a state of T cell dysfunction called T cell exhaustion[84–86]. However, what determines whether PD1 mediates exhaustion or apoptosis in certain contexts is still an active area of research. One model suggests that the interaction between PI3K signalling and the mitochondrial B cell lymphoma-extra large (BCL-X$_L$) protein is a critical control point at which PD1-mediated P13K inhibition reduces BCL-X$_L$ and promotes apoptosis[25,83]. Beyond regulating conventional T cells, PDL1 on APCs can control T_{reg} cell differentiation and suppressive activity[87]. Unfortunately, tumour cells can exploit this mechanism by upregulating PD1 ligands to induce T cell exhaustion and generate a tumour microenvironment that facilitates tumour growth and invasion[88].

PD1/PDL1 blockade in cancer. Once the PD1 axis was implicated in the negative regulation of T cells, preclinical work examined whether inhibitors of this pathway could be used for cancer treatment and biomarker discovery. First, overexpression of PDL1 or PDL2 in cancer cell lines was found to constrain the CD8+ T cell cytotoxic antitumour response, whereas tumours were rejected in mice without functional PD1 (REFS[89,90]). Second, blockade of PD1 suppressed the growth of transplanted myeloma cells in syngeneic animals[90]. Conversely, transplanted cells overexpressing PDL1 or PDL2 in syngeneic mice allowed for increased tumour colonization, burden and invasiveness[90]. Neutralizing the PD1 axis using mAbs[89,91] or secreted PD1 extracellular domains[92] reversed these effects and enhanced T cell cytotoxicity towards tumour cells[90] (FIG. 4). Rescuing CD8+ T cell cytotoxicity by PD1 blockade depends on the expression of CD28 as PD1-mediated immunomodulation is lost in the context of CTLA4Ig, B7 blockade or CD28 conditional-knockout mice[92]. In addition, reinvigorated T cells in the peripheral blood of patients with lung cancer following PD1 blockade were shown to express CD28 (REF.[93]). PD1 inhibition not only augments antitumoural immunity but also limits haematogenous seeding of B16 melanoma and CT26 colon carcinoma metastases in mouse models[94]. Thus, PD1/PDL1 blockade can both enhance tumour
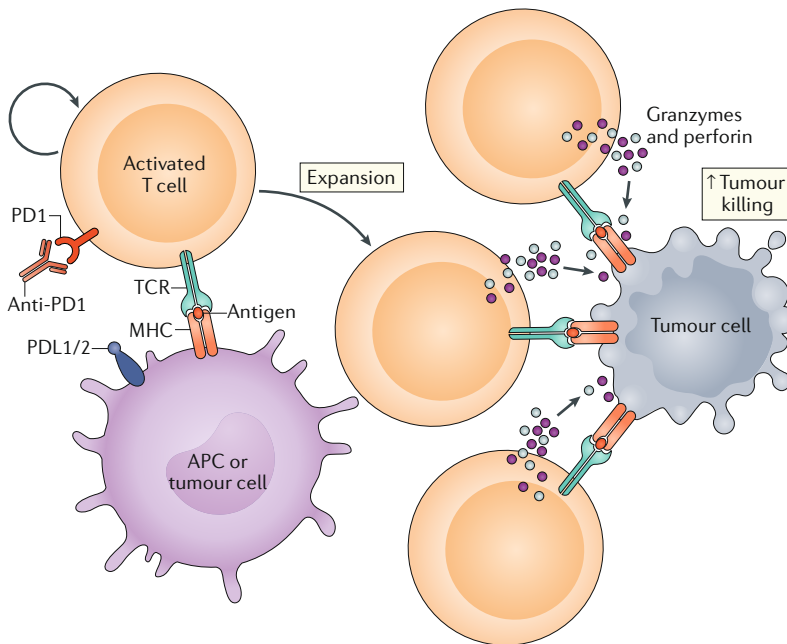
Fig. 4 | **Mechanisms of PD1 axis inhibition.** Activated T cells express programmed cell death 1 (PD1), which engages with its specific ligand (PDL1 or PDL2) to dampen activation. Blocking of the PD1 axis through the administration of an anti-PD1 (or anti-PDL1 or anti-PDL2) antibody prevents this inhibitory interaction and unleashes antitumoural T lymphocyte activity by promoting increased T cell activation and proliferation, by enhancing their effector functions and by supporting the formation of memory cells. Consequently, more T cells bind to tumour antigens presented on tumour cells by MHC molecules via their T cell receptors (TCRs). This ultimately leads to the release of cytolytic mediators, such as perforin and granzyme, causing enhanced tumour killing. APC, antigen-presenting cell. Adapted from ©2019 Fritz, J. M. & Lenardo, M. J. Originally published in *J. Exp. Med.* https://doi.org/10.1084/jem.20182395 (REF.[135]).

cytolysis and limit metastasis. Apart from a role of PD1 and its ligands in cancer treatment, multiple studies have also shown a negative correlation between human tumour expression of proteins involved in the PD1 axis and prognosis, indicating the utility of these proteins as potential biomarkers[95–97].

Following preclinical success, mAbs designed to counteract negative immunoregulation by the PD1 axis were developed and efficacy was shown in clinical trials[98]. Development was initiated by Medarex (ultimately purchased by Bristol-Myers Squibb) in 2001 (REF.[99]). In 2010, a phase I trial demonstrated that PD1 blockade was well tolerated and could promote antitumoural responses[100]. In 2014, the humanized and fully human anti-PD1 mAbs pembrolizumab and nivolumab (both IgG4) became the first FDA-approved PD1-targeted therapeutics for refractory and unresectable melanoma[101–104]. In a head-to-head comparison, pembrolizumab showed better 6-month progression-free survival than ipilimumab and conferred an overall survival benefit[105,106]. Clinical trials of nivolumab demonstrated an overall survival of 72.9% at 1 year compared with 42.1% survival in the group of patients treated with the chemotherapeutic dacarbazine[104]. In 2015, pembrolizumab was approved for the treatment of PDL1-expressing non-small-cell lung carcinoma because it provided a 4.3-month increase in progression-free survival compared with platinum-based chemotherapeutics and was more effective than the chemotherapeutic paclitaxel[107,108]. Increased PDL1 expression on the target tumour was associated with improved responses to PD1 axis blockade[109]. Additional successful clinical trials expanded the use of pembrolizumab to head and neck squamous cell carcinoma[110], Hodgkin lymphoma[111], urothelial carcinoma[112], gastric/gastro-oesophageal junction cancer[113] and tissue-agnostic carcinoma with a high degree of microsatellite instability[114]. Following approval in tissue-agnostic cancers with microsatellite instability, pembrolizumab became the first drug to be approved based on a molecular biomarker rather than by cancer site. However, the immunosuppressive microenvironment of different tissues makes it hard to predict which patients will benefit[115,116]. Similar to prembrolizumab, the use of nivolumab has since been extended to renal cell carcinoma[117], head and neck squamous cell carcinoma[118], urothelial carcinoma[119], hepatocellular carcinoma[120], Hodgkin lymphoma[121] and colorectal cancer with a high degree of microsatellite instability[122]. As was seen with anti-CTLA4 therapy, long-term survival analyses demonstrate a long-lasting immune-mediated survival benefit following PD1 blockade[123]. However, the reason why PD1 blockade has demonstrated broader clinical utility than anti-CTLA4 treatment has remained elusive. It is hypothesized that the difference may be because the PD1 axis is frequently co-opted by tumours via ligand expression, whereas CTLA4 represents a broader immunoregulatory circuit[74,124].

PDL1 is also targetable by specific antibodies that have proven effective treatments in multiple forms of cancer. In 2016, the first PDL1-targeted humanized mAb, atezolizumab (an IgG4 antibody), was approved for treatment of urothelial carcinoma. An overall response rate of 15% was deemed statistically significant based on historical control data, although responses were dependent on tumour PDL1 expression status[125]. Unfortunately, additional trial data have not demonstrated that atezolizumab has clinical efficacy beyond the standard of care in urothelial carcinoma, although it is less toxic than traditional chemotherapy[126]. Indications have since expanded to include the treatment of non-small-cell lung carcinoma[127], triple-negative breast cancer[128] and small-cell lung cancer[129]. Additional anti-PDL1 human mAbs, avelumab and durvalumab, entered the market in 2017 (REF.[98]). Avelumab is used for the treatment of Merkel cell carcinoma[130], urothelial carcinoma[131] and advanced renal cell carcinoma[132]. Duvalumab is used for urothelial carcinoma[133] and non-small-cell lung cancer[134]. Therefore, similar to PD1, blockade of PDL1 has been effective in difficult-to-treat forms of cancer.

Adverse effects of checkpoint blockade. Blocking a naturally occurring central immune checkpoint unleashes powerful immune effector mechanisms that may not respect the normal boundaries of immune tolerance to self-tissues[135]. *Ctla4-* and *Pdcd1*-knockout mice provided a glimpse into the spectrum of autoimmune responses that occur in humans during immune checkpoint blockade therapy[19,77–79]. Human loss-of-function mutations in *CTLA4* and its interacting regulatory protein, *LRBA*, also mirror the immune-related side effects observed with anti-CTLA4 therapy[13,24]. On the basis of a meta-analysis of trial data sets, immune-related adverse events are estimated to occur in 15–90% of patients[55]. More severe events requiring intervention are observed in 30% and 15% of patients treated with CTLA4 and PD1 axis inhibitors, respectively[136]. The common immune feature of toxicity is the loss of naive T cells and the accumulation of overactive memory T cells that invade peripheral organs, such as the gastrointestinal tract and lungs, and cause inflammatory damage. Keratinized and non-keratinized mucosa appear to be the most susceptible, as approximately 68% and 40% of treated patients exhibit pruritis and mucositis, respectively[137,138]. Anti-CTLA4 therapy carries an increased risk of severe autoimmune complications compared with therapies targeting the PD1 axis, as was observed in knockout mice and in clinical studies[19,77–80,139]. In addition, data from dose-escalation trials support the claim that anti-CTLA4 agents elicit dose-dependent responses not seen with therapies targeted at the PD1 axis[107,139]. Toxicities affecting the gastrointestinal tract and brain are more common with anti-CTLA4 therapy, whereas patients treated with PD1 axis-targeted therapies are at higher risk of hypothyroidism, hepatoxicity and pneumonitis[137]. However, as the number of indications treated with checkpoint blockade increases and more patients are treated, rarer side effects in a wider spectrum of organs and heterogeneous responses have manifested[137]. For example, hyperprogression of disease has been observed in a minority of patients with various tumour types treated with PD1 inhibitors[140–142]. Most recently, it was shown that the PD1 inhibitor nivolumab can lead to the rapid progression of disease in patients

with adult T cell leukaemia/lymphoma, providing evidence for a role of tumour-resident T_{reg} cells in the pathogenesis of this lymphoma[143]. Multiple immune-related response criteria have been developed to better categorize patient responses to checkpoint blockade. In addition, these criteria aim to distinguish progression from pseudoprogression, a phenomenon in which patients treated with CTLA4 or PD1 inhibitors experience a period of progression followed by rapid tumour clearance[144,145]. Overall, checkpoint blockade leads to autoimmune toxicities with a therapy-specific pattern of organ involvement, as predicted by the phenotypes of animals genetically deficient for checkpoint molecules.

Interestingly, preclinical immune checkpoint therapy studies did not demonstrate major adverse effects in vivo and, thus, were not great predictors of human toxicities[146]. This is thought to be due to the short time frame of these studies and the inbred nature of mouse strains[146]. Recently developed humanized mouse models represent a platform that better recapitulates side effects due to checkpoint therapy[146,147]. Nevertheless, toxicity associated with immune checkpoint blockade is tolerated better than the toxicities associated with traditional chemotherapeutics, making these therapies attractive for quality of life reasons beyond their survival benefit[98,148].

Recent research has aimed to improve the side-effect profiles and clinical response of immune checkpoint blockade through the modification of existing antibodies and the engineering of novel delivery methods. It was recently shown that abnormal CTLA4 recycling and subsequent lysosomal degradation was a mechanism that contributes to toxicities and reduced drug effectiveness. Modified pH-sensitive antibodies that do not interfere with LRBA-mediated CTLA4 recycling were shown to limit adverse events and improve clinical outcomes in established tumours in mouse models, which may ultimately broaden clinical utility[149,150]. Additional research has focused on developing biomaterials for the localized administration of checkpoint inhibitors[151]. For example, compared with systemic delivery, transdermal patch delivery of anti-PD1 antibodies was better tolerated and unleashed a more robust antitumoural response in a mouse model of melanoma[151]. A broad field of research is currently aimed at discovering novel methods to reduce toxicities associated with checkpoint therapy and to increase clinical benefit in a greater variety of tumours.

Clinical management of drug-related toxicities is the same for all checkpoint drugs, and toxicities are graded according to the 2009 National Cancer Institute Common Terminology Criteria for Adverse Events severity scale[137,152]. Mild (grade 1) toxicities are not typically treated. In the setting of grade 2 or 3 adverse events, checkpoint inhibitors are discontinued until symptoms and laboratory-value abnormalities resolve. Glucocorticoids are also used to effectively control immune hyperactivity. Infliximab and other immunosuppressive agents can be used when glucocorticoids fail. Life-threatening (grade 4) toxicities necessitate the complete discontinuation of therapy and the use of life-saving measures, as required. Active monitoring of symptoms and laboratory parameters is recommended in order to prevent death due to checkpoint blockade (grade 5).

Current research is aimed at identifying predictive biomarkers for organ-specific toxicities due to checkpoint therapy. For example, neutrophil activation, as measured by increased expression of the biliary glycoprotein CEACAM1 and the cell surface glycoprotein CD177, correlates with gastrointestinal-related side effects in patients treated with ipilimumab[153]. Increases in eosinophil counts and release of the pro-inflammatory cytokine IL-17 are associated with toxicity regardless of the organ affected[154,155]. Pharmacogenomic profiling (using genetic information to predict responses to drugs) may provide more insight into the relevant genes and pathways mediating toxicity[137]. Ultimately, the hope is that genetic, biochemical or metabolic profiling could either pre-screen or rapidly detect individuals likely to experience the most severe adverse reactions to checkpoint therapy.

Adoptive T cell transfer therapy

Adoptive T cell (ATC) therapy, in which autologous or allogenic T cells are infused into patients with cancer, has shown considerable promise in recent years. The viability of this type of therapy was first shown by Southam et al. in 1966, when half of the patients with advanced cancer demonstrated tumour regression following co-transplantation with patient-derived leukocytes and autologous tumour cells[156]. Allogenic haematopoietic stem cell transplants for leukaemia represented the first effective adoptive transfer approach deployed clinically, and clinical improvement was shown to be mediated by a T cell graft versus tumour response[157].

ATC with tumour-infiltrating lymphocytes. ATC therapy using tumour-infiltrating lymphocytes (TILs) for the treatment of metastatic melanoma was pioneered at the National Cancer Institute in the late 1980s[158]. Lymphocytes isolated from a cancer biopsy were greatly expanded with IL-2 and then reinfused intravenously into the same patient with a large bolus of IL-2. The objective response rate was 34%; however, the median duration of response was only 4 months and few patients experienced a complete response[159]. Later studies incorporating lymphodepletion before ATC therapy in 93 patients with metastatic melanoma were more successful, with complete tumour regression in 20 (22%) patients, 19 of whom were still in complete remission 3 years after treatment[160]. The screening and enriching for neoantigen-specific TILs, made possible by high-throughput technologies, recently demonstrated promise in a patient with metastatic breast cancer[161]. In addition, knockdown of the gene encoding cytokine-inducible SH2-containing protein (*Cish*), a negative regulator of TCR signalling, was shown to boost the antitumoural response of ATC therapy in mouse models[162]. However, in order for TIL-based ATC therapy to elicit durable responses (FIG. 5), effector T cells with antitumour activity must be present in the tumour, which is not the case for many cancer types[163]. Other innovative approaches to tweak T cell activity and proliferation may allow for a greater palette of treatments to be developed.
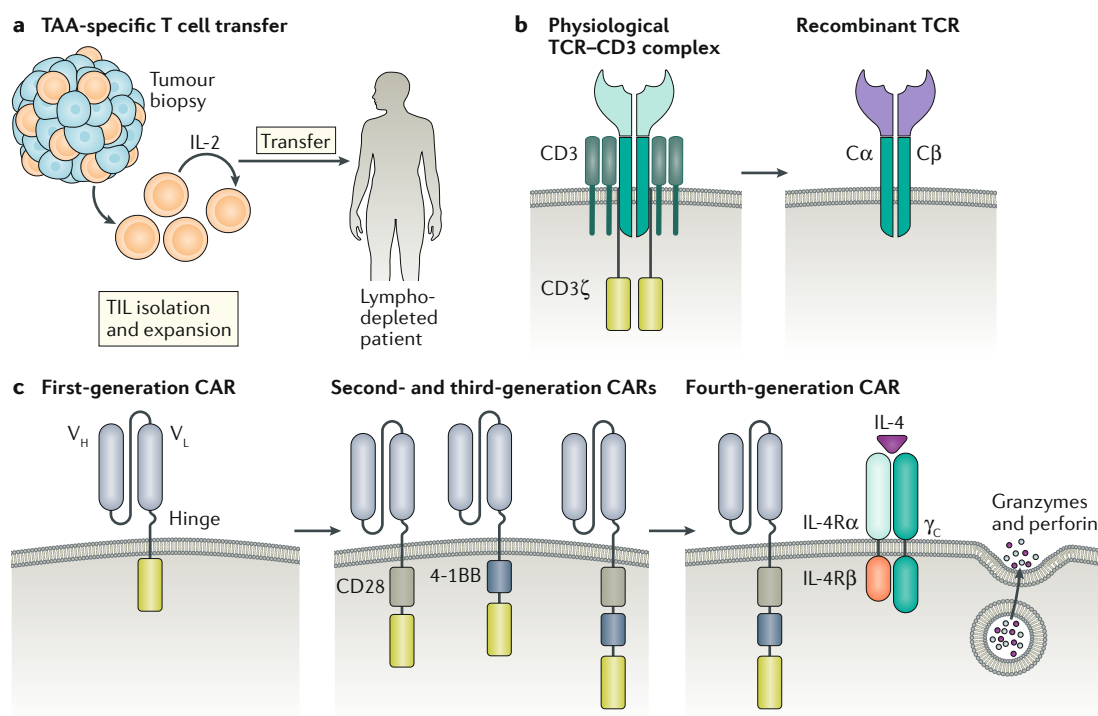
Fig. 5 | **Adoptive T cell therapy. a** | Tumour-infiltrating lymphocytes (TILs) are isolated from a patient tumour biopsy and expanded ex vivo with IL-2. TILs are then infused into a patient who has undergone lymphodepletion to provide a niche for the transferred TILs to expand, act as effector cells and generate immunological memory. As the T cells were derived from the tumour, it is assumed that a good proportion can recognize tumour-associated antigens (TAAs) or neoantigens. **b** | The physiological T cell receptor (TCR) complex gains its specificity from polymorphic α- and β-glycoprotein chains that have an antigen-binding portion and a conserved domain that associate with and signal through a group of non-polymorphic proteins, CD3 γ, δ, ε and ζ. Bioengineering of the TCR α- and β-glycoprotein antigen-binding domain (purple), while preserving the conserved domains (Cα and Cβ), allows for the development and expansion of T lymphocytes with specificity to tumour neoantigens. **c** | Originally, chimeric antigen receptors (CARs) were composed of an extracellular single-chain fragment of an antibody variable region coupled to a CD3 ζ-signalling domain. Poor expansion and functionality of these first-generation CARs led to the development of second and third-generation CARs containing intracellular modules from co-stimulatory molecules (CD28 and/or 4-1BB) that provide additional signals necessary to fully activate the T cell. Subsequent generations of CAR T cells contain further modifications to improve antitumour efficacy. For example, fourth-generation 'armoured' CAR T cells have been engineered to secrete pro-inflammatory cytokines, such as IL-12, to overcome immunosuppression in the tumour microenvironment. The chimeric cytokine receptor 4αβ, comprising the ectodomain of IL-4Rα fused to the IL-2/IL-15Rβ chain, signals in response to IL-4, an abundant cytokine in numerous tumour types. V_H, variable heavy chain; V_L, variable light chain.

Engineered lymphocytes for ATC. The challenges associated with expanding tumour-specific T cells in vitro led to the development of TCR-engineered lymphocytes (FIG. 5). However, these cells are limited to responding to tumour antigens presented by the MHC (also known as human leukocyte antigen (HLA) in humans) rather than surface antigens on tumour cells[163]. However, synthetic chimeric antigen receptors (CARs) can bypass MHC restriction and direct specific cytotoxicity to a target molecule on the surface of the malignant cell. Isolated T cells from the patient (or allogeneic donor) are genetically modified to express CARs and then expanded and infused into the patient. This overcomes the problem that tumour cells often downregulate MHC molecules, which leaves the cell unable to present antigen to conventional T cells[164]. CARs comprise an antigen-binding domain, most often from the variable regions of antibodies, linked to signalling domains of the TCR and various co-stimulatory molecules (FIG. 5). Given the domain modularity of cell surface signalling proteins,

mixes and matches of extracellular targeting domains and internal signal transduction domains can be assembled using protein engineering. This offers many options to tailor CARs to specific tumours. The first generation of CAR T cells relied only on the CD3 ζ-chain to simulate TCR signalling[165], but this design was ineffective in clinical trials owing to limited T cell proliferation and cytokine production[166,167]. Subsequent generations of CAR T cells have been engineered to include domains from CD28, CD40 ligand and other positive regulators of T cell activation to potentiate activation and cytotoxicity in vivo[168–171]. An engineered single-chain PD1 blocker has also demonstrated similar enhanced efficacy to second-generation CAR T cells with solely a CD28 domain[172]. Even though CAR T cells are typically engineered using retroviral transduction, recent work has used CRISPR–Cas9 technology. CRISPR–Cas9 can be used to edit the TCR germline sequence directly, which could lead to more uniform CAR T cell generation and, ultimately, better efficacy[173].

A limitation to the development of CAR T cell therapies is the requirement for a distinct tissue-restricted target antigen on the tumour cell surface. For example, CAR T cells designed with specificity for the cell surface molecule CD19, which is expressed by all B cells, have been successful in the treatment of B cell malignancies. The first clinical deployment of second-generation CD19-specific CAR T cells led to durable responses in chronic lymphocytic leukaemia[174]. Additional clinical trials of CD19-specific second-generation CAR T cells in B cell acute lymphoblastic leukaemia (B-ALL) led to remission in all patients with B-ALL who were tested[175]. A follow-up report on patients with B-ALL enrolled in this clinical trial showed complete remission of disease in 44 of 53 (83%) patients with a median follow-up of 29 months[176]. Similar successes were reported for patients with diffuse large B cell lymphoma[177], leading to FDA approval for these B cell malignancies in 2017.

The clinical success of CAR T cell therapy for the treatment of B-ALL and diffuse large B cell lymphoma is due, in part, to targeting the CD19 antigen, an ideal candidate owing to its high expression in certain B cell malignancies and specificity to the B cell lineage. Crossover targeting of normal CD19$^+$ B cells does not hamper therapy or cause severe side effects. However, even as an ideal target, CD19 antigen loss is a common cause of treatment failure. CD22 is another antigen commonly expressed by malignant cells in B-ALL and has shown promise as a target for CAR T cell therapy in a phase I trial[178]. Other targets, especially tumour neoantigens, are currently being investigated for haematological malignancies that do not express CD19, as well as for solid tumours[179,180]. B cell maturation antigen (BCMA)-targeted CAR T cell therapy is poised for FDA approval for multiple myeloma in 2020 on the basis of promising preclinical and clinical data[181,182]. However, owing to reported patient relapses, the investigation of additional target antigens continues. A preclinical study recently identified another target antigen, GPRC5D, with comparable efficacy and toxicity to BCMA-targeted CAR T cell therapy[183]. Thus far, CAR T cell therapy has only been modestly successful for solid tumours[184–186] and innovative approaches to improve therapy are underway[179]. A recently identified pan-cancer target, B7-H3 (also known as CD276), has demonstrated success in multiple paediatric solid tumour models[187]. In addition to directly acting as cytolytic agents, CAR T cells can also target the unhospitable tumour microenvironment and revive exhausted T cells[188,189]. For example, a new generation of 'armoured' CAR T cells engineered to produce IL-12 can overcome immunosuppression by T_{reg} cells and myeloid cells in the tumour environment, promote CD8$^+$ T cell cytolytic activity[190] and enhance myeloid cell recruitment and antigen presentation[191,192]. Preclinical models using IL-12-expressing CAR T cells that target the conserved extracellular domain of mucin 16 (MUC16^{ecto}) have shown promising results in models of ovarian cancer, a tumour with poor prognosis in advanced stages[193,194]. A phase I clinical trial is currently in progress for patients with ovarian, fallopian or primary peritoneal cancer[195].

The efficacy of CAR T cells may also be strengthened through co-expression of a chimeric cytokine receptor (4αβ) that stimulates proliferation in response to IL-4, a cytokine that is usually abundant in the tumour microenvironment. Preliminary studies have shown that this approach works for CAR T cells directed against different tumour-associated antigens (TAAs)[196] and clinical trials are underway in head and neck cancer[197]. In addition, overexpression of the transcription factor JUN was shown to confer resistance to CAR T cell exhaustion[198]. Overall, CAR T cells have been successful for the treatment of B cell malignancies and it will be exciting to continue research on this new treatment modality for intractable types of cancer.

Limitations and adverse effects of ATCs. Toxicities can arise from CAR T cell therapy and affect many different organ systems with a range of severity[199]. Patients most commonly experience cytokine release syndrome (CRS) and neurotoxicity[200]. CRS results from the powerful activation and proliferation of CAR T cells in vivo and typically appears quickly after cell transfer. The symptoms are often mild and flu-like but can also be severe and life-threatening, involving hypotension, high fever, capillary leakage, coagulopathy and multisystem organ failure. Serious neurological events can also occur, such as CAR T cell-related encephalopathy syndrome, typically characterized by confusion and delirium, but sometimes also associated with seizures and cerebral oedema[199]. Glucocorticoids are the first-line treatment for milder forms of CRS and CAR T cell-related encephalopathy syndrome. Tocilizumab, a humanized anti-IL-6 antibody, is a highly effective second-line treatment for CRS caused by CAR T cell therapy[201]. Other side effects of CD19-specific CAR T cell therapy include lymphopenia and hypogammaglobulinaemia[202], which can be effectively managed with intravenous immunoglobulin therapy, similar to the treatment that patients with primary B cell immunodeficiencies receive[203]. The mechanisms behind these side effects are unclear and further research may yield ways to avoid or minimize toxicity. Recent development of a novel murine model of CRS demonstrated that it is not mediated by CAR T cell-derived IL-6 but rather by recipient macrophages that secrete IL-6, IL-1 and nitric oxide. Therefore, IL-1 blockade represents a possible novel intervention in the armamentarium against CRS[204]. Moreover, a clinical study of low-affinity CD19-specific CAR T cells demonstrated reduced toxicity and enhanced efficacy[205]. Additional efforts to reduce toxicity involve the engineering of CAR T cells with multiple receptor specificities[206] and reducing the half-life of cellular toxicity by using mRNA-based methods that allow for transient receptor expression[207] or including suicide cassettes that can be activated by exogenous agents to clonally delete the infused cells[208].

The ATC approach necessitates a patient-specific therapy design, its cost can be prohibitive, patient access to the treatment is limited and manufacturing is challenging. In the United States, the CAR T cell therapies tisagenlecleucel and axicabtagene ciloleucel have a

direct cost of US$475,000 and US$373,000 per patient, respectively[209]. However, these values do not take into account the additional costs associated with treating the severe adverse effects common to CAR T cell therapy, which are estimated to increase drug-associated costs by US$30,000 or more[209]. In comparison with CAR T cell therapy, checkpoint blockade has a price tag of approximately US$12,500 per month[210]. Patient access to CAR T cell therapies also represents a major problem as there are only a few laboratories certified to generate CAR T cells and only a few specialized tertiary care centres able to administer this therapy[211]. Lastly, variability in the manufacturing of CAR T cells and a lack of standard practices can contribute to heterogeneous outcomes[211].

Cancer vaccines

Cancer vaccines prompt the immune system to protect the body from cancer and fall into two categories, prophylactic and therapeutic. Prophylactic vaccines against hepatitis B and human papillomavirus have been instrumental in reducing the incidence of hepatocellular carcinoma and cervical cancer, respectively[212]. These are classic vaccines used to prevent infection by oncogenic viruses. By contrast, therapeutic vaccines aim to harness the immune system to eliminate disease-causing cells that are already neoplastic[212]. An early example of this is the use of the bacillus Calmette–Guérin vaccine, comprising attenuated *Mycobacterium bovis*, which is generally used as a prophylactic tuberculosis vaccine but has also been repurposed as a primitive therapeutic vaccine for bladder cancer[213].

Historically, the discovery of TAAs[214], which are highly expressed on tumour cells and to a lesser extent on normal tissues, opened the door for further therapeutic vaccine-based approaches. However, as TAAs are often recognized by the immune system as 'self', viral antigens and neoantigens that are unique to a malignancy may be more suited as vaccine targets.

Early vaccination approaches in the 1970s were based on autologous tumour vaccines and involved the administration of patient-derived tumour cells together with an adjuvant or virus in order to activate polyclonal immune responses to TAAs[215]. For example, autologous tumour cells infected with Newcastle disease virus have been used in one type of cancer vaccine that has demonstrated success in preclinical models of metastatic lymphoma and melanoma[216,217]. Modified Newcastle disease virus-based vaccines have been engineered to express granulocyte–macrophage colony-stimulating factor (GM-CSF) in attempts to enhance efficacy[218]. Synergism of vaccine approaches with checkpoint blockade agents has also been demonstrated in some preclinical studies of melanoma[46,219]. Numerous autologous tumour vaccines are being investigated in phase II and phase III trials but have yet to receive FDA approval. This approach suffers from multiple limitations, most notably the difficulty in obtaining patient-derived tumour cells in certain cancer types[212]. Newer approaches include the development of personalized recombinant cancer vaccines informed by next-generation sequencing of genomic DNA from tumours.

Development of personalized recombinant cancer vaccines. Vaccines that elicit responses to tumour-derived neoantigens should induce more robust immune responses and cause fewer autoimmune-related toxicities than vaccines based on self-derived TAAs, as the T cells that are activated by such a vaccine would not have undergone negative selection during development. These factors, as well as the ability to identify neoantigens through next-generation sequencing of genomic DNA from tumours, has shifted the focus to investigating the clinical feasibility of making personalized recombinant vaccines that target neoantigens. However, although a higher mutational burden in the tumour has been shown to correlate with greater immunogenicity and survival after checkpoint blockade[66,220], only a small percentage of neoantigens spontaneously generate immune responses in patients with cancer[221]. Sahin and colleagues showed that neoantigens identified through next-generation sequencing can generate antitumour responses in vivo; in mice that were vaccinated with 50 different neoantigens, 16 were immunogenic[222,223]. Interestingly, most neoantigens induced cytokine responses from CD4+ T cells rather than CD8+ T cells, suggesting that neoantigens are selected for MHC class II binding[222,223]. Other preclinical studies demonstrated effective CD4+ and CD8+ T cell responses to neoantigen vaccines in various cancer types[223–227]. However, recent preclinical work has also highlighted the non-overlapping role of neoantigen responses mediated by CD4+ and CD8+ T cells[228].

To design and manufacture a personalized vaccine for clinical use, computer-based algorithms are used to identify which tumour-derived peptides could potentially form a suitable TAA or tumour neoantigen with the patient's MHC alleles (FIG. 6). There are several different strategies to formulate neoantigen-based vaccines, including as synthetic peptides, mRNA, viral and DNA plasmids or antigen-loaded dendritic cells, and it is difficult to directly compare how each strategy influences immunogenicity[229,230]. In one trial that tested a multi-peptide vaccine that included up to 20 personal neoantigens, 4 of 6 patients with melanoma who entered the study with stage III disease experienced complete responses with no recurrence 25 months post vaccination, and the other 2 patients with progressive disease subsequently underwent anti-PD1 therapy that resulted in complete tumour regression[231]. Further, of the 97 different neoantigens that were tested for immunogenicity in this study, 60% elicited CD4+ T cell responses whereas 15% elicited CD8+ T cell responses. Another clinical trial, which tested an RNA vaccine that encoded 10 peptides representing personalized TAAs in 13 patients with advanced melanoma, achieved similar results[232].

Pitfalls and adverse effects of cancer vaccines. Although these early cancer vaccine experiments have been promising, challenges remain. An individual tumour can harbour thousands of somatic mutations and predicting which neoantigens can elicit strong antitumour responses remains an imperfect art. However, the current methods, consisting of validating mRNA expression of the mutation in tumour cells and using software/

Tissue samples → DNA sequencing → Identification of neoantigens and HLA screening → Vaccine development → Administration of vaccine → Immune monitoring
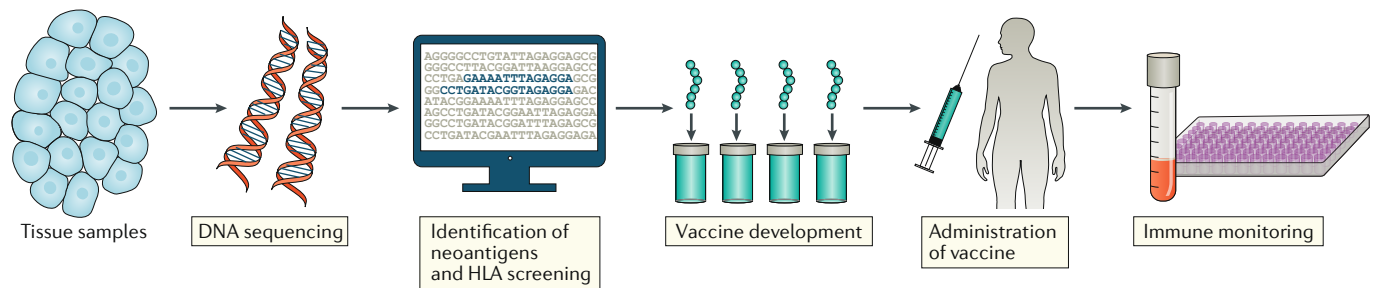
Fig. 6 | **Personalized vaccine development.** Healthy tissue and tumour tissue from a patient with cancer are submitted for DNA sequencing and bioinformatic analyses to identify gene variants that encode peptides that are specific to the tumour (neoantigens). Prediction algorithms are then used to screen for neoantigens that are likely to stably bind to the patient's MHC (also known as HLA in human) molecules and their expression is validated by sequencing tumour mRNA. Multiple predicted neoantigens are then formulated into vaccines, which are administered to the patient together with adjuvants. Post treatment, the patient is regularly monitored for neoantigen-specific immune responses and tumour growth.

databases to predict peptide–MHC binding, have been surprisingly effective in clinical trials to date[229]. However, this success has been biased towards MHC class I-specific neoantigens as prediction for MHC class II molecules presents unique challenges. For example, the increased diversity of MHC class II molecules and the structural nature of their open binding pocket make discerning a predictable binding motif difficult[233]. Taken together, these differences between MHC classes highlight the particular need for new MHC class II prediction algorithms. Other challenging factors to consider are the time and cost associated with developing bespoke vaccines. Currently, development and production of these vaccines takes approximately 4 months, and, although the downtime can be used to initiate other types of treatment, shortening the time span to personalized treatment is critical. For rapidly growing or metastatic tumours, months might matter. Ongoing efforts to improve design and manufacturing could shorten the production time to several weeks[229].

Overall, the comprehensive identification of somatic mutations, and the evaluation of peptides derived from these mutations to elicit immune responses, has renewed interest in vaccination strategies for cancer treatment. Even though early clinical trials are promising, extrapolation of these findings could be misleading and advanced clinical trials will ultimately determine the efficacy of personalized vaccine therapy. Nonetheless, cancer vaccines are prototypical 'single patient and single disease' precision medications and would have been in the realm of science fiction just a few decades ago. Further research and technological developments will no doubt lead to greater precision and effectiveness and also provide a better understanding of the mechanisms of antitumoural immune responses.

Emerging cancer immunotherapeutics

The molecular diversity of genetic changes that transform cells in human cancers creates a plethora of diseases involving specific tissue types and cancer mechanisms. Given the exciting advances in cancer immunotherapy, various modifications to current immunotherapeutic approaches are being developed and tested to address the complexity of cancer immunopathogenesis and cancer targetability.

Abscopal responses
A phenomenon in which the therapeutic effect of radiation is extended beyond the boundaries of the tissue that was treated

Combination therapies. Following the clinical success of checkpoint blockade monotherapy, combination therapies that couple agents with distinct mechanisms of action have augmented treatment success in various cancers. For example, ipilimumab and nivolumab combination therapy conferred a significant survival benefit in patients with metastatic melanoma and advanced renal cell carcinoma, leading to FDA approvals for these conditions[234,235]. The synergism of anti-CTLA4 and anti-PD1 therapies is not surprising because CTLA4 and PD1 regulate antitumoural immunity in a complementary manner[8]. Crosstalk between the CTLA4 and PD1 pathways, mediated by CD80 and PDL1 dimerization, provides additional insight into the mechanism behind the success of dual therapy[236,237]. However, as expected, combination checkpoint therapy also increases the risk of medication-induced toxicities[235].

Combining radiation therapy with checkpoint blockade is another treatment option for recalcitrant tumours. The immunomodulatory effect of radiotherapy alone represents a double-edged sword. Mechanistically, radiotherapy increases the diversity of antitumoural T cell responses by exposing novel neoantigens at the same time as blunting the immune response through the induction of PDL1 expression on tumour cells[238]. Therefore, and on the basis of preclinical data, combining radiotherapy with blockers of the PD1 axis represents an attractive synergistic combination[239]. Patients with metastatic disease may represent a target population for deploying this combination as abscopal responses to radiotherapy are boosted by checkpoint blockade for many tumour types[238,240]. Overall, dual checkpoint blockade and radiation–checkpoint polytherapy represent promising avenues for synergistic therapeutic responses because these drug combinations display unique and complementary pharmacodynamics.

New targets for checkpoint blockade. Research is also directed at newly discovered negative regulators of T cell activation, including lymphocyte activation gene 3 (LAG3), T cell immunoglobulin 3 (TIM3), V-domain immunoglobulin suppressor of T cell activation (VISTA), B7-H3 and T cell immunoreceptor with immunoglobulin and immunoreceptor tyrosine-based inhibitory motif domains (TIGIT), as adjuvant cancer drugs[241–243]. LAG3

T helper 2 (T_H2) cell skewing
Biasing of CD4⁺ T helper cells towards a phenotype essential for humoral immunity.

is an inhibitory ligand that reduces T cell activation by blocking CD4 contact sites on MHC class II proteins and is expressed on activated T cells and T_{reg} cells. It prevents the overexpansion of the T cell compartment by inducing cell cycle arrest[244]. Like PD1, LAG3 is a marker of T cell exhaustion, which portends a poorer prognosis when expressed on TILs[245]. Multiple strategies of blockade have been developed, including a LAG3:Ig fusion protein and LAG3-targeted mAbs[246]. In clinical trials in patients with renal cell carcinoma and pancreatic adenocarcinoma, these drugs did not succeed as monotherapies even though they increased the frequency of tumour-specific T cells[246]. However, when combined with paclitaxel for metastatic breast cancer, 50% of patients treated with LAG3:Ig responded to treatment[247]. Recent research has demonstrated that fibrinogen-like protein 1 (FGL1) activates LAG3 independently of binding MHC class II molecules and interference with this interaction is essential for unleashing potent antitumoural effects[248].

TIM3 is another negative regulator of the T cell response. Rather than inhibiting cell cycle progression like LAG3, it regulates apoptosis following galectin 9 binding[249]. Its upregulation could represent a mechanism of resistance to anti-PD1 therapy, making combination therapy an attractive option to boost the effectiveness of anti-PD1 therapy. In addition, TIM3 expression correlates with poor prognosis in non-small-cell lung cancer and follicular lymphoma, suggesting a role in cancer progression[250]. Similar to TIM3, VISTA is another molecule shown to be associated with resistance to current checkpoint inhibitors and has demonstrated synergism with anti-PD1 therapy in mouse models[251,252].

B7-H3 represents another targetable negative regulator of the T cell response. It is highly expressed in many tumour types, including non-small-cell lung carcinoma, prostate cancer, pancreatic cancer, ovarian cancer and colorectal cancer[241,243]. Enoblituzumab, a humanized mAb targeting B7-H3, was effective at inducing antitumoural responses in a phase I study of patients with various tumour types[253]. Dual-affinity retargeting (DART) proteins that bind to B7-H3 and CD3, as well as radioactive iodine-conjugated B7-H3 mAbs, represent additional ways to modulate this pathway and are in early-phase clinical testing[254,255].

Lastly, TIGIT, which contains two immunoreceptor tyrosine-based inhibitory motifs in its intracellular domain and dampens T cell hyperactivation, is being investigated as a checkpoint target. It is more robustly expressed in TILs than in peripheral cells, making it an attractive target owing to its increased specificity compared with other checkpoint molecules[243]. Preclinical evidence demonstrates that TIGIT blockade augments the effect of pre-existing checkpoint inhibitors and reinvigorates tumour-specific exhausted T cells[250,256]. Currently, blockade of immune checkpoints other than CTLA4 or the PD1 axis have not yet shown major clinical benefits as single agents but rather may increase the effectiveness of pre-existing treatments.

Although the blocking of immune checkpoint molecules releases potent antitumoural responses, the stimulation of T cell co-stimulatory receptors, including inducible co-stimulator (ICOS), tumour necrosis factor receptor superfamily member 4 (TNFRSF4; also known as CD134), tumour necrosis factor receptor superfamily member 9 (TNFRSF9; also known as 4-1BB), glucocorticoid-induced tumour necrosis factor receptor (GITR) and CD27, can also amplify the effect of existing immunotherapies, as shown preclinically and in early-stage clinical studies[168,170,171,241–243,257]. ICOS is a member of the CD28 family of co-stimulatory molecules that mediates context-dependent cytokine responses with an emphasis on T helper 2 (T_H2) cell skewing[258]. ICOS stimulation by vaccines modified to express ICOS ligand exhibited synergism with treatment with CTLA4-blocking antibodies preclinically[259]. ICOS upregulation following treatment with currently approved anti-CTLA4 and anti-PD1 therapies may represent a biomarker of active antitumoural responses because it associates with favourable outcomes[260].

TNFRSF4 is another co-stimulatory molecule for which preclinical evidence indicates a role in deploying robust antitumoural responses in sarcoma, melanoma and breast cancer[261,262]. Data suggest that targeting TNFRSF4 amplifies anti-PD1 therapy because TNFRSF4 agonism can upregulate PDL1 expression[263]. In addition to synergism with checkpoint blockade, TNFRSF4 upregulation within CAR T cells by transfection represents a way to augment tumour cytotoxicity[170]. Agonism of additional TNFR family members, such as TNFRSF9, GITR and CD27, is being tested as adjuvant therapy in phase I/II trials for various tumour types, with promising results[243]. Therefore, agonism of positive T cell co-stimulatory signals, in concert with the existing checkpoint inhibitors or CAR T cells, represents a novel therapeutic avenue to boost antitumoural immunity.

Concluding remarks

Cancer immunotherapy focused on T cells has emerged as a powerful tool in the armamentarium against cancer. Nevertheless, it took many years of basic science discoveries and subsequent clinical translation to unequivocally demonstrate the power of modulating the immune system to treat cancer. Further research that investigates the regulation of T cells and other immune cells, for example APCs and natural killer cells, may allow us to enhance the power of this approach. In 'difficult to treat' tumours, the effect sizes observed in clinical trials of checkpoint blockade agents, ATC transfer therapies and cancer vaccines have been far higher than the most effective chemotherapeutic agents. Although immune-related adverse effects are common, these innovative immune-targeting therapies are better tolerated than traditional chemotherapeutic agents. The burgeoning field of cancer immunotherapy continues to grow as indications for currently approved therapies expand and the search for novel druggable targets continues. The cancer immunotherapy success stories we have recounted highlight the intrinsic connection between basic science research and clinical practice. They also illustrate how a bench-to-bedside approach, built upon a solid basic science foundation, can be successful in fighting one of humanity's most dreaded diseases.

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