Regulatory T cells in the treatment of disease

Amir Sharabi, Maria G Tsokos, Ying Ding, Thomas R Malek, David Klatzmann, George C Tsokos

Regulatory T cells in the treatment of disease

Amir Sharabi[1,2], Maria G. Tsokos[1], Ying Ding[3], Thomas R. Malek[3], David Klatzmann[4,5,6]* and George C. Tsokos[1]**

Abstract | Regulatory T (T_{reg}) cells suppress inflammation and regulate immune system activity. In patients with systemic or organ-specific autoimmune diseases or those receiving transplanted organs, T_{reg} cells are compromised. Approaches to strengthen T_{reg} cell function, either by expanding them ex vivo and reinfusing them or by increasing the number or capacity of existing T_{reg} cells, have entered clinical trials. Unlike the situation in autoimmunity, in patients with cancer, T_{reg} 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 T_{reg} cells specifically in tumour sites are being investigated to promote antitumour immunity and regression. Here, we describe the current progress in modulating T_{reg} cells in autoimmune disorders, transplantation and cancer.

[1]*Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA.*
[2]*Sackler Faculty of Medicine, Tel-Aviv University, Tel-Aviv, Israel.*
[3]*Department of Microbiology and Immunology, The Diabetes Research Institute, Miller School of Medicine, University of Miami, Miami, FL, USA.*
[4]*Sorbonne Université, UPMC Univ Paris 06, UMRS 959, Immunology-Immunopathology-Immunotherapy (i3), Paris, France.*
[5]*INSERM, UMRS 959, Immunology-Immunopathology-Immunotherapy (i3), Paris, France.*
[6]*Assistance Publique — Hôpitaux de Paris, Hôpital Pitié-Salpêtrière, Biotherapy and Département Hospitalo-Universitaire Inflammation-Immunopathology-Biotherapy (i2B), Paris, France.*

**e-mail:*
isharabiamir@gmail.com; david.klatzmann@upmc.fr; gtsokos@bidmc.harvard.edu

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 (T_{reg}) cells). The critical role of T_{reg} cells in the development of autoimmunity has been highlighted by the multi-organ autoinflammatory syndrome that develops in FOXP3-deficient mice[1,2] and the immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome seen in humans[3,4], which occurs in individuals who harbour mutations in *FOXP3*.

Approaches to increase the number and function of T_{reg} cells could clearly benefit patients with autoimmune disorders. Some of these approaches are now in clinical trials. One such effort involves adoptive T_{reg} cell therapy[5–7] and includes the potential to engineer antigen specificity into the transferred T_{reg} cells; in another, low-dose interleukin (IL)-2 is administered to selectively expand T_{reg} cell populations, a strategy that could be applied to many patients with autoimmune diseases[8–10]. In addition, strategies to reduce the function and/or number of T_{reg} cells are being investigated to promote antitumour immunity.

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

Biology of T_{reg} cells

Developmental heterogeneity

T_{reg} cells are marked by the transcriptional regulator FOXP3 and constitute approximately 5–10% of peripheral $CD4^+$ T cells in humans and mice[11]. FOXP3 is a reliable marker for mouse T_{reg} cells. Even though FOXP3 is an intracellular protein, genetic reporter mice have facilitated rigorous purification of viable T_{reg} cells for molecular and cellular analysis. In humans, T_{reg} cells are characterized by constitutive expression of the transcription factor FOXP3, high expression of the low-avidity IL-2 receptor (IL-2R) α chain (CD25) and low levels of the IL-7R α-chain (CD127)[12,13] (Supplementary Box 1). However, FOXP3 is a less reliable marker for the identification of T_{reg} cells in humans because some activated effector T cells (T_{eff} cells) also express this molecule, albeit transiently and often at a somewhat low level[14–16].

FOXP3 controls many functions of T_{reg} cells. FOXP3 stabilizes the T_{reg} cell lineage in part by reinforcing a gene expression programme that is required for the suppressive function of T_{reg} cells[2,17,18]. T_{reg} cell populations expand in response to IL-2, and this cytokine is required for T_{reg} cell survival. FOXP3 represses IL-2 expression, which is produced by T_{eff} cells during immune reactions, and, thus, T_{reg} cells are dependent on paracrine IL-2.

T_{reg} cells are classified into two major groups on the basis of their developmental origins. One population of T_{reg} cells, designated thymus-derived T_{reg} (tT_{reg}) cells, develops in the thymus. Another population of T_{reg} 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 T_{reg} cells are designated induced T_{reg} (iT_{reg}) cells, and when this occurs in vivo, these cells are designated peripherally induced T_{reg} (pT_{reg}) cells (Supplementary Box 2; Supplementary Fig. 1).

Unlike tT_{reg} cells, which constitute a stable population of suppressor cells, pT_{reg} cells have substantial plasticity and may convert into T_{eff} cells, which are characterized by the production of interferon-γ (IFNγ) and IL-17. This feature allows for a cellular response that adapts to conditions within specific tissue sites. For example, pT_{reg} cells are abundant in the gut mucosa, where they promote tolerance to a normal microbiota. However, during an infection, the inflammatory environment may convert pT_{reg} cells into T helper 1 cells (T_H1 cells) or T helper 17 cells (T_H17 cells) to promote an immune response[19].

A two-step model has been proposed whereby signalling from the T cell receptor (TCR), the co-stimulatory molecule CD28 and IL-2R promotes tT_{reg} cell development[20] (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[21–23]. NFAT and FOXO bind directly to the promoter, conserved non-coding region 2 (*CNS2*) and *CNS3* of the *FOXP3* gene to drive FOXP3 expression and T_{reg} cell development[18,24,25]. These cells then differentiate into IL-2-responsive T_{reg} cell progenitors ($CD4^+CD25^+CD122^{high}GITR^{high}FOXP3^-$ T cells). In the second step, IL-2R signalling promotes these $FOXP3^-$ T_{reg} cell progenitors to further develop into fully functional mature $FOXP3^+$ T_{reg} cells by activation of signal transducer and activator of transcription 5 (STAT5)[20,21]. 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 tT_{reg} 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 T_{reg} cell precursors to IL-2 stimulation[26]. Another important aspect of tT_{reg} cell development is that the phosphoinositide 3-kinase (PI3K) pathway, which mediates signalling downstream of the TCR, is attenuated in T_{reg} cells relative to T_{eff} cells owing to the upregulation of PTEN in T_{reg} cells; PTEN is the main negative regulator of the PI3K pathway[27]. Whereas transforming growth factor β (TGFβ) is essential for pT_{reg} cell development, its role in tT_{reg} cell development is less clear, as mice lacking TGFβ signalling did not have a numerical defect in tT_{reg} cells[28]. Other studies, however, suggest that TGFβ, stimulated by thymic apoptosis, is an essential factor for *Foxp3* transcription and tT_{reg} cell generation in the thymus[29–31].

Functions and phenotypic heterogeneity

Multiple mechanisms have been ascribed to mouse and human T_{reg} 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. T_{reg} cells also promote the conversion of dendritic cells (DCs) to a tolerogenic state through surface expression of cytotoxic T lymphocyte protein 4 (CTLA4)[32,33], 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[34,35]. In turn, DCs, particularly those chronically exposed to antigen and rendered tolerogenic, may promote T_{reg} cell expansion and function (see below).

The ectoenzymes CD39 and CD73 are highly expressed on the surface of some T_{reg} cells and these two enzymes together convert extracellular ATP to adenosine[36,37], which inhibits the proliferation of T_{eff} cells and suppresses the function of DCs[38]. In addition, the high level of CD25 on T_{reg} cells sequesters IL-2 within the microenvironment and therefore hampers IL-2-dependent activation of $CD8^+$ T cells and natural killer (NK) cells[39–41]. Lastly, intratumoural T_{reg} cells also exhibit direct cytotoxicity towards NK cells and autoreactive $CD8^+$ T cells, which mediate tumour cell clearance[42,43].

Two main subsets of mature T_{reg} cells have been defined on the basis of distinctive phenotypes and gene expression. These are resting or naive T_{reg} (nT_{reg}) cells and activated or effector T_{reg} (eT_{reg}) cells, including tissue-resident T_{reg} cells (BOX 1). eT_{reg} cells differ critically from nT_{reg} cells in that the former often express increased levels of immunosuppressive molecules, particularly IL-10, and have increased surface expression of tissue-seeking chemokine receptors[44]. This property, in conjunction with downregulation of CC-chemokine receptor 7 (CCR7) and L-selectin (also known as CD62L; these two protein are markers of naive cells), promotes the migration of highly functional T_{reg} cells into tissues[45]. TCR repertoire analysis has shown numerous major clonotype expansions in eT_{reg} cells from deep tissue draining lymph nodes; these clonotypes are absent or reduced in activated

Self-tolerance
The inability to respond to self-antigens.

$CD4^+$ T cells
T cells that recognize peptides presented by major histocompatibility complex class II molecules and provide help to B cells to produce antibodies or to $CD8^+$ cells to produce cytotoxic responses.

Effector T cells
(T_{eff} cells). Short-lived activated cells that defend the body in an immune response.

T helper 1 cells
(T_H1 cells). Cells that produce interleukin 2, interferon-γ and tumour necrosis factor and are pro-inflammatory.

T helper 17 cells
(T_H17 cells). Cells that produce interleukin-17 and play an important role in maintaining mucosal barriers and contributing to pathogen clearance at mucosal surfaces; they also propagate autoimmune and inflammatory pathology.

Co-stimulatory molecule
A membrane-bound or secreted product that is required for co-stimulation. This second signal (in addition to T cell receptor engagement) from an antigen-presenting cell to a T cell allows the T cell to become activated and produce cytokines. CD28 (on T cells) is the best known example.

Antigen-presenting cells
(APCs). Cells that display antigen complexed with major histocompatibility complex molecules on their surfaces, which they present to T cells.

Dendritic cells
(DCs). Cells that are named for their surface projections (which resemble the dendrites of neurons). They continuously sample the environment for antigen, which they process and present to T cells.

$CD8^+$ T cells
Cytotoxic T cells that recognize peptides presented by major histocompatibility complex class I molecules.

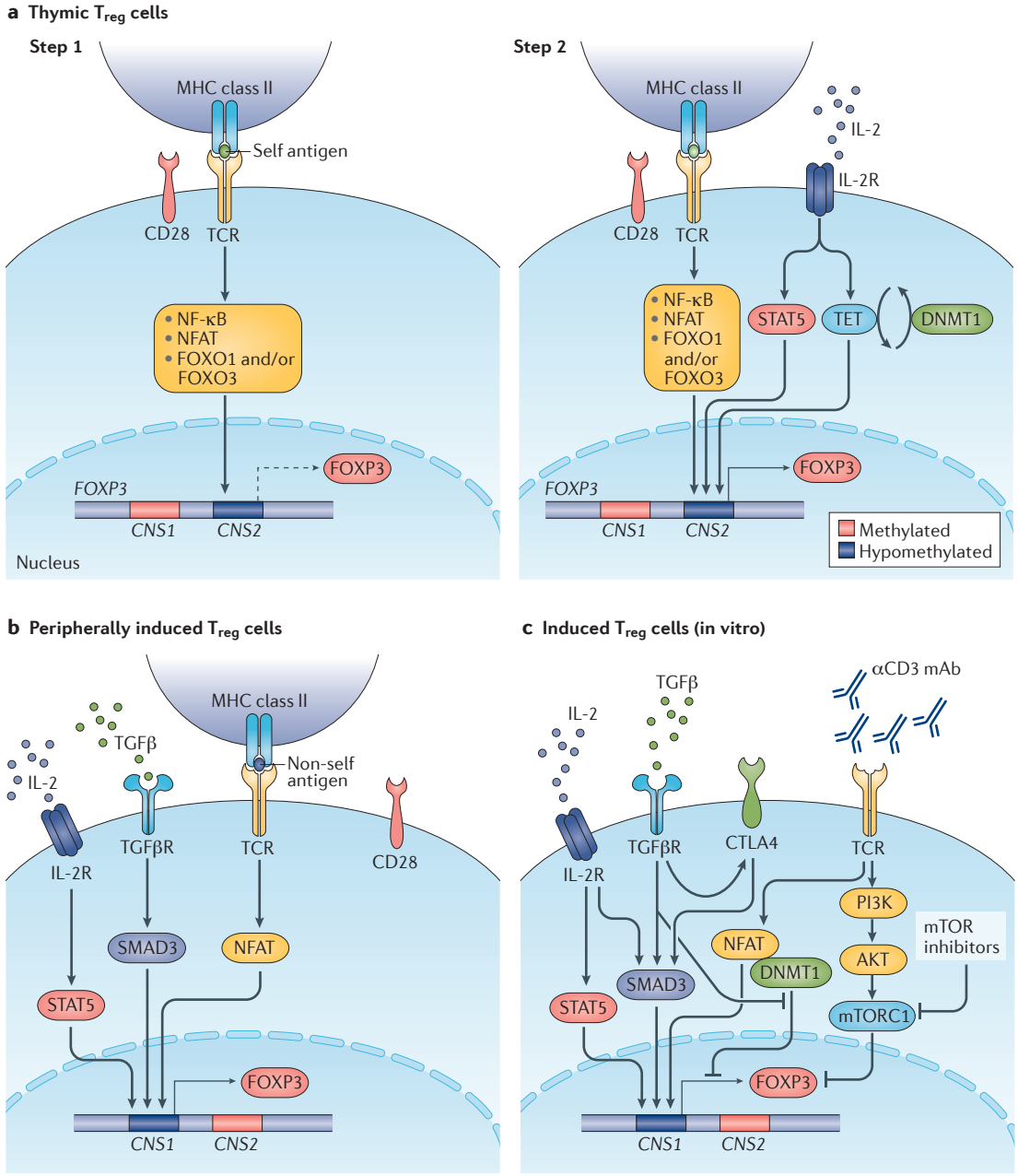


Figure 1 | **Steps in T_{reg} cell development in vivo and in vitro. a** | The development of regulatory T (T_{reg}) cells in the thymus occurs in steps and requires the expression of the T cell receptor (TCR) followed by the expression of the interleukin 2 receptor (IL-2R). When the TCR and self-antigens interact at a moderate level of avidity (just below the threshold for negative selection), the α-chain of IL-2R (CD25) is expressed and accumulates on the cell surface; IL-2 then plays a central role in T_{reg} cell development independently of the TCR. The expression of forkhead box P3 (FOXP3) increases following the TCR–self-peptide–major histocompatibility complex (MHC) interaction. Transcription factors downstream of TCR engagement become activated and bind to the gene promoter and conserved non-coding region 2 (*CNS2*) of *FOXP3*. The expression of FOXP3 is maintained in IL-2-responsive T_{reg} cells. Signalling through IL-2R preserves the hypomethylated status of *CNS2* by recruiting TET. IL-2R signalling also activates signal transducer and activator of transcription 5 (STAT5) to further increase FOXP3 expression. **b** | T_{reg} cells that are generated in vivo outside the thymus arise from naive $CD4^+$ T cells in response to transforming growth factor-β (TGFβ) and IL-2 in peripheral tissues. The expression of FOXP3 in these cells derives mainly from TCR interactions with non-self-antigen–MHC and IL-2R signalling, which affect *CNS1*, with minor contributions from TGFβ signalling. **c** | FOXP3 expression in in vitro-induced T_{reg} (iT_{reg}) cells is activation-dependent and short-lived. The addition of TGFβ or IL-2 to the media results in *CNS1* hypomethylation, as DNA (cytosine-5)-methyltransferase 1 (DNMT1) is excluded from the nucleus. Both cytokines activate SMAD3 and STAT5, which increase FOXP3 expression. Mechanistic or mammalian target of rapamycin (mTOR) inhibitors may contribute to the activation of T_{reg} cells in vitro. The *CNS2* in iT_{reg} cells is only partially hypomethylated, and therefore, FOXP3 expression is somewhat unstable. CTLA4, cytotoxic T lymphocyte protein 4; mAb, monoclonal antibody; mTORC1, mTOR complex 1; NF-κB, nuclear factor-κB; NFAT, nuclear factor of activated T cells; PI3K, phosphoinositide 3-kinase.

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 T_{reg} cells**

Regulatory T (T_{reg}) cells constitute a higher percentage of the T cells present in tissues than in the peripheral blood, and most of the tissue-resident T_{reg} cells have an effector or memory phenotype[299] and could be classified as effector T_{reg} cells. Moreover, gene expression patterns in tissue-resident T_{reg} cells are distinctly dependent on the hosting tissue. For example, an intestinal peripherally induced T_{reg} cell subset expresses RORγt 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 T_{reg} cells express peroxisome proliferator-activated receptor-γ to regulate insulin sensitivity[301,302]. Visceral adipose tissue T_{reg} 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[303]. Some strains of gut microorganisms can induce T_{reg} cells in the intestine[304]. Deletion of skin-resident T_{reg} cells in mice leads to atopic dermatitis[305]. The biology and characteristics of human tissue-resident T_{reg} cells have been reviewed[306,307].

memory T_{reg} cells from superficial lymph nodes and in nT_{reg} cell populations, further suggesting that eT_{reg} cells actively control autoimmunity[46]. Thus, eT_{reg} cells exhibit characteristics important for re-establishing tolerance in autoimmune-inflamed tissues. Correspondingly, T_{reg} cell-based therapies that promote eT_{reg} cells are likely to be more efficacious in tissues that are undergoing an immune or autoimmune attack[47].

In many ways, the heterogeneity of T_{reg} cells mirrors that reported for T_H cells, and one might simply view T_{reg} cells as activated T cells that suppress rather than promote immunity. An important parallel between T_{reg} cells and T_H cells is that key transcriptional regulators that are expressed in T_H cells are also expressed by T_{reg} cells and are associated with the suppression of the corresponding T_H cell subset. For example, T_{reg} cells that suppress T_H1 cells express T-BET (also known as T-box transcription factor TBX21), the signature transcription factor of T_H1 cells[48]. Likewise, T_{reg} cells that suppress T helper 2 cells (T_H2 cells), T_H17 cells and T follicular helper cells (T_{FH} cells) express GATA3, STAT3, and BCL6, respectively, which are key transcriptional regulators associated with each of these T_H subsets[49–51]. The precise mechanism of how T_{reg} cell subsets affect a particular type of T_H cell response is not well understood. However, it is likely that both T_{reg} cells and T_H cells respond to similar environmental cues, which promote T_{reg} cells to migrate to and counteract inflammatory responses promoted by the corresponding T_H cell subset, thus preventing undesired bystander suppression[52].

TCR and IL-2R signalling

T_{reg} cell homeostasis in the periphery depends critically on TCR and IL-2R signals. In mice with conditional TCR ablation in T_{reg} cells, nT_{reg} cell numbers were readily maintained, whereas eT_{reg} cell numbers were substantially reduced[53]. Thus, maintenance of the eT_{reg} cell population depends on recent TCR stimulation. In addition, when placed in competitive, IL-2-limited environments, T_{reg} cells that have intrinsically impaired IL-2R signalling compete poorly with wild-type T_{reg} cells, showing clearly that IL-2 is important for their maintenance in secondary lymphoid tissues[45]. Recently IL-2-activated T_{reg} cells in vivo, defined as those T_{reg} cells that contain activated STAT5, are nT_{reg} cells, indicating that this T_{reg} cell subset is especially dependent on IL-2 for maintenance[45,54]. IL-2 also promotes eT_{reg} cell survival, as terminally differentiated ($KLRG1^+$) eT_{reg} cell numbers are reduced in mice with intrinsic defects in IL-2R signalling[55]. IL-2 promotes T_{reg} cell survival in part by upregulating the transcription of the anti-apoptotic molecules BCL2 and MCL1 (REF.[56]).

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 T_{reg} cells in the periphery. This indicates that IL-2 is a potent growth factor for T_{reg} 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_{eff} 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 T_{reg} cells while the IL-2-dependent activation of T_{eff} cells remained impaired[57,58]. The high level of expression of CD25 on mouse and human T_{reg} cells, relative to other lymphoid cells, is one key reason why T_{reg} 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[9,10] and will be discussed in more detail below, as these approaches are in clinical trials.

Key molecules that control T_{reg} cells

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

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[59].

In T_{eff} cells, mTOR signalling is high and increases the inflammatory response; mTOR activity is low in T_{reg} cells[60]. Indeed, deletion of mTOR in all conventional $CD4^+$ T cells results in a phenotype reminiscent of T_{reg} cells[61]. However, a certain level of mTORC1 activity is necessary for T_{reg} cells to meet their metabolic demands, which occurs through cholesterol and lipid metabolism, specifically through the mevalonate pathway[62]. mTORC1 is essential to T_{reg} cell function, and when mTORC1 is conditionally knocked out in T_{reg} cells, mice develop a severe lymphoproliferative autoimmune condition[62]. In T_{eff} cells, mTOR upregulates genes associated with the pentose phosphate pathway and the glucose transporter

T helper 2 cells
(T_H2 cells). They promote allergic responses and provide help to B cells. Cells that can also promote resolution of inflammation and produce interleukin 4 (IL-4), IL-5, IL-6 and IL-10.

T follicular helper cells
(T_{FH} cells). Antigen-experienced $CD4^+$ T cells found in the periphery within B cell follicles of secondary lymphoid organs such as lymph nodes, spleens and Peyer's patches.

GLUT1 (also known as SLC2A1), thereby facilitating glucose entry into the cells and subsequent glycolysis. By contrast, in T_{reg} cells, in a manner similar to that observed in memory $CD8^+$ cells, mTOR is required not for glycolysis but rather for lipogenesis to maintain the functional capacity of these cells[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_{reg} cell function. Thus, although complete deletion of mTORC1 results in T_{reg} cell dysfunction and autoimmunity, unrestrained mTORC1 activity also disrupts T_{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[65]. PIP3 is highly expressed in T_{reg} cells and regulates their differentiation[66]. It has been shown that the NRP1–semaphorin 4A (SEMA4A) axis stabilizes T_{reg} cell function by restraining PI3K and potentiating PTEN activity[67]. Conditional deletion of PTEN in T_{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[27].

Protein phosphatase 2A (PP2A), a serine/threonine phosphatase, is crucial for T_{reg} cell function. In mice, the catalytic activity of PP2A in T_{reg} cells is substantially higher than in conventional T cells, and conditional deletion of PP2A in T_{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.[68]).

Other molecules, such as NOTCH, other transcription factors and chromatin remodellers (Supplementary Box 3), are also key to T_{reg} cell function. Understanding the molecular and/or biochemical events that govern T_{reg} cell function is vital to the development of new T_{reg} cell-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_{reg} cells in key human diseases

Monogenic diseases

Several monogenic conditions illustrate the importance of functioning T_{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[4,69].

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 $FOXP3^+$ 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')[70,71]. Deficiency of or mutations in *STAT5B*[72,73], *CTLA4* (REF.[74]) or *LRBA*[75] also result in autoimmune phenotypes.

Systemic lupus erythematosus

T_{reg} cells have been logically implicated in the pathophysiology of systemic autoimmune diseases, although their numbers and function have been reported to be variable[76]. 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_{reg} cells and the conditions and performance of functional assays[76–79]. The proportion of activated T_{reg} cells is reduced during the early phases of the disease, whereas the $CD45RA^-FOXP3^{low}$ non-T_{reg} cell population is increased in patients with active SLE[80]. In addition, T cells from patients with SLE have low levels of phosphorylated STAT5 upon stimulation with IL-2, suggesting an inherent T_{reg} cell defect[81]. A small study reported decreased numbers of T_{reg} cells in the kidneys of patients with lupus nephritis and anti-neutrophil cytoplasmic antibody-associated vasculitis[82]; this finding suggests that T_{reg} cells are involved in the repair of tissue damage and that approaches to direct T_{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_{reg} cells is reduced during the early phases of the disease[83].This numerical deficiency is exacerbated by their reduced ability to increase the expression of FOXP3 in response to IL-2 (REF.[84]) and the resistance of T_{eff} cells to suppression[85].

Psoriasis is an inflammatory skin disease in which the dermis and subcutaneous tissue are infiltrated with T_H1 cells, T_H17 cells and other subsets of T cells that produce pro-inflammatory cytokines including TNF, IL-6, IFNγ and IL-17 (REF.[86]). In patients with psoriasis, T_{reg} cells, particularly those that express CCR5 (REF.[87]) and are present in the skin[88] or the bone marrow[89], display poor function. More importantly, the local skin inflammatory milieu promotes T_{reg} cell plasticity and differentiation into IL-17-producing cells[90].

Myasthenia gravis is characterized by worsening muscle weakness — the pathology originates from autoreactive T cells, which provide help to B cells to produce anti-acetylcholine receptor (ACHR) antibodies, which block the ACHR. T_{reg} cells from patients with myasthenia gravis display decreased expression of FOXP3 and compromised suppressive function[91,92], which could reflect reduced IL-2–STAT5 signalling[93]. Finally, the expression of CTLA4, which has been linked to myasthenia gravis in genome-wide studies[94], is reduced on T_{reg} cells from patients with this disease[95].

T_{reg} cells residing in the intestine of patients with inflammatory bowel disease (IBD) exhibit a normal phenotype and are functional in assays in vitro[96]. The number of T_{reg} cells in the lamina propria under any condition of intestinal inflammation is increased, whereas the number is decreased in the periphery[97,98]. It should be noted that T_{reg} cells that are present in the lamina propria of patients with IBD do not suppress T_{eff} cells because the TGFβ signalling pathway is defective in the intestinal

mucosa[99]. 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[100]. Substantial insights have been gained from the study of the contribution of the microbiota[101], 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[102]. Experiments in a mouse model of multiple sclerosis showed that T_{reg} cells are generated from encephalitogenic T cells that infiltrate the central nervous system[103], but these cells fail to control autoimmune inflammation[104]. In patients with multiple sclerosis, the number of T_{reg} cells is variable[105], but these cells have compromised regulatory function[106].

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[107,108]. Inhibition of mTOR can simultaneously suppress T_{eff} 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)[109,110].

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[111,112] (CCL2–CCR4 (REF.[113]), CCL5–CCR5, hypoxia-mediated CCL8–CCR10 (REFS[114,115]) and CXCL12–CXCR4), local expansion of tT_{reg} cells[111,112,116] 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[117,118]. Another possibility is that pT_{reg} cells that are generated de novo from conventional $CD4^+$ cells in the tumour microenvironment[119–121], 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 mouse[122] and human[123] tumours. The second challenge to this theory is that intratumoural T_{reg} cells originate from tT_{reg} cells, which recognize self-antigens specific to the organ of cancer origin[124,125]. 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[126,127]. High levels of IDO[128] and adenosine[129] 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[118,130] and, more importantly, with advanced-stage disease and decreased survival in patients with cancer[131]. Furthermore, a reduced $CD8^+$ T cell to T_{reg} cell ratio in the tumour site is predictive of poor clinical outcome[132]. 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[114,115] and stimulating metastasis via receptor activator of NF-κB ligand (RANKL; also known as TNFSF11) signalling[133]. Some studies indicated a better prognosis for colorectal cancers with $FOXP3^+$ T cell infiltrates[134]. However, a recent study showed that certain colorectal cancers contain an abundance of $FOXP3^{low}CD45RA^-$ non-T_{reg} cells, which secrete pro-inflammatory cytokines, and that these patients have a better prognosis than those with a predominant $FOXP3^{high}CD45RA^-$ T_{reg} cell population[135]. This difference in FOXP3 expression could account for the conflicting data, as the distinction between $FOXP3^{low}$ cells and $FOXP3^{high}$ 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, granzyme, galectin 1, adenosine, prostaglandin E2 (PGE_2) 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[136]. 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[67]. 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[10] 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 autoimmune 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 purification[137]. The first therapeutic evaluation of ex vivo-expanded polyclonal T_{reg} cells was performed in haematopoietic 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 GVHD[138,139]. 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 GVHD[140,141] 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 mofetil[142]. 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 efficacy[143].

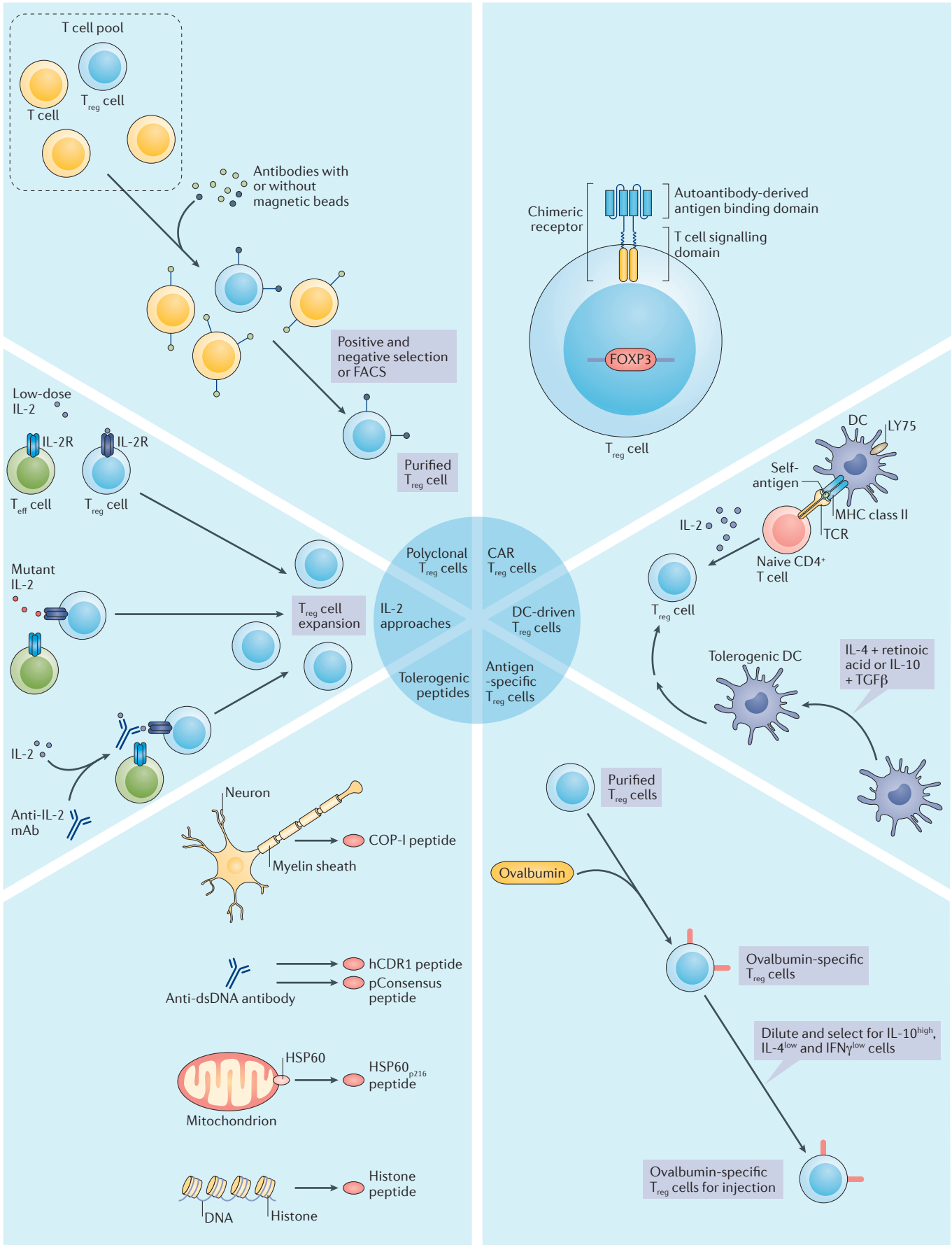
It is estimated that, at the onset of T1D, approximately half of the β-cell mass remains functional and produces insulin but will be destroyed within a year. The administration of potent immunosuppressant drugs shortly after the onset of T1D can block the autoimmune process and prolong the 'honeymoon' period of the disease, but this occurs at the expense of severe side effects[144,145]. 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 T1D[146], with clinical results lasting at least 1 year[147]. 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 treatment[148].

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 enticing[149–151]. 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 expression[152]. 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 pathway[153], and several studies in mice deficient in negative regulators of this pathway showed functional impairment of T_{reg} cells and systemic autoimmunity[27,68,154]. Human T_{reg} cells expand efficiently in the presence of the mTOR inhibitor rapamycin[155]. In SLE, activated mTOR in T cells accounts for several abnormalities, including the downregulation of the TCR companion signalling molecule CD3ζ, the expansion of T_H17 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 populations[60,156,157]. Administration of rapamycin has been reported to improve clinical outcomes in lupus-prone mice[158] and patients with SLE[159] (TABLE 2). Moreover, rapamycin can block the production of antiphospholipid antibodies in lupus-prone mice[160] and improve renal allograft survival in patients with antiphospholipid syndrome[161]. 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 months[162]. Clinical indices revealed a major improvement in disease, concurrent with a reduced need for steroids. Sirolimus expanded the T_{reg} cell and $CD8^+$


T cell pool
T cell
T_{reg} cell
Antibodies with or without magnetic beads
Positive and negative selection or FACS
Purified T_{reg} cell
Low-dose IL-2
IL-2R
IL-2R
T_{eff} cell
T_{reg} cell
Mutant IL-2
T_{reg} cell expansion
IL-2
Anti-IL-2 mAb
Polyclonal T_{reg} cells
CAR T_{reg} cells
IL-2 approaches
DC-driven T_{reg} cells
Tolerogenic peptides
Antigen -specific T_{reg} cells
Chimeric receptor
Autoantibody-derived antigen binding domain
T cell signalling domain
FOXP3
T_{reg} cell
DC
LY75
Self-antigen
MHC class II
TCR
IL-2
Naive CD4+ T cell
T_{reg} cell
Tolerogenic DC
IL-4 + retinoic acid or IL-10 + TGFβ
Neuron
COP-I peptide
Myelin sheath
Anti-dsDNA antibody
hCDR1 peptide
pConsensus peptide
HSP60
$HSP60_{p216}$ peptide
Mitochondrion
Histone peptide
DNA
Histone
Purified T_{reg} cells
Ovalbumin
Ovalbumin-specific T_{reg} cells
Dilute and select for IL-10^{high}, IL-4^{low} and IFNγ^{low} cells
Ovalbumin-specific T_{reg} cells for injection

◂ Figure 2 | **Therapeutic approaches to alter T_{reg} cells in autoimmune diseases and transplantation.** The therapies are in clockwise order. Polyclonal regulatory T (T_{reg}) cells can be isolated by sorting for cells with characteristic surface markers by fluorescence-activated cell sorting or by using magnetic beads as shown. These methods have been evaluated in patients with type 1 diabetes or undergoing haematopoietic stem cell transplantation. Chimeric antigen receptor (CAR) T_{reg} cells are T cells that have been genetically engineered to contain *FOXP3* and an antigen receptor that consists of the antigen binding domain of an autoantibody and an intracellular signalling domain. Proof of concept studies have been done in xenogeneic graft-versus-host disease (GVHD), xenogeneic skin transplantation and haemophilia. Dendritic cells (DCs) that express lymphocyte antigen 75 (LY75) and present self-antigen, or that become tolerogenic because of cytokine exposure, can induce antigen-specific T_{reg} cells. The latter has been tried in patients with rheumatoid arthritis, in which the tolerogenic DCs were incubated with citrullinated peptides. Peptides and copolymers with amino acid sequences that are similar to those of self-antigens can induce T_{reg} cells and ameliorate disease manifestations in patients with multiple sclerosis or systemic lupus erythematosus (SLE). Finally, the highly sensitive interleukin 2 receptor (IL-2R) on T_{reg} cells makes low doses of IL-2 sufficient for preferential T_{reg} cell expansion. This approach has been tested in hepatitis C virus-induced vasculitis, GVHD and SLE. The biological activity of IL-2 can be increased by forming immune complexes of IL-2 with anti-IL-2 monoclonal antibodies (mAbs), which selectively stimulate T_{reg} cells. COP-I, copolymer-I; dsDNA, double-stranded DNA; FACS, fluorescence-activated cell sorting; FOXP3, forkhead box P3; HSP60, heat shock protein 60; IFNγ, interferon-γ; MHC, major histocompatibility complex; TCR, T cell receptor; T_{eff} cell, effector T cell; TGFβ, transforming growth factor-β.

memory T cell populations and inhibited IL-4 and IL-17 production by $CD4^+$ and double-negative T cells after 12 months[162].

Concurrent blockade of OX40L (also known as TNFSF4) and mTOR prevented graft rejection in a non-human primate GVHD model[163]. In a complementary approach, murine acute GVHD could be diminished with administration of FTY720, a sphingosine-1-phosphate (S1P) receptor antagonist[164] that promotes the activity of PP2A, thereby reducing mTOR activity[68]. It is noteworthy that FTY720 is currently being studied in clinical trials to prevent acute GVHD (including EudraCT 2004-000655-41). In a different approach, patients with acute GVHD who were treated with mTOR inhibitors together with a glycolipid (α-galactosylceramide (α-GalCer) in the form of RGI-2001) that promotes NK T cell function had an increased number of T_{reg} cells and a reduced immune response[165].

In a negative feedback loop, FOXP3 expression is compromised when mTORC2 is activated, because mTORC2 prevents FOXO1 from entering the nucleus and stabilizing FOXP3 expression. Pyrazolopyrimidine derivative drugs, which promote the nuclear translocation of FOXO1 and simultaneously inhibit PI3K, are worthy of clinical investigation because they target both upstream and downstream components of mTORC2 signalling[166].

Other molecules that limit mTOR activity work by diverse mechanisms of action but include S1P receptor blockers[167,168], antioxidants such as metformin and *N*-acetyl cysteine[169,170] and calmodulin kinase type II and type IV inhibitors[111,112]. It remains to be seen whether any of them will generate clinical traction.

Other modalities include targeting histone acetylation with inhibitors of specific histone deacetylases (such as HDAC6, which is expressed at higher levels in T_{reg} cells than in conventional T cells). These inhibitors have been shown to selectively increase T_{reg} cell function in lupus-prone mice and protect them from lupus nephritis[171,172]. In addition, mice deficient in HDAC6 had T_{reg} cells with improved suppressive activity, poor antibody responses to antigen[173] and curtailed autoimmune and inflammatory responses[174].

Antigen-specific T_{reg} cell therapies

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[177] 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 TCR 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[178,179]. 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 $HLA\text{-}A2^+$ T cells in mice[179]. Furthermore, HLA-A2 CAR T cells were shown to alleviate alloimmune-mediated xenogeneic skin injury[180].

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

Table 1 | **Current clinical trials studying T_{reg} cells in transplantation and autoimmunity**

Target tissue or condition	T_{reg} cell type administered	Treatment groups	Trial phase	Sponsoring institute	Clinical trial identifier
Transplantation					
Liver	Alloantigen T_{reg} cells	• i.v. 50×10^6 cells; once • i.v. 200×10^6 cells; once • i.v. 800×10^6 cells; once	I	NIAID	NCT02188719
Kidney	Alloantigen T_{reg} cells	• i.v. 300×10^6 cells; once • i.v. 900×10^6 cells; once	I	UCSF	NCT02244801
Kidney	Alloantigen T_{reg} cells	i.v. 400×10^6 cells; once	I/II	NIAID	NCT02711826
Liver	Alloantigen T_{reg} cells	i.v. 400×10^6 cells; once	I/II	NIAID	NCT02474199
Liver	Autologous T_{reg} cells	• i.v. 1×10^6 cells per kg; once • i.v. 5×10^6 cells per kg; once	I/II	Guy's and St Thomas' NHS Foundation Trust, UK	NCT02166177
Islet cell	Autologous T_{reg} cells	• i.v. 400×10^6 cells; once • i.v. $1{,}600 \times 10^6$ cells; once	I	University of Alberta, Canada	NCT03444064
Refractory chronic GVHD	Alloantigen T_{reg} cells	• Three escalating doses; one dose per month • i.v. 0.17×10^6 cells per kg • i.v. 0.33×10^6 cells per kg • i.v. 0.66×10^6 cells per kg	I/II	Azienda Ospedaliera Universitaria di Bologna Policlinico S. Orsola Malpighi, Italy	NCT02749084
Refractory chronic GVHD	Alloantigen T_{reg} cells	• i.v. 0.5×10^6 cells per kg; once • i.v. 1.0×10^6 cells per kg; once • i.v. 3.0×10^6 cells per kg; once	I/II	Instituto de Medicina Molecular, Portugal	NCT02385019
Refractory chronic GVHD	Alloantigen T_{reg} cells	i.v. $1–3 \times 10^6$ cells; unknown regimen	II	University Hospital Regensburg, Germany	EudraCT 2012-002685-12
Refractory chronic GVHD	Alloantigen T_{reg} cells	i.v. $3–10 \times 10^6$ cells; unknown regimen	II	University Hospital Regensburg, Germany	EudraCT 2016-003947-12
Refractory chronic GVHD	Alloantigen T_{reg} cells	i.v.; unknown dose and regimen	II	CHU-ULG, Belgium	EudraCT 2012-000301-71
Autoimmune disorders					
SLE (skin)	Autologous T_{reg} cells	• i.v. 100×10^6 cells; once • i.v. 400×10^6 cells; once • i.v. $1{,}600 \times 10^6$ cells; once	I	NIAID	NCT02428309
Pemphigus vulgaris	Autologous T_{reg} cells	• i.v. 250×10^6 cells; once • i.v. $1{,}000 \times 10^6$ cells; once	I	NIAID	NCT03239470
Type 1 diabetes	Autologous T_{reg} cells	i.v. 3×10^6 cells; once, followed by IL-2 at 1×10^6 IU per day, for 5 days, in weeks 1 and 6	I	UCSF	NCT02772679

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).

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[10]. 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[57], resulting in high levels of STAT5 phosphorylation and specific enhancement of the gene activation programme downstream of IL-2R signalling[183]. Also, IL-2 was shown to block the differentiation of naive $CD4^+$ T cells into pro-inflammatory T_H17 cells[184] and to favourably influence the balance between T_{FH} 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.[188]) (and later for IL-2R signalling[189,190]) 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 immune effector responses to treat cancer[193], but it had numerous and severe side effects[10].

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

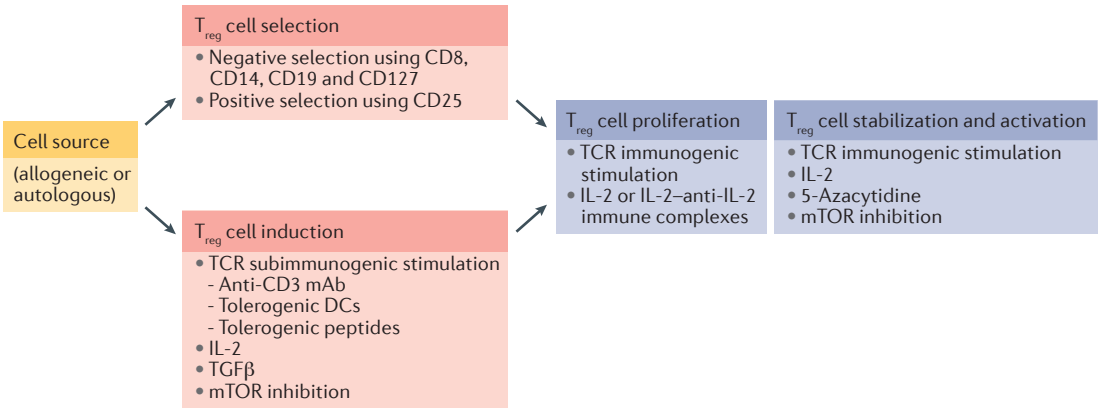


Figure 3 | **The production of therapeutic T_{reg} cells.** There are a number of protocols for regulatory T (T_{reg}) cell production in the clinical setting. The steps include choosing an appropriate cell source as well as methods for T_{reg} cell production (either by T_{reg} cell selection or T_{reg} cell induction), proliferation, stabilization and activation. T cells can be sourced from the patient (autologous) or from another donor (allogeneic). T_{reg} cells can be generated by one of two options. One protocol uses cell surface markers to identify the non-$CD4^+$ T cells (CD8, CD14, CD19 and/or CD127) for negative selection, followed by positive selection of the cells with surface expression of the α-chain of the IL-2 receptor (IL-2R) (CD25) to generate polyclonal T_{reg} cells. Alternatively, to induce T_{reg} cells, the T cell receptor (TCR) is stimulated in the presence of IL-2 and transforming growth factor-β (TGFβ). The amount of TCR stimulation should be low enough to preferentially stimulate T_{reg} cells. Exposure to IL-2, TGFβ and mechanