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Regulatory T cells in the treatment of disease

Amir Sharabi1,2*, Maria G. Tsokos1, Ying Ding3, Thomas R. Malek3, David Klatzmann4,5,6* and George C. Tsokos1*

Abstract | Regulatory T (Treg) cells suppress inflammation and regulate immune system activity. In patients with systemic or organ-specific autoimmune diseases or those receiving transplanted organs, Treg cells are compromised. Approaches to strengthen Treg cell function, either by expanding them ex vivo and reinfusing them or by increasing the number or capacity of existing Treg cells, have entered clinical trials. Unlike the situation in autoimmunity, in patients with cancer, Treg cells limit the antitumour immune response and promote angiogenesis and tumour growth. Their immunosuppressive function may, in part, explain the failure of many immunotherapies in cancer. Strategies to reduce the function and/or number of Treg cells specifically in tumour sites are being investigated to promote antitumour immunity and regression. Here, we describe the current progress in modulating Treg cells in autoimmune disorders, transplantation and cancer.

The immune system employs numerous mechanisms to regulate T cell-dependent immune responses. Downregulation of an immune response following infection is critical to avoid uncontrolled clonal expansion and excessive cytokine production, and reducing the immune response to self-antigens is necessary to prevent organ damage in autoimmune diseases. On the flip side, the suppression of the immune response that occurs in patients with cancer is detrimental, as it allows unchecked tumour growth.

Much of our understanding of these processes comes from uncovering the cellular and molecular mechanisms by which responses to self-antigens are regulated. Numerous different cells harbour suppressive activity that contributes to self-tolerance (Supplementary Box 1). The most important cells in the suppression of self-reactive T cells are the CD4+ T cells that express forkhead box P3 (FOXP3) (herein referred to as regulatory T (Treg) cells). The critical role of Treg cells in the development of autoimmunity has been highlighted by the multi-organ autoinflammatory syndrome seen in humans3,4, which occurs in individuals with mutations in FOXP3, high expression of Treg cells and is now being exploited to treat autoimmune diseases and cancer. We discuss the biology and function of Treg cells and then highlight the current therapeutic approaches being investigated to either empower them or limit their suppressive capacity and expansion.

Biology of Treg cells

Developmental heterogeneity

Treg cells are marked by the transcriptional regulator FOXP3 and constitute approximately 5–10% of peripheral CD4+ T cells in humans and mice11. FOXP3 is a reliable marker for the identification of Treg cells in humans4–10. In addition, strategies to reduce the function and/or number of Treg cells are being investigated to promote antitumour immunity.

In this Review, we focus on therapies that target Treg cells and are now being exploited to treat autoimmune diseases and cancer. We discuss the biology and function of Treg cells and then highlight the current therapeutic approaches being investigated to either empower them or limit their suppressive capacity and expansion.

 approaches to increase the number and function of Treg cells could clearly benefit patients with autoimmune disorders. Some of these approaches are now in clinical trials. One such effort involves adoptive Treg cell therapy5,7 and includes the potential to engineer antigen specificity into the transferred Treg cells; in another, low-dose interleukin (IL)-2 is administered to selectively expand Treg cell populations, a strategy that could be applied to many patients with autoimmune diseases4–10. In addition, strategies to reduce the function and/or number of Treg cells are being investigated to promote antitumour immunity.

Biological pathways

The immune system employs numerous mechanisms by which responses to self-antigens are regulated. Numerous different cells harbour suppressive activity that contributes to self-tolerance (Supplementary Box 1). The most important cells in the suppression of self-reactive T cells are the CD4+ T cells that express forkhead box P3 (FOXP3) (herein referred to as regulatory T (Treg) cells). The critical role of Treg cells in the development of autoimmunity has been highlighted by the multi-organ autoinflammatory syndrome that develops in FOXP3-deficient mice12 and the immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome seen in humans13, which occurs in individuals who harbour mutations in FOXP3.

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Biological pathways

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FOXP3 controls many functions of Treg cells. FOXP3 stabilizes the Treg cell lineage in part by reinforcing a gene expression programme that is required for the suppressive function of Treg cells. Treg cell populations expand in response to IL-2, and this cytokine is required for Treg cell survival. FOXP3 represses IL-2 expression, which is produced by Treg cells during immune reactions, and thus, Treg cells are dependent on paracrine IL-2.

Treg cells are classified into two major groups on the basis of their developmental origins. One population of Treg cells, designated thymus-derived Treg (tTreg) cells, develops in the thymus. Another population of Treg cells develops when conventional peripheral CD4+ T cells become activated by antigen and encounter certain environmental signals that promote FOXP3 expression and suppressive function. When this occurs in vitro, these Treg cells are designated induced Treg (iTreg) cells, and when this occurs in vivo, these cells are designated peripherally induced Treg (pTreg) cells (Supplementary Box 2; Supplementary Fig. 1).

Unlike tTreg cells, which constitute a stable population of suppressor cells, pTreg cells have substantial plasticity and may convert into T effector cells, which are characterized by the production of interferon-γ (IFNγ) and IL-17. This feature allows for a cellular adaption that adapts to conditions within specific tissue sites. For example, pTreg cells are abundant in the gut mucosa, where they promote tolerance to a normal microbiota. However, during an infection, the inflammatory environment may convert pTreg cells into T helper 1 cells (T(H)1 cells) or T helper 17 cells (T(H)17 cells) to promote an immune response.

A two-step model has been proposed whereby signalling from the T-cell receptor (TCR), the co-stimulatory molecule CD28 and IL-2R promotes Treg cell development (Fig. 1). The first step depends on the engagement of TCR and CD28, which together define archetypal immune stimulation, whereby TCRs with high affinity for self-antigens interact with self-peptide-major histocompatibility complex (MHC) class II complexes on antigen-presenting cells (APCs) to initiate fairly strong TCR signalling that activates nuclear factor-κB (NF-κB), nuclear factor of activated T cells (NFAT), and forkhead box protein O (FOXO) transcription factors in CD4+CD8+ thymocytes. NFAT1 and FOXO bind directly to the promoter, conserved non-coding region 2 (CNS2) and CNS3 of the FOXP3 gene to drive FOXP3 expression and Treg cell development. These cells then differentiate into IL-2-responsive Treg cell progenitors (CD4+CD25+CD122+GITR+FOXP3+ T cells). In the second step, IL-2R signalling promotes these FOXP3+ Treg cell progenitors to further develop into fully functional mature FOXP3+ Treg cells by activation of signal transducer and activator of transcription 5 (STAT5). STAT5 has been proposed to initiate the demethylation of CNS2, which would help facilitate FOXP3 transcription. In addition to these primary signalling events, other molecules promote Treg cell maturation. Some of these include TNF receptor superfamily (TNFRSF) members, such as GITR (also known as TNFRSF18), OX40 (also known as TNFRSF4) and TNFR2 (also known as TNFRSF1B), which function as co-stimulatory molecules to sensitize Treg cell precursors to IL-2 stimulation.

Another important aspect of Treg cell development is that the phosphoinositide 3-kinase (PI3K) pathway, which mediates signalling downstream of the TCR, is attenuated in Treg cells relative to T effector cells owing to the upregulation of PTEN in Treg cells. PTEN is the main negative regulator of the PI3K pathway. Whereas transforming growth factor β (TGFβ) is essential for pTreg cell development, its role in tTreg cell development is less clear, as mice lacking TGFβ signalling did not have a numerical defect in tTreg cells. Other studies, however, suggest that TGFβ, stimulated by thymic apotosis, is an essential factor for Foxp3 transcription and Treg cell generation in the thymus.

**Functions and phenotypic heterogeneity**

Multiple mechanisms have been ascribed to mouse and human Treg cells that suppress autoreactive T cells. One prominent mechanism is the secretion of proteins, such as IL-10, IL-35 and TGFβ, which suppress pro-inflammatory responses. Treg cells also promote the conversion of dendritic cells (DCs) to a tolerogenic state through surface expression of cytotoxic T lymphocyte protein 4 (CTLA4), which down-modulates the expression of the co-stimulatory molecules CD80 and CD86 on APCs and stimulates DCs to produce the immunosuppressive enzyme indoleamine 2,3-dioxygenase (IDO). Lymphocyte activation gene 3 (LAG3), an immune checkpoint receptor, and neuropilin 1 (NRP1) have also been suggested to promote tolerogenic DCs.

In turn, DCs, particularly those chronically exposed to antigen and rendered tolerogenic, may promote Treg cell expansion and function (see below).

The ectoenzymes CD39 and CD73 are highly expressed on the surface of some Treg cells and these two enzymes together convert extracellular ATP to adenosine, which inhibits the proliferation of T effector cells and suppresses the function of DCs. In addition, the high level of CD25 on Treg cells sequesters IL-2 within the microenvironment and therefore hampers IL-2-dependent activation of CD8+ T cells and natural killer (NK) cells. Lastly, intratumoural Treg cells also exhibit direct cytotoxicity towards NK cells and autoreactive CD8+ T cells, which mediate tumour cell clearance.
Natural killer (NK) cells

Cytotoxic lymphocytes critical to the innate immune system that provide rapid responses to viral infection and respond to tumour formation. They express an array of activating and inhibitory receptors and produce interferon-γ.
Box 1 | Tissue-resident Treg cells

Regulatory T (Treg) cells constitute a higher percentage of the T cells present in tissues than in the peripheral blood, and most of the tissue-resident Treg cells have an effector or memory phenotype and could be classified as effector Treg cells. Moreover, gene expression patterns in tissue-resident Treg cells are distinctly dependent on the hosting tissue. For example, an intestinal peripherally induced Treg cell subset expresses ROR-γt and can produce interleukin(IL)-17 (REF.300). These two molecules are hallmarks of T helper 17 cells, which are present in large numbers in the intestine. Similarly, visceral adipose tissue Treg cells express peroxisome proliferator-activated receptor-y to regulate insulin sensitivity. Visceral adipose tissue Treg cells also express high levels of the IL-33 receptor (IL-33R, also known as IL1RL1), which is required for their accumulation in visceral adipose tissue. Some strains of gut microorganisms can induce Treg cells in the intestine. Deletion of skin-resident Treg cells in mice leads to atopic dermatitis. The biology and characteristics of human tissue-resident Treg cells have been reviewed.

In many ways, the heterogeneity of Treg cells mirrors that reported for T effector (Teff) cells, and one might simply view Treg cells as activated T cells that suppress rather than promote immunity. An important parallel between Treg cells and Teff cells is that key transcriptional regulators that are expressed in Treg cells are also expressed by Teff cells and are associated with the suppression of the corresponding Treg cell subset. For example, Treg cells that suppress Teff cells express T-BET (also known as T-box transcription factor, TBX21), the signature transcription factor of Teff cells. Likewise, Treg cells that suppress T helper 2 cells (Treg cells) express GATA3, STAT3, and BCL6, respectively, which are key transcriptional regulators associated with each of these Teff subsets.

The precise mechanism of how Treg cell subsets affect a particular type of Teff cell response is not well understood. However, it is likely that both Treg cells and Teff cells respond to similar environmental cues, which promote Treg cells to migrate to and counteract inflammatory responses promoted by the corresponding Teff cell subset, thus preventing undesired bystander suppression.

Mechanistic target of rapamycin (mTOR) integrates cell signalling pathways and metabolic inputs and enables specific cell responses. mTOR is a serine/threonine-protein kinase in the PI3K-related kinase family that forms the catalytic subunit of two distinct protein complexes, known as mTOR complex 1 (mTORC1) and mTORC2. mTORC2 phosphorylates and activates AKT, a key effector of PI3K signalling that promotes cell survival, proliferation and growth through the phosphorylation and inhibition of several key substrates, which include transcription factors and kinases.

In Treg cells, mTOR signalling is high and increases the inflammatory response; mTOR activity is low in Treg cells. Indeed, deletion of mTOR in all conventional CD4+ T cells results in a phenotype reminiscent of Treg cells. However, a certain level of mTORC1 activity is necessary for Treg cells to meet their metabolic demands, which occurs through cholesterol and lipid metabolism, specifically through the mevalonate pathway. mTORC1 is essential to Treg cell function, and when mTORC1 is conditionally knocked out in Treg cells, mice develop a severe lymphoproliferative autoimmune condition. In Treg cells, mTOR upregulates genes associated with the pentose phosphate pathway and the glucose transporter is especially dependent on IL-2 for maintenance. IL-2 also promotes eTreg cell survival, as terminally differentiated (KLRC1+) eTreg cell numbers are reduced in mice with intrinsic defects in IL-2R signalling. IL-2 promotes Treg cell survival in part by upregulating the transcription of the anti-apoptotic molecules BCL2 and MCL1 (REF.9).

Administration of IL-2 to mice (alone or as an IL-2–anti-IL-2 antibody complex to increase its bioavailability) promotes the proliferation of Treg cells in the periphery. This indicates that IL-2 is a potent growth factor for Treg cells and raises the possibility that it can be used as a means to directly expand these suppressive cells in the context of autoimmune diseases. The conundrum had been that IL-2 is well known to be a growth factor for T effector cells as well. Thus, the use of IL-2 in autoimmune diseases was thought to be contraindicated because IL-2 might also expand pathogenic self-reactive T cells. However, studies of genetically modified mice in which IL-2R signalling was impaired, but not abrogated, showed that low IL-2R signalling readily promoted the development and homeostasis of Treg cells while the IL-2-dependent activation of effector cells remained impaired. The high level of expression of CD25 on mouse and human Treg cells, relative to other lymphoid cells, is one key reason why Treg cells exhibit high sensitivity to low levels of IL-2. This was used to conceptualize the first investigation of IL-2 in the context of autoimmune diseases and will be discussed in more detail below, as these approaches are in clinical trials.

Key molecules that control Treg cells

The list of molecules, including transcription factors, kinases and phosphatases, that are recognized to be important for the function of Treg cells is growing. These molecules offer additional targets for therapeutic exploitation.

TCR and IL-2R signalling

Treg cell homeostasis in the periphery depends critically on TCR and IL-2R signals. In mice with conditional TCR ablation in Treg cells, nTreg cell numbers were readily maintained, whereas eTreg cell numbers were substantially reduced. Thus, maintenance of the eTreg cell population depends on recent TCR stimulation. In addition, when placed in competitive, IL-2–limited environments, Treg cells that have intrinsically impaired IL-2R signalling compete poorly with wild-type Treg cells, showing clearly that IL-2 is important for their maintenance in secondary lymphoid tissues. Recently IL-2–activated Treg cells in vivo, defined as those Treg cells that contain activated STAT5, are nTreg cells, indicating that this Treg cell subset...
GLUT1 (also known as SLC2A1), thereby facilitating glucose entry into the cells and subsequent glycolysis. By contrast, in T\textsubscript{reg} cells, in a manner similar to that observed in memory CD8\textsuperscript{+} cells, mTOR is required not for glycolysis but rather for lipogenesis to maintain the functional capacity of these cells\textsuperscript{63,64}. These findings illustrate how mTORC1 integrates signalling from the TCR, IL-2 and nutrient availability to modulate lipogenic pathways that are essential for T\textsubscript{reg} cell function. Thus, although complete deletion of mTORC1 results in T\textsubscript{reg} cell dysfunction and autoimmunity, unrestrained mTORC1 activity also disrupts T\textsubscript{reg} cell homeostasis.

PTEN is a lipid phosphatase that negatively regulates PI3K by dephosphorylating phosphatidylinositol (3,4,5)-triphosphate (PIP3), the dominant signalling molecule produced by the kinase activity of PI3K\textsuperscript{65}. PIP3 is highly expressed in T\textsubscript{reg} cells and regulates their differentiation\textsuperscript{66}. It has been shown that the NRP1–semaphorin 4A (SEM4A) axis stabilizes T\textsubscript{reg} cell function by restraining PI3K and potentiating PTEN activity\textsuperscript{67}. Conditional deletion of PTEN in T\textsubscript{reg} cells in mice resulted in a systemic lymphoproliferative autoimmunity that resembled systemic lupus erythematosus (SLE) with high titres of anti-nuclear antibodies and immune complex glomerulonephritis. Mechanistically, all these changes were attributed to increased mTORC2 and AKT activity\textsuperscript{27}.

Protein phosphatase 2A (PP2A), a serine/threonine phosphatase, is crucial for T\textsubscript{reg} cell function. In mice, the catalytic activity of PP2A in T\textsubscript{reg} cells is substantially higher than in conventional T cells, and conditional deletion of PP2A in T\textsubscript{reg} cells leads to severe multi-organ autoimmune pathology. FOXP3 promotes the generation of ceramide, which activates the catalytic subunit of PP2A, which in turn inactivates mTORC1 but not mTORC2 (REF.\textsuperscript{68}).

Other molecules, such as NOTCH, other transcription factors and chromatin remodelers (Supplementary Box 3), are also key to T\textsubscript{reg} cell function. Understanding the molecular and/or biochemical events that govern T\textsubscript{reg} cell function is vital to the development of new T\textsubscript{reg}-based therapeutic approaches. Manipulation of kinase or phosphatase activity, preferably in a cell-specific manner, may offer, as discussed below, opportunities to modulate inflammatory and autoinflammatory responses.

**T\textsubscript{reg} cells in key human diseases**

**Monogenic diseases**

Several monogenic conditions illustrate the importance of functioning T\textsubscript{reg} cells to human immune homeostasis. The prototypical example is FOXP3 deficiency, which results in IPEX syndrome, a disease that appears within the first several years of life in males and requires bone marrow transplantation\textsuperscript{49,50}.

Patients with CD25 deficiency present with both autoimmunity and immunodeficiency and a disease that is clinically similar to IPEX syndrome. CD25 deficiency does not affect the number of FOXP3\textsuperscript{+} cells in the circulation but impairs their suppressive function by decreasing IL-10 production and increasing IL-2 availability (loss of the ‘IL-2 sink’\textsuperscript{76,71}). Deficiency of or mutations in STAT5B\textsuperscript{72,73}, CTLA4 (REF.\textsuperscript{74}) orLRBA\textsuperscript{75} also result in autoimmune phenotypes.

**Systemic lupus erythematosus**

T\textsubscript{reg} cells have been logically implicated in the pathophysiology of systemic autoimmune diseases, although their numbers and function have been reported to be variable\textsuperscript{6}. This variability most likely reflects the heterogeneity of the disease and the small size of the studied cohorts. The clinical heterogeneity is confounded by diversity in experimental protocols, markers to identify T\textsubscript{reg} cells and the conditions and performance of functional assays\textsuperscript{76–79}. The proportion of activated T\textsubscript{reg} cells is reduced during the early phases of the disease, whereas the CD45RA FOXP3\textsuperscript{low} non-T\textsubscript{reg} cell population is increased in patients with active SLE\textsuperscript{80}. In addition, T cells from patients with SLE have low levels of phosphorylated STAT5 upon stimulation with IL-2, suggesting an inherent T\textsubscript{reg} cell defect\textsuperscript{81}. A small study reported decreased numbers of T\textsubscript{reg} cells in the kidneys of patients with lupus nephritis and anti-neutrophil cytoplasmic antibody-associated vasculitis\textsuperscript{82}; this finding suggests that T\textsubscript{reg} cells are involved in the repair of tissue damage and that approaches to direct T\textsubscript{reg} cells to the kidney could be therapeutically useful.

**Organ-specific autoimmune diseases**

In type 1 diabetes (T1D), inflammatory cells infiltrate the pancreatic islets and destroy the insulin-producing cells. The number of T\textsubscript{reg} cells is reduced during the early phases of the disease\textsuperscript{83}. This numerical deficiency is exacerbated by their reduced ability to increase the expression of FOXP3 in response to IL-2 (REF.\textsuperscript{84}) and the resistance of T\textsubscript{reg} cells to suppression\textsuperscript{85}.

Psoriasis is an inflammatory skin disease in which the dermis and subcutaneous tissue are infiltrated with the pancreatic islets and destroy the insulin-producing cells. The number of T\textsubscript{reg} cells is reduced during the early phases of the disease\textsuperscript{83}. This numerical deficiency is exacerbated by their reduced ability to increase the expression of FOXP3 in response to IL-2 (REF.\textsuperscript{84}) and the resistance of T\textsubscript{reg} cells to suppression\textsuperscript{85}.

Myasthenia gravis is characterized by worsening muscle weakness — the pathology originates from autoimmune T cells, which provide help to B cells to produce anti-acetylcholine receptor (ACHR) antibodies, which block the ACHR. T\textsubscript{reg} cells from patients with myasthenia gravis display decreased expression of FOXP3 and compromised suppressive function\textsuperscript{86,87}, which could reflect reduced IL-2– STAT5 signalling\textsuperscript{88}. Finally, the expression of CTLA4, which has been linked to myasthenia gravis in genome-wide studies\textsuperscript{89}, is reduced on T\textsubscript{reg} cells from patients with this disease\textsuperscript{90}.

T\textsubscript{reg} cells residing in the intestine of patients with inflammatory bowel disease (IBD) exhibit a normal phenotype and are functional in assays in vitro\textsuperscript{90}. The number of T\textsubscript{reg} cells in the lamina propria under any condition of intestinal inflammation is increased, whereas the number is decreased in the periphery\textsuperscript{97,98}. It should be noted that T\textsubscript{reg} cells that are present in the lamina propria of patients with IBD do not suppress T\textsubscript{reg} cells because the TGF\textsubscript{β} signalling pathway is defective in the intestinal...
mucosa. The regulation of the inflammatory response in the gut and the balance between T_{reg} cells, T_{eff} cells and innate lymphoid cells are complex. Substantial insights have been gained from the study of the contribution of the microbiota, as has added complexity.

Multiple sclerosis is a chronic inflammatory demyelinating disease of the central nervous system, and myelin-specific autoreactive T_{eff} cells initiate a chronic autoimmune response within the central nervous system. Experiments in a mouse model of multiple sclerosis showed that T_{reg} cells are generated from ependymal T cells that infiltrate the central nervous system, but these cells fail to control autoimmune inflammation. In patients with multiple sclerosis, the number of T_{reg} cells is variable, but these cells have compromised regulatory function.

Transplantation
In transplanted organs, where the active alloimmune responses take place, T_{reg} cells that develop in response to antigen presented directly by the donor APCs or by self APCs expand and gradually infiltrate the transplanted organ. Yet, early after transplantation, T_{reg} cells fail to suppress the alloimmune inflammatory response. Inhibition of mTOR can simultaneously suppress T_{reg} cell function and improve the ability of T_{reg} cells to control the inflammatory response. Donor alloantigen-specific T_{reg} cells are more effective and have substantially less nonspecific immunosuppression than polyclonal T_{reg} cells. Polyclonal T_{reg} cells can suppress T_{eff} cells of various specificities (dominant suppression), and they can suppress T_{eff} cells generated in response to alloantigens other than that for which they were originally induced (bystander suppression).

Cancer
T_{reg} cell numbers are increased in the circulation and within tumour sites of various tumour types in humans and mice. Mechanisms that lead to intratumoural T_{reg} cell accumulation include increased recruitment through the interaction of chemokine receptor-expressing activated T_{reg} cells and the chemokines that are produced in the tumour microenvironment (CCL2–CCR4, CCL5–CCR5, hypoxia-mediated CCL8–CCR10, and CXCL12–CXCR4), local expansion of T_{reg} cells and higher resistance of T_{reg} cells than T_{eff} cells to the reactive oxygen species in the tumour microenvironment, which results in a relative increase in T_{reg} cell number. Another possibility is that T_{reg} cells that are generated de novo from conventional CD4+ T cells in the tumour microenvironment, but this has been challenged by TCR profiling studies, which did not show that the TCR repertoires in intratumoural T_{reg} cells and conventional CD4+ T cells are largely overlapping in carcinogen-induced mice and human tumours. The second challenge to this theory is that intratumoural T_{reg} cells originate from T_{reg} cells, which recognize self-antigens specific to the organ of cancer origin. Finally, tumour-generated metabolites may favour intratumoural retention and survival of T_{reg} cells over T_{eff} cells. Specifically, the increased glycolytic activity of cancer cells may create a glucose-deprived, lactic acid-enriched and fatty acid-enriched microenvironment, which favours T_{reg} cell survival, as these cells utilize fatty acid oxidation and oxidative phosphorylation to generate energy, whereas T_{eff} cells utilize aerobic glycolysis and anabolism for their bioenergetics needs. High levels of IDO and adenosine in the cancer microenvironment may be additional supportive mechanisms for intratumoural T_{reg} cell generation and function.

The intratumoural accumulation of T_{reg} cells has been associated with metastatic disease in several mouse tumour models and, more importantly, with advanced-stage disease and decreased survival in patients with cancer. Furthermore, a reduced CD8+ T cell to T_{reg} cell ratio in the tumour site is predictive of poor clinical outcome. Collective experimental and clinical evidence supports the notion that intratumoural T_{reg} cells facilitate tumour growth and progression by suppressing antitumour immune responses, promoting tumour angiogenesis and stimulating metastasis via receptor activator of NF-κB ligand (RANKL; also known as TNFSF11) signalling.

Some studies indicated a better prognosis for colorectal cancers with FOXP3+ T cells infiltrates. However, a recent study showed that certain colorectal cancers contain an abundance of FOXP3+CD45RA− non-T_{reg} cells, which secrete pro-inflammatory cytokines, and that these patients have a better prognosis than those with a predominant FOXP3+CD45RA+ T_{reg} cell population. This difference in FOXP3 expression could account for the conflicting data, as the distinction between FOXP3+ and FOXP3+ cells by immunohistochemistry could have been difficult. Tumour-resident T_{reg} cells may suppress cytotoxic immune responses by contact-dependent (involving CTLA4, programmed cell death 1 ligand 1 (PD-L1), LAG3, NRP1, CD39 or CD73) or contact-independent (involving IL-10, TGFβ, granzyne, galec tin 1, adenosine, prostaglandin E2 (PGE2) or IDO) mechanisms or may acquire unique tumour-specific immunoregulatory mechanisms, as recently reported for specialized tissue-resident T_{reg} cells that were epigenetically reprogrammed to express tissue-appropriate molecules. In this way, tumours may behave as newly formed tissues with specialized immunoregulatory microenvironments. For example, a recent report indicates that the SEMA4A–NRP1 pathway is utilized by T_{reg} cells to potentiate their function exclusively in tumours and not in other tissues. Understanding the tumour-specific mechanisms that T_{reg} cells utilize for their function will help design more effective and less toxic cancer immunotherapies.

Therapies: autoimmunity and transplants
As there is a homeostatic balance between the regulatory and effector arms of the immune response, any autoimmune or inflammatory disease marks the failure of the regulatory arm to efficiently control the effector arm and thus implies T_{reg} cell insufficiency. Importantly, this does not necessarily indicate a T_{reg} cell numerical or functional deficiency but only defective overall performance. Current treatment of autoimmunity or inflammatory diseases focuses on the reduction of the effector...
arm of the immune response with nonspecific immunosuppressant drugs. The discovery of T_{reg} cells and the understanding of the balance between the effector and regulatory arms of the immune response have opened the path to approaches to expand the overall capacity of T_{reg} cells to tilt the balance against the inflammatory process. Now, drugs and biologics are needed to improve T_{reg} cell performance (FIG. 2).

**Polyclonal T_{reg} cell therapies**

In the absence of a specific means to activate and expand T_{reg} cells in vivo, their therapeutic potential was first explored as cell therapy (TABLE 1). This raised the important question of how to purify these cells efficiently without contamination by T_{eff} cells. Indeed, the best marker to characterize T_{reg} cells, FOXP3, is a nuclear transcription factor and as such is not suitable to purify viable cells by flow cytometry, and the CD25 membrane marker that is constitutively and highly expressed by most T_{reg} cells is also transiently expressed by T_{eff} cells. Initial clinical trials used magnetic bead sorting of T_{reg} cells with the expectation that this process would enrich for cells expressing high levels of CD25, including T_{reg} cells. The advent of clinical grade fluorescence-activated cell sorting allowed the addition of low expression of CD127 as an additional marker, which improved purification137. The first therapeutic evaluation of ex vivo-expanded polyclonal T_{reg} cells was performed in hematopoietic stem cell transplantation (HSCT) and T1D using polyclonal T_{reg} cells (FIG. 3).

Preclinical data in mice demonstrated that T_{reg} cells control alloimmune responses. Indeed, T_{reg} cell depletion exacerbated graft-versus-host disease (GVHD) after allogeneic HSCT, and T_{reg} cell repletion controls GVHD138,139. This preclinical information provided the needed rationale to investigate T_{reg} cell-based therapies to control GVHD in humans. A number of small-scale efforts that gauge toxicity and clinical efficacy have been reported, including the administration of autologous peripheral blood T_{reg} cells expanded in vitro to patients with acute or chronic GVHD140,141 and the administration of partially human leukocyte antigen (HLA)-matched T_{reg} cells from cord blood in conjunction with a classical GVHD prophylaxis regimen that consists of cyclosporine, sirolimus or mycophenolate mofetil142.

In solid organ transplantation, the ONE study (performed by a consortium of institutions supported by the European Union) has investigated the safety and efficacy of T_{reg} cells administered in a dose-escalating approach (from 0.5 million to 3.0 million cells per kg body weight) in order to examine both safety and potential efficacy143.

It is estimated that, at the onset of T1D, approximately half of the β-cell mass remains functional and produces insulin but will be destroyed within a year. The administration of potent immunosuppressant drugs shortly after the onset of T1D can block the autoimmune process and prolong the ‘honeymoon’ period of the disease, but this occurs at the expense of severe side effects144,145. This information strongly supports the exploitation of immune intervention at this time of the disease by boosting the T_{reg} cell compartment. A first trial reported that infusion of CD4^+CD25^{high}CD127^- T_{reg} cells prolonged the honeymoon phase of patients with recently diagnosed T1D146, with clinical results lasting at least 1 year147. Another study sorted CD4^+CD25^{high}CD127^- T_{reg} cells, labelled them with deuterated glucose and injected them into patients with recent-onset T1D. Labelled cells were detected at least 1 year later, and some of the patients maintained endogenous insulin production for 2 years after treatment148.

These trials, which used large doses of polyclonal T_{reg} cells in HSCT and T1D, have demonstrated that T_{reg} cell injections are safe; that, despite extensive proliferation during ex vivo production, some of the cells can survive for long periods of time; and that there is evidence for potential therapeutic efficacy. In parallel, further clinical evaluation of ex vivo-expanded T_{reg} cell therapies is warranted, which also calls for improvements in T_{reg} cell purification and production.

The stability of these in vitro-generated T_{reg} cells after they are infused into the patient is of obvious importance. The possibility of devising approaches to maintain the FOXP3 locus in a demethylated state is particularly enticing149–151. In one report, hydrogen sulfide maintained the expression of the methylcytosine dioxygenases TET1 and TET2, which catalyse conversion of 5-methylcytosine to 5-hydroxymethylcytosine in the Foxp3 gene, to establish a T_{reg} cell-specific hypomethylation pattern and stable FOXP3 expression152. Drugs that imitate the action of hydrogen sulfide may prove of value to stabilize T_{reg} cells and expand their function.

**T_{reg} cell-enhancing drugs**

As discussed above, FOXP3 inhibits the PI3K–mTOR pathway153, and several studies in mice deficient in negative regulators of this pathway showed functional impairment of T_{reg} cells and systemic autoimmunity154. Human T_{reg} cells expand efficiently in the presence of the mTOR inhibitor rapamycin155. In SLE, activated mTOR in T cells accounts for several abnormalities, including the downregulation of the TCR companion signalling molecule CD3ɛ, the expansion of T_{reg}17 cell and double-negative T cell (express neither CD4 nor CD8) populations, which have been shown to contribute to tissue inflammation, and the contraction of T_{reg} cell populations156,157. Administration of rapamycin has been reported to improve clinical outcomes in lupus-prone mice158 and patients with SLE159 (TABLE 2). Moreover, rapamycin can block the production of antiphospholipid antibodies in lupus-prone mice159 and improve renal allograft survival in patients with antiphospholipid syndrome160. Rapamycin is a promising drug for the treatment of patients with systemic autoimmunity and other inflammatory conditions because it normalizes numerous T cell functions, including those of T_{reg} cells. A single-arm, open-label, phase I/II trial administered the mTOR inhibitor sirolimus (2 mg per day) to patients with active SLE who were unresponsive to, or intolerant of, conventional medications for 12 months161. Clinical indices revealed a major improvement in disease, concurrent with a reduced need for steroids. Sirolimus expanded the T_{reg} cell and CD8^+
The antigen specificity of T_{reg} cells is poorly understood. TCR analysis indicates that the T_{reg} cell repertoire is quite diverse; even the repertoire of activated eT_{reg} cells\(^{175}\), which have probably developed in response to stimulation with self-antigens\(^{46}\), is diverse. It is, however, not known whether protection from autoimmunity is performed by T_{reg} cells that recognize tissue-specific or ubiquitous self-antigens. The therapeutic efficacy of antigen-specific T_{reg} cells should be higher than that of polyclonal T_{reg} cells (reviewed previously\(^{176}\)). The advent of antigen-specific T_{eff} cells that express a chimeric antigen receptor (CAR) and are used to treat patients with cancer\(^{117}\) has instigated the consideration of developing antigen-specific T_{reg} cell therapies (FIG. 3).

Different sources of cells and different means to generate antigen specificity can be envisioned. Indeed, it is possible to turn T_{eff} cells into T_{reg} cells by overexpressing FOXP3. Likewise, the transduction of a TCR that recognizes an autoantigen, together with FOXP3, should generate antigen-specific artificial T_{reg} cells. The best CAR constructs for efficient targeting and activation of T_{reg} cells will have to be identified, as has been done for the CAR T_{eff} cells\(^{116}\). Alternatively, purified antigen-specific T_{reg} cells could also be used.

Proof-of-concept studies in animal models of disease using CAR T_{reg} cells in which the CAR recognizes specific antigens have suggested the plausibility of this approach in clinical settings\(^{179,172}\). However, the identification of an appropriate antigen in human autoimmune diseases is challenging, particularly for conditions in which more than one antigen is involved. This issue can be somewhat easily solved in the context of transplantation or immune responses to therapeutic proteins. HLA-A2 is a frequently mismatched alloantigen in transplantation. Human HLA-A2-specific CAR T_{reg} cells were better than T_{reg} cells expressing an irrelevant CAR at preventing xenogeneic GVHD caused by HLA-A2\(^+\) T cells in mice\(^{115}\). Furthermore, HLA-A2 CAR T cells were shown to alleviate alloimmune-mediated xenogeneic skin injury\(^{110}\).

Investigators have also considered the construction of T_{reg} cells with a TCR that recognizes factor VIII in order to suppress the production of neutralizing antibodies in patients with haemophilia\(^{181}\); these patients often develop immune responses to injected factor VIII. These proof-of-concept studies pave the way to clinical evaluation of CAR T_{reg} cells in humans.

**IL-2**

High doses of IL-2 were initially used to promote T_{eff} cell function against tumours, but this approach was undermined by side effects\(^{182}\). The realization that T_{reg} cells have higher affinity receptors for IL-2 (owing to the expression of CD25) and therefore stronger IL-2R-mediated...
signalling than T_{eff} cells suggested that administration of IL-2 at a lower dose than used for T_{eff} cells should promote T_{reg} cell expansion and function\(^{18}\). The demonstrated safety profile of IL-2 and its capacity to specifically activate T_{reg} cells at low doses\(^{10}\) have delivered early clinical benefits.

Low-dose IL-2. In addition to the expression of the high-affinity receptor, T_{reg} cells are also intrinsically more sensitive to IL-2 than are T_{eff} cells\(^{16}\), resulting in high levels of STAT5 phosphorylation and specific enhancement of the gene activation programme downstream of IL-2R signalling\(^{18}\). Also, IL-2 was shown to block the differentiation of naïve CD4^+ T cells into pro-inflammatory T_{H}17 cells\(^{184}\) and to favourably influence the balance between T_{H}1 cells and follicular regulatory T cells\(^{185}\).

Before T_{reg} cells were discovered, delivery of IL-2 with a vaccinia virus containing the IL2 gene to MRL/lpr mice resulted in prolonged survival and shrinkage of the double-negative T cell population, particularly the population that produces IL-17 (REFS\(^{186,187}\)). The dual nature of IL-2 signalling was uncovered in 1993, when mice deficient for IL-2 (REF\(^{186}\)) (and later for IL-2R signalling\(^{188,189}\)) were shown to develop lethal autoimmunity and inflammation rather than immunodeficiency. T_{reg} cells were later found to be defective in these mice and responsible for the phenotype\(^{191}\). Simultaneously, investigators who had used IL-2 to treat patients with cancer noticed a major expansion of T_{reg} cells\(^{192}\). At that time, IL-2 was an approved marketed drug for activating immunity effector responses to treat cancer\(^{193}\), but it had numerous and severe side effects\(^{195}\).

The Klatzmann group initiated the first proof-of-concept evaluation of low-dose IL-2 for the treatment of autoimmune diseases in 2006. Patients with hepatitis C virus (HCV)-induced vasculitis were known to have decreased numbers of T_{reg} cells, which was corrected after treatment with B cell depletors\(^{194}\) or antivirals\(^{195}\) in complete responders but not in nonresponders or partial

<table>
<thead>
<tr>
<th>Target tissue or condition</th>
<th>T_{reg} cell type administered</th>
<th>Treatment groups</th>
<th>Trial phase</th>
<th>Sponsoring institute</th>
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<td>NCT03444064</td>
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<td>i.v. 3 escalating doses; one dose per month</td>
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<td>i.v. 1.0 × 10^6 cells per kg; once</td>
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<tr>
<td></td>
<td></td>
<td>i.v. 1,600 × 10^6 cells; once</td>
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<td>i.v. 1,000 × 10^6 cells; once</td>
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<td>I</td>
<td>UCSF</td>
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</table>

CHU-ULG, Centre Hospitalier Universitaire de Liège; GVHD, graft versus host disease; i.v., intravenous; IU, international units; NIAID, National Institute of Allergy and Infectious Diseases (USA); SLE, systemic lupus erythematosus; T_{reg}, cell, regulatory T cell; UCSF, University of California, San Francisco (USA).
responders. This suggested that T<sub>reg</sub> cell stimulation drives the clinical response. Daily doses of IL-2 that were about 30-fold lower than those used in patients with cancer were administered to patients with HCV-induced vasculitis and resulted in expansion of T<sub>reg</sub> cells, without affecting T<sub>eff</sub> cells, and in substantial clinical improvement<sup>6</sup>. In a parallel study, administration of low-dose IL-2 to patients who had developed GVHD and were resistant to steroids resulted in a preferential expansion of T<sub>reg</sub> cells and clinical improvement<sup>9</sup>. Indeed, very low doses of IL-2 have even been used to prevent the development of GVHD<sup>197</sup>. Low-dose IL-2 has been used in patients with alopecia areata, characterized by autoimmune-mediated hair loss, and showed impressive hair regrowth in a subset of patients<sup>198</sup>. Importantly, this study documented that the IL-2 administration expanded T<sub>reg</sub> cell populations not only in the blood but also at the hair follicles, the site of the autoimmune process.

Several reports have demonstrated clinical improvement and expansion of the T<sub>reg</sub> cell population in patients with SLE following administration of low-dose IL-2 (REFS<sup>199–201</sup>). A caveat to this observed success is early evidence that the IL-2–IL-2R–STAT5 signalling pathway in T cells is compromised in many patients with SLE<sup>202</sup>, and, thus, administration of IL-2 to those patients is unlikely to improve the disease. However, because IL-2 has the potential to reverse several pathogenic processes involved in the development of SLE, including poor T<sub>reg</sub> cell function, increased IL-17 production, increased T<sub>H1</sub> cell activity and an expanded population of double-negative T cells<sup>203</sup>, controlled studies could reveal clinical usefulness.

As IL-2 can activate T<sub>eff</sub> cells or T<sub>reg</sub> cells in a dose-dependent manner and its side effects are also dose-dependent, defining the therapeutic window for safe and specific activation of T<sub>reg</sub> cells is crucial. On the basis of a dose-finding double-blind placebo-controlled study<sup>200</sup>, a dose of 1–2 million international units (MIU) per injection is used in an ongoing phase IIb trial in SLE and T1D. This dose allows for a 100–200% increase in T<sub>reg</sub> cell numbers after five injections.

Results of early clinical trials have confirmed the excellent safety profile of low-dose IL-2 and, other than local reactions at the injection sites, have shown no severe side effects. Low-dose IL-2 has been tried in a variety of autoimmune diseases and has had clinical benefit linked to increased numbers of T<sub>reg</sub> cells (D.K., unpublished observations). Ongoing registered clinical trials addressing the clinical efficacy of low-dose IL-2 are listed in TABLE 3.

**Modified IL-2.** In more inflammatory settings, such as in rheumatoid arthritis or during flares of various autoimmune diseases, it may be necessary to achieve more than a 200% increase in T<sub>reg</sub> cell numbers. Intense work has therefore been put into generating mutant IL-2 proteins that have increased specificity for T<sub>reg</sub> cells over T<sub>eff</sub> cells<sup>204</sup>. Another approach to improve the potential therapeutic utility of IL-2 is to increase its half-life. The half-life of IL-2 after subcutaneous injection is a few hours, although its effect on T<sub>reg</sub> cells is dose-dependent and lasts much longer. Current trials often use IL-2 in a series of three to five injections repeated over weeks or single weekly injections. Pegylation<sup>205</sup> or fusion of IL-2 with
carrier proteins such as immunoglobulin fragments206 or albumin207,208 increases its half-life (FIG. 3). However, these approaches risk rendering the recombinant fusion proteins immunogenic or even able to stimulate T req cells. The trade-off between ease of use and risks now needs to be investigated.

Complexing IL-2 with antibodies that affect its binding to the trimeric or dimeric IL-2Rs has also been explored209. Some of these complexes preferentially expand T req cell populations and improve disease in lupus-prone mice205. Although the initial development used murine antibodies, the identification of human antibodies or the humanization of murine antibodies that can confer increased specificity of IL-2 complexes for T req cells would allow their use in the clinic. Human antibodies that favour the expansion of T eff cells over T req cells have been described210, and recently, a fully human anti-IL-2 antibody, which stabilizes IL-2 in a conformation that results in the preferential STAT5 phosphorylation of T req cells in vitro and selective expansion of T req cells in vivo, has been developed211.

**Dendritic cells**

Lymphocyte antigen 75 (LY75; also known as DEC-205 and CD205) belongs to the lectin family of surface receptors that function as antigen uptake and processing receptors for tolerogenic DCs212. Thus, cognate antigen presentation by immature LY75+ DCs to naive CD4+ T cells can promote the development of functional T req cells that have the canonical T req cell signature (including expression of chemokine receptors, CTLA4 and IL-10)41. In these T req cells, the CNS2 region of the FOXP3 promoter is hypomethylated and allows for stable expression of FOXP3 and prolonged cell survival213. In the preclinical setting, LY75+ DCs promoted tolerance through the generation of T req cells and protected mice from the development of T1D and experimental autoimmune encephalomyelitis (EAE), which result from the administration of tetramers composed of region of a murine anti-DNA antibody, were beneficial in treating lupus-prone mice, and both approaches involved the induction of both CD8+ and CD4+ T req cells225,226.

Tolerogenic DCs can be generated by exposing DCs to either IL-4 and retinoic acid or IL-10 and TGFβ. DCs from patients with rheumatoid arthritis cultured under such conditions led to the development of T req cells and T eff,2 cells in culture, both of which produced immunosuppressive cytokines216. This approach has been tried in patients with rheumatoid arthritis in a phase I clinical trial wherein the patients were treated with autologous tolerogenic DCs that were differentiated ex vivo from peripheral blood mononuclear cells in the presence of IL-4, granulocyte–macrophage colony-stimulating factor (GM-CSF) and an NF-kB inhibitor and treated with citrullinated peptides (Rheumavax). These tolerogenic DCs were then injected intradermally to 18 patients with rheumatoid arthritis who carried the shared HLA-DR epitope, which recognizes the citrullinated peptides. The treatment was safe and biologically active, and 1 month after the injection, the number of T req cells decreased, and serum levels of inflammatory cytokines and chemokines (IL-15, IL-29, CXCL1 and CXCL11) were reduced217.

**Tolerogenic peptides**

Myelin basic protein (MBP) is an antigen derived from myelin sheaths. A polypeptide known as copolymer-1 (COP-1), which contains the four immunogenic amino acids found in MBP, was designed and proved clinically efficient in treating patients with multiple sclerosis218. In the murine model of EAE, administration of COP-1 was associated with induction of T req cells219.

A human anti-double-stranded DNA monoclonal antibody was the basis for the design of the hCDR1 peptide, which, when administered to mice and humans with lupus, resulted in clinical improvement and induction of FOXP3-expressing T cells220,221 that also expressed BCL-XL (REFS 222,222). In a phase II clinical trial wherein patients with SLE were treated with four different doses of hCDR1, the peptide was safe and well tolerated with some promise of clinical benefit224.

Both histone peptides derived from a histone autoepitope and the pConsensus peptide, which is based on T cell determinants in the variable chain heavy (VH) region of a murine anti-DNA antibody, were beneficial in treating lupus-prone mice, and both approaches involved the induction of both CD8+ and CD4+ T req cells225,226.

A peptide derived from heat shock protein 60 (HSP60), designated HSP60216, was shown to be tolerogenic in a murine model for rheumatoid arthritis. HSP60216-specific CD8+ T req cells were induced following the administration of tetramers composed of HSP60216 and the murine MHC class Ib molecule Qa1. These cells used perforin and IL-15 to suppress pathogenic T eff cells and T eff,17 cells, reduce the production of collagen-specific autoantibodies and inhibit the development of arthritis in these mice227.

A different approach to developing tolerogenic peptides for rheumatoid arthritis utilizes the citrullination of peptides, as anti-citrullinated peptide antibodies are a hallmark of this disease. Thus, a multi-epitope peptide was designed that contained sequences of citrullinated human autoantigens (such as type II collagen, vimentin, fibrinogen and filaggrin). This compound (Cit-ME) induced T req cells and improved clinical indices in a rat adjuvant-induced arthritis model228.
A general limitation of using tolerogenic peptides is the fact that the autoimmune response spreads to different epitopes, even those located on the same protein\(^{236}\), and, thus, the peptide may lose efficacy over time.

**Limitations and considerations**

Although therapies that promote \(T_{reg}\) cells have great potential, there are some caveats to their prospective use. Information generated in mice may not be readily transferable to humans because human and murine \(T_{reg}\) cells are not identical. For instance, some human \(T_{reg}\) cell subsets are not suppressive, whereas murine \(T_{reg}\) cells are functionally more homogeneous. Patients with established autoimmune disease already harbour high numbers of autoreactive \(T_{eff}\) cells and memory \(T\) cells and have an inflammatory milieu that can be difficult for \(T_{reg}\) cells to overcome. For instance, in patients with rheumatoid arthritis, the inflammatory cytokines (such as IL-6 and TNF) that are present in their synovial fluid make the \(T_{eff}\) cells resistant to suppression by \(T_{reg}\) cells\(^{236}\). Also, the \(T_{eff}\) cells from patients with SLE are more resistant to suppression by \(T_{reg}\) cells in vitro\(^{236,237}\). Under such conditions, it would take a high number of autoantigen-specific \(T_{reg}\) cells to suppress those pathogenic cells. Projecting from data from the mouse models, it would be wise first to get rid of the autoreactive \(T_{eff}\) cells and only then administer \(T_{reg}\) cells. Approaches to increase the sensitivity of \(T_{eff}\) cells to the suppressive effects of \(T_{reg}\) cells are also needed.

For cell therapies, \(T_{reg}\) cells that are expanded ex vivo are not necessarily stable nor long-lived. Epigenetic changes to the expanded \(T_{reg}\) cells could make them stable. In the setting of ex vivo \(T_{reg}\) cell expansion and transfusion, it is still difficult to demonstrate \(T_{reg}\) cell purity and potency. It is unlikely that all of the expanded cells will be \(T_{reg}\) cells or that their suppressive function in vitro will necessarily be reproduced in vivo. As with any cell-based immunotherapy, treatment with \(T_{reg}\) could induce an inflammatory reaction following the cell infusion (cytokine release syndrome).

For treatment with IL-2, determining the exact IL-2 'low dose' for human studies may prove difficult because the therapeutic window may vary according to the inflammatory context, and, in some patients, it may stimulate the \(T_{eff}\) cells.

**Therapies: cancer**

As discussed above, \(T_{reg}\) cells enter tumours, and except for in cancers that are driven by inflammation, \(T_{reg}\) cells promote tumour growth and progression through multiple inhibitory pathways. Therefore, several approaches have been considered to deplete \(T_{reg}\) cells, limit their entry into the tumour tissue and/or disrupt their function (Table 4).

**Nonspecific \(T_{reg}\) cell targeting**

Traditional chemotherapeutics, such as cyclophosphamide and others, have been shown to reduce the number and immunosuppressive function of \(T_{reg}\) cells through various mechanisms\(^{115}\). Low-dose cyclophosphamide (known as metronomic chemotherapy) was reported to deplete \(T_{reg}\) cells by inhibiting proliferation and inducing apoptosis and to decrease their function by reducing FOXP3 and GITR expression\(^{232,233,234}\). In addition, tyrosine kinase inhibitors (sunitinib, sorafenib and imatinib) have been shown to inhibit intratumoural \(T_{reg}\) cell expansion and function\(^{117,235,236}\). However, although these approaches can limit \(T_{reg}\) cell proliferation and function, they are not an ideal choice, as they are not specific to tumour-associated \(T_{reg}\) cells.

**Blocking \(T_{reg}\) cell trafficking into tumours**

Tumours have microenvironments rich in cytokines and chemokines that promote the accumulation of \(T_{reg}\) cells, which express high levels of chemokine receptors\(^{237,238}\).
Blocking the interactions between chemokines and their receptors — such as that of CCL22 and CCR4, which constitutes the dominant intratumoural T<sub>reg</sub> cell recruitment mechanism in numerous tumours — reduces intratumoural accumulation of T<sub>reg</sub> cells and suppresses tumour growth in mice. Similarly, intraperitoneal injection of an anti-CCR10 immunotoxin in mice with orthotopically implanted human ovarian carcinoma cells that showed hypoxia-induced overexpression of CCL28 (the ligand for CCR10) resulted in complete intratumoural T<sub>reg</sub> cell depletion and reduced tumour growth. Clinical studies conducted with a humanized anti-CCR4 antibody (mogamulizumab, KW-0761), which depletes CCR4<sup>+</sup> T<sub>reg</sub> cells by antibody-dependent cell-mediated cytotoxicity (ADCC), showed intratumoural T<sub>reg</sub> cell depletion and antitumour activity with minimal to moderate toxicity. However, a recent update of two clinical phase I/II trials reported unclear long-term antitumour effects. Occasional serious side effects were reported as well.

Comparative flow cytometric and RNA sequencing analysis of T<sub>reg</sub> cells and conventional T cells in tumour and normal tissues and in the circulation of patients with breast carcinoma showed that CCR4, in addition to being highly expressed in intratumoural T<sub>reg</sub> cells, is also expressed in T<sub>reg</sub> cells in the peripheral blood and in activated T<sub>eff</sub> cells, although in somewhat lesser amounts. It is therefore possible that the CCR4-depleting antibody affects the survival of peripheral T<sub>reg</sub> cells, which leads to unwanted effects, and affects the survival of T<sub>eff</sub> cells as well, which limits antitumour responses. Indeed, both T<sub>reg</sub> cell and T<sub>eff</sub> cell numbers were decreased in patients who received anti-CCR4 antibody treatment in a clinical study. These data highlight the need to identify molecules that are specific to tumour-dwelling T<sub>reg</sub> cells to use as therapeutic targets. Notably, two recent studies showed that intratumoural T<sub>reg</sub> cells from various human tumours express CCR8 at levels that are much higher than those in peripheral T<sub>reg</sub> cells, or conventional T cells in the periphery of tumours, indicating that CCR8 is a novel promiscuously targetable molecule. CCR8 is the receptor for the CCL1 and CCL18 chemokines, both of which are differentially upregulated in intratumoural myeloid cells.

**T<sub>reg</sub> cell depletion**

Numerous specific intratumoural T<sub>reg</sub> cell depletion strategies have been investigated and reviewed extensively. Antibodies directed to CD25 (daclizumab, basiliximab and LMB-2 (REFs 245–249), a single-chain variable fragment (scFv) fused to exotoxin A of *Pseudomonas* spp.), have been used to kill T<sub>reg</sub> cells by ADCC and complement-mediated cytotoxicity. Also, a fusion protein containing IL-2 and diphtheria toxin protein (denileukin difitox, Ontak) was designed to induce direct cytotoxicity. Preclinical and clinical studies using a combination of anti-CD25 antibodies and DC vaccines reported beneficial effects, but clinical studies with denileukin difitox had mixed results. Notably, patients with metastatic melanoma treated with denileukin difitox showed no clinical benefit and severe autoimmune side effects. Depletion of T<sub>reg</sub> cells from HSCT to treat relapses of leukaemia in patients who did not develop GVHD during the first transplant produced better outcomes. These data suggest that global T<sub>reg</sub> cell depletion has variable efficacy and the potential to induce systemic complications.

**Immune checkpoint inhibitors**

Immune checkpoint molecules are upregulated in T<sub>reg</sub> cells and thus could be targeted to modulate T<sub>reg</sub> cell function. A widely used approach to activate antitumour immunity is immune checkpoint blockade with antibodies against CTLA4 (ipilimumab and tremelimumab), which induce tumour regression and improve survival of patients with metastatic melanoma. Although CTLA4 targeting was initially aimed to reactivate T<sub>reg</sub> cells, CTLA4 is also highly expressed on T<sub>reg</sub> cells, and CTLA4 targeting induces T<sub>reg</sub> cell depletion in the tumour microenvironment by ADCC. Furthermore, CTLA4 antibody binding to T<sub>reg</sub> cells contributed, independently of its T<sub>eff</sub> cell binding, to the antitumour activity of this molecule, thus leading to a synergistic maximal antitumour effect in a mouse melanoma model. Intriguingly, however, conditional CTLA4 depletion in T<sub>reg</sub> cells increased the immunosuppressive functions of T<sub>reg</sub> cells in adult mice, indicating that the role of CTLA4 in T<sub>reg</sub> cells needs to be further investigated. Undesirable autoimmune manifestations in a subset of patients treated with anti-CTLA4 antibodies indicated systemic loss of T<sub>reg</sub> cell activity.

Anti-CTLA4 antibodies have complimentary activity with therapies targeting anti-PD-1 (nivolumab), another checkpoint inhibitor expressed by T<sub>reg</sub> cells, and their combined use is more beneficial than either agent alone. Furthermore, because anti-CTLA4 and anti-PD-1 therapies benefit only a small subset of patients with cancer and may have undesirable effects, such as the development of autoimmune manifestations, other molecules that are expressed by T<sub>reg</sub> cells with superior immunosuppressive activity, such as T cell immunoreceptor with immunoglobulin and ITIM domains (TIGIT), LAG3 and T cell immunoglobulin mucin domain 3 (TIM3; also known as HAVCR2), are being considered and evaluated in early-phase clinical trials. TIM3 targeting may be more advantageous than CTLA4 and PD-1 targeting, because expression of TIM3 is restricted to intratumoural T cells, and hence its inhibition is less likely to interfere with T cell functions in other sites; TIM3-deficient mice do not exhibit autoimmune disorders.

**Engagement of TNFRs**

GITR belongs to the group of TNFRs that provides co-stimulatory signalling to increase T cell activation and is induced in T cells upon activation, but GITR is also highly expressed (higher than in the periphery) in intratumoural T<sub>reg</sub> cells. Ligation of GITR with an agonistic antibody was shown to suppress T<sub>reg</sub> cell activity, reduce T<sub>reg</sub> cell numbers and decrease T<sub>reg</sub> cell lineage stability selectively in tumours and also limit tumour growth in mice, particularly when administered together with CTLA4 (REF. 249) or PD-1 inhibitors. GITR ligation, similar to CTLA4 blockade, also improves T<sub>eff</sub> cell functions and renders T<sub>reg</sub> cells resistant to inhibition by T<sub>reg</sub> cells. Mice treated with an
anti-GITR antibody showed dramatically reduced tumour growth and the intratumoural Treg cells in these mice had decreased expression of HELIOS (also known as IKZF2, a transcription factor that maintains Treg cell stability and function) and increased IFNγ production. These data provide a mechanistic explanation for the reduction of Treg cell lineage stability by GITR ligation and reinforce GITR as a therapeutic target to reverse the immunosuppressive function of intratumoural Treg cells. Although side effects have been reported with GITR ligation\(^{271}\), these may be avoided by using low doses of anti-GITR antibody, which are sufficient for Treg cell conversion in mice\(^{272}\). In addition, the anti-GITR antibody has been reported to increase the number of Treg cells in the periphery\(^{273,274}\).

Another TNF receptor family member, OX40, has similar pattern of expression and functional properties to GITR and, upon ligation, leads to impaired Treg cell function and improved Treg cell responses\(^{275}\). On the basis of the promising results from the animal studies, human GITR and OX40 agonists, as well as agonists for other members of the TNFR family (CD27 and 4-1BB) are currently being evaluated in clinical trials as monotherapies or in combination with other agents\(^{275,276,277}\).

Two newly developed antagonistic antibodies that target TNFR2, which is expressed by the most suppressive intratumoural Treg cells and by human cancers, disabled the ability of TNFR2 to bind TNF and activate NF-κB, killed Treg cells and also induced killing of tumour cells\(^{279}\). Interestingly, TNFR2 can exert bidirectional control on Treg cells because it can induce their proliferation and activation through the NF-κB, AP1 and MAPK pathways\(^{280}\). TNFR2 agonists have been used as a novel strategy to induce Treg cell expansion in vitro and to inhibit GVHD in vivo\(^{281-283}\). Therefore, TNFR2 is an exciting molecular target for the development of Treg cell-based immunomodulatory therapies to treat both cancer and autoimmune diseases using antagonistic or agonistic antibodies respectively.

### IDO inhibitors

IDO, an enzyme with two isoforms (IDO1 and IDO2), converts tryptophan to kynurenine, resulting in depletion of tryptophan, a molecule that is central to T cell proliferation and differentiation. Lack of tryptophan and upregulation of kynurenine diminishes T cell proliferation and survival and induces the differentiation of T cells into Treg cells\(^{287-290}\). In the tumour microenvironment, IDO is produced by plasmacytoid DCs and by tumour cells in response to inflammatory stimuli, such as IFNγ, which leads to decreased Treg cell responses and increased Treg cell responses. IDO1 is more widely expressed than IDO2 and is overexpressed and activated in many human cancers.
often (but not always) conferring a poor prognosis\textsuperscript{286,287}. IDO inhibitors (indoximod (NLG-8189), navoximod (NLG-919), epcadostat (INCB024360), BMS-986205 and others) are currently being evaluated in various clinical trials, mostly in combination with standard chemotherapy or with other immunotherapies, such as checkpoint inhibitors or tumour vaccines. The preclinical and clinical development of IDO inhibitors have been reviewed\textsuperscript{286}. Preliminary studies have shown that the combination of IDO and PD-1 inhibitors is fairly safe although not entirely without side effects. Interestingly, it was reported that combined treatment of mice with melanoma with an IDO inhibitor and a tumour vaccine induced conversion of \(T_{reg}\) cells into \(T_{eff}\)-like cells in the tumour-draining lymph node and increased \(T_{eff}\) cell activation and antitumour efficacy, suggesting that the combined use of IDO inhibitors and tumour vaccines could be an alternative strategy to deactivate \(T_{reg}\) cells by converting them to \(T_{eff}\)-like cells\textsuperscript{288}.

\(T_{reg}\) cell-derived cytokines

In another approach, the effects of the anti-inflammatory cytokines (TGF\(\beta\), IL-10 and IL-35) that are secreted at high levels by \(T_{reg}\) cells in the tumour microenvironment were blocked using neutralizing antibodies. Blockade of TGF\(\beta\) expressed on the surface of \(T_{reg}\) cells improved the anti-melanoma immune response\textsuperscript{282} and suppressed metastasis of pancreatic tumours in mice\textsuperscript{113,289-291}. Anti-IL-35 limited tumour growth in multiple mouse models of human cancer\textsuperscript{284}.

Novel intratumoural \(T_{reg}\) cell targets

NRP1, a receptor for the immune cell-expressed ligand SEMA4A, is selectively and highly expressed in intratumoural \(T_{reg}\) cells and correlates with poor prognosis in patients with melanoma\textsuperscript{292}. NRP1 regulates \(T_{reg}\) cell function and survival in the inflammatory tumour environment through ligation with SEMA4A, and targeting this axis can inhibit intratumoural \(T_{reg}\) cell function\textsuperscript{293}. NRP1-deficient intratumoural \(T_{reg}\) cells lose their immunosuppressive function and produce IFN\(\gamma\) (a \(T_{eff}\) cell cytokine) while retaining FOXP3 expression, thus exhibiting a phenotype that was named \(T_{reg}\) cell fragility (REF\textsuperscript{295}). Mice lacking NRP1 in \(T_{reg}\) cells or treated with blocking antibodies to NRP1 or SEMA4A exhibit delayed tumour growth and have no autoimmune responses. Furthermore, IFN\(\gamma\) produced by NRP1-deficient intratumoural \(T_{reg}\) cells drives the fragility of adjacent NRP1-expressing \(T_{reg}\) cells, thereby amplifying the effect and leading to tumour regression; IFN\(\gamma\) receptor inactivation abrogates the anti-PD-1 tumour response, as shown in a fibrosarcoma mouse model\textsuperscript{296}. These findings indicate that NRP1 is a potential therapeutic target, which could reverse the immunosuppressive function of intratumoural \(T_{reg}\) cells and improve the efficacy of checkpoint blockade therapies while maintaining peripheral tolerance.

Another recent study found that the c-REL subunit of the canonical NF-\(\kappa\)B pathway is required for the maintenance of the active status of \(T_{reg}\) cells\textsuperscript{297}. REL genetic ablation or degradation by pentoxifylline, a US Food and Drug Administration-approved drug, downregulates HELIOS and impairs the molecular identity and suppressive function of \(T_{reg}\) cells, indicating that c-REL can be targeted to inhibit \(T_{reg}\) cell function\textsuperscript{298}. Pentoxifylline did not reduce growth of established tumours as a monotherapy but potentiated the effect of PD-1 blockade and was well tolerated in mice and patients.

Oxygen-sensing propyl hydroxylases promote tumour growth by inducing \(T_{reg}\) cell function and limiting \(T_{eff}\) cell function. Genetic or pharmacological inhibition of the oxygen-sensing propyl hydroxylases limits tumour metastasis to the lungs\textsuperscript{293}.

Because \(T_{reg}\) cells in tumour tissues diversify to adapt to the tumour microenvironment, characterization of the immune features of the tumour-infiltrating cells has gained wide attention for both predicting clinical outcomes and deciding which immunotherapy may work the best\textsuperscript{296,297}. The immune landscape of each tumour should probably dictate the immune approach most expected to be of clinical value.

**Conclusions and future directions**

Immunotherapy has been well established in the treatment of autoimmune diseases and is expected to substantially advance the treatment of cancer. In autoimmune diseases, most of the established approaches address the over-reactive autoimmune response and have been successful in organ-specific diseases including rheumatoid arthritis, IBD, psoriasis and others. However, parallel approaches have been discouraging for systemic autoimmune diseases. Similarly, checkpoint inhibitors have made substantial contributions in the treatment of at least certain types of tumour.

Restoring or empowering the regulatory component of the immune system has gained substantial traction, as it may provide an alternative approach to the manipulation of the effector component and could even be used as a primary therapeutic approach for autoimmune and transplantation-related diseases, as well as cancer.

In most organ-specific and systemic autoimmune diseases, the numbers of \(T_{reg}\) cells and their functional status are not universally decreased, suggesting that, although \(T_{reg}\) cell malfunction is dominant and drives disease pathology in certain patients, it does not do so in others. Simply speaking, any trial attempting to empower \(T_{reg}\) cells in all patients who present with an autoimmune disease may be subject to type 2 (false negative) errors, and a treatment that could be beneficial for a subset of patients could be erroneously rejected. A cohort of patients who have a deficit in \(T_{reg}\) cell number should be identified and \(T_{reg}\) cell-enhancing medications should be administered to only these patients. Similarly, elimination of \(T_{reg}\) cells in patients with cancer may benefit only those in whom \(T_{reg}\) cells outnumber \(T_{eff}\) cells in the tumour.

Expansion of \(T_{reg}\) cells ex vivo before reinfusion has been considered extensively in early clinical trials. This particular approach is confounded by a number of problems inherent to cell therapies or specifically to \(T_{reg}\) cells. Cell therapy, outside a research setting or a specialized centre, presents myriad logistical and financial burdens that may preclude its applicability to common
autoimmune diseases or patients receiving transplant organs. $T_{reg}$ cells may be expanded ex vivo, but their instability may be a serious limiting factor. After being expanded in vitro in high concentrations of IL-2, they will probably die after being infused into patients, where the concentrations of IL-2 are low. Methods to stabilize $T_{reg}$ cells — for example, by increasing the methylation status of the FOXP3 locus or increasing the effect of FOXP3 on its target genes (for example, by suppressing special AT-rich sequence-binding protein 1 (SATB1), a genome organizer that is expressed in T cells and regulates chromatin structure and gene expression) — should be explored.

Although it has not been documented in patients, one possible issue with $T_{reg}$ cell therapy is that the newly expanded $T_{reg}$ cells in the periphery easily convert to effector cells, and if the percentage of these cells is high, there may be a flare of the inflammatory process in the individual recipient.

The possibility of further engineering $T_{reg}$ cells (transfected with the FOXP3 gene) with TCRs that recognize known antigens or autoantigens and are fused to the intracellular domains of signalling proteins that are able to confer a regulatory phenotype is tantalizing. This approach could be of great value in patients with haemophilia or receiving transplant organs. It could be equally valuable to patients with organ-specific autoimmune diseases in whom one or a few culprit autoantigens are known to be involved, but this approach would be more cumbersome in patients with systemic autoimmune diseases. Again, this approach should be limited to patients in whom a $T_{reg}$ cell numerical deficiency drives autoimmune pathology.

Expansion or empowerment of $T_{reg}$ cells in vivo is more realistic and, if successful, could benefit many patients whose care is not linked to major centres. Low-dose IL-2 has been claimed to be helpful in uncontrolled studies, and ongoing controlled studies should determine the clinical usefulness of this drug. There are a number of issues that may surface with administering low-dose IL-2 to patients with autoimmune diseases. First, the short half-life of IL-2 (the drug itself or its biological effect), which may be even shorter in patients with autoimmune diseases, could be problematic. Next, the therapeutic window between low-dose and high-dose IL-2 is very narrow, and this may cause side effects in some patients. Last, but equally important, is the possibility that IL-2 does not elicit a signalling response in $T_{reg}$ cells from patients with autoimmune diseases as it does in healthy individuals. The problem of the short half-life of IL-2 has been recognized, and a number of fusion molecules have been developed. The poor IL-2-elicited signalling response has been largely ignored, but approaches that involve co-engagement of other surface molecules (for example, SLAMF3 (also known as LY9)) may offer ways to restore the defective generation of phosphorylated STAT5. In this article, we have discussed molecules, such as mTOR, that limit $T_{reg}$ cell function and can be targeted with adjuvant drugs to IL-2 to increase the function of $T_{reg}$ cells. PP2A activity enhancers (such as ceramide) may increase PP2A activity, which is needed for the proper function of $T_{reg}$ cells.

The challenges to suppressing $T_{reg}$ cells in tumours loom larger than those to expand their function. Obviously, we need to better understand the nature and development of $T_{reg}$ cells in tumours. Some of the obvious big questions are what molecules enable $T_{reg}$ cells to enter tumours, do tumour cells or the tumour microenvironment further propagate $T_{reg}$ cell stability and function, and do $T_{reg}$ cells develop from naive CD4+ T cells after they are inside the tumour. Current technologies involving single-cell RNA sequencing of tumour cells and infiltrating inflammatory cells are expected to provide these much-needed insights. Systemic inhibition of $T_{reg}$ cells likely carries the risks of not depleting $T_{reg}$ cells in the tumour and of unleashing an autoinflammatory response similar to those already reported in patients receiving checkpoint inhibitors. Biologics directed against chemokines and/or their receptors that enable $T_{reg}$ cell entry into the tumour should gain traction. Alternatively, approaches to deliver $T_{reg}$ cell-disabling or $T_{reg}$ cell-depleting biologics to the tumour in a specific manner could be therapeutically important.

An exciting new concept comes from data that detail the role of $T_{reg}$ cells present in organs targeted by the inflammatory response and their unexpected ability to repair damaged tissues (BOX 2). Although purely speculative at this point, it is exciting to consider delivering engineered $T_{reg}$ cells to the kidneys of patients with lupus nephritis or to the pancreas of patients with TID.

Although expanding and empowering $T_{reg}$ cells to treat autoimmune disease are actively being explored, suppressing them in cancer is still in the nascent stage. The expected reward if $T_{reg}$ cells can be manipulated is high given the currently used side-effect-laden indiscriminate immunosuppressant drugs in the treatment of autoimmune diseases and the toxic drugs used in patients with cancer.

**Box 2 | $T_{reg}$ cells in wound repair and tissue regeneration**

Lymphocytes, including CD4+ and CD8+ T cells, are recruited to sites of inflammation and promote tissue injury. Regulatory T ($T_{reg}$) cells also accumulate in sites of inflammation, such as the skeletal muscle after injury, where they promote the switch from an inflammatory to a regenerative state and persist for at least 1 month. These $T_{reg}$ cells express high levels of amphiregulin (AREG), an epithelial growth factor (EGF) family protein, which promotes muscle regeneration by activating the EGF receptor (EGFR) signalling axis. $T_{reg}$ cells that express AREG can also protect lungs from infection-induced damage. AREG controls the immune response by regulating $T_{reg}$ cell function. Involvement of AREG-EGFR signals in $T_{reg}$ cell-mediated tissue regeneration is also observed in skin injury and promotes wound healing. Furthermore, skin $T_{reg}$ cells preferentially reside close to hair follicle stem cells (HFSCs) and help HFSC-mediated hair regeneration. Paracrine effects of $T_{reg}$ cells were also shown to promote cardiomyocyte proliferation during pregnancy and after myocardial infarction.

T helper 1 ($T_h$1) cells and $T_h$17 cells have been shown to promote neuroinflammation, but remyelination is compromised in the absence of lymphocytes, indicating that some lymphocytes are pro-regenerative in the brain. Indeed, $T_{reg}$ cells directly promote remyelination independently of immunomodulation, and when $T_{reg}$ cells are deleted, the process is impaired.

The ability of $T_{reg}$ cells to contribute to tissue repair expands their importance far beyond controlling the immune response and suggests that these cells can be used to reverse advanced tissue damage caused by immune or non-immune processes.


This study shows that tumor-derived CD25+ regulatory T cells in cancers: a systematic review and meta-analysis. J. Exp. Ther. Med. 6, 154 (2017).


This study shows that the presence of FoxP3+ regulatory T cells in colorectal cancers indicates a significantly better prognosis than the presence of predominantly FoxP3− regulatory T cells. This study has brought attention to the subpopulation of FoxP3− regulatory T cells with low FoxP3 expression, which should not be deleted when applying immunotherapeutic regimens.


This study shows that the presence of FoxP3+ regulatory T cells in cancer immune microenvironments is associated with a significantly better prognosis than the presence of predominantly FoxP3− regulatory T cells. This study shows that the presence of FoxP3+ regulatory T cells in cancer immune microenvironments is associated with a significantly better prognosis than the presence of predominantly FoxP3− regulatory T cells.
beta-cell function in type 1 diabetes in children. CD4+CD25highCD127- regulatory T cells preserves the antiphospholipid syndrome.

T reg cell development. Independent and complementary events required for epigenetic changes and Foxp3 expression are cyclosporine.

Disease onset and respond to rapamycin in lupus-prone and antiphospholipid antibody production precede T regulatory cells in adults transplanted with umbilical cord blood. Transplantation 2012; 93:1817–1820.


This is one of the first studies to suggest that autoreactivity can be controlled through metabolism.


This is the first study to show that low-dose IL-2 has clinical benefit in patients with GHD linked to the expansion of T cells.


This paper shows that NRP1 is required to maintain intratumoral Treg stability and function and that NRP1-deficient Treg cells produce IFNγ, which promotes Treg cell fragility and boosts anti-tumor activity.


This is a novel study that explains the abundance of lung metastases by various tumours. Low oxygen pressure promotes the development of Treg cells, which promote tumour growth.


Copelletta, D. et al. PPAR-gamma is a major driver of the accumulation and phenotype of adipose tissue Treg cells. Nature 486, 549–555 (2012). This study shows that Treg cells that infiltrate tissues and become tissue-resident T cell express transcription factors that are master regulators of the specific tissue. PPAR is a master regulator of adipose tissue.


Malhotra, N. et al. RORα-expressing T regulatory cells restrain allergic skin inflammation. Sci. Immunol. 3, eaao6292 (2018). This study shows that expression of retinoid-related orphan receptor-α (RORα) in skin-resident Treg cells is important for restraining allergic skin inflammation.


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Competing interests
G.C.T. is a consultant for Johnson & Johnson and a science advisory board member for Alpro and Silicon Therapeutics (appointments that are not related to the work discussed herein). D.K. is an inventor on a patent application claiming low-dose IL-2 for therapy of autoimmune diseases, which is owned by his academic institution and licensed to ILTOO Pharma; D.K. advises for and holds shares in ILTOO Pharma. The University of Miami and T.R.M. have a patent pending (WO2016022671A1) on IL-2/CD25 fusion proteins that has been licensed exclusively to Bristol-Myers Squibb and have a collaboration and sponsored research & licensing agreement with Bristol-Myers Squibb. A.S., M.G.T. and Y.D. declare no competing interests.

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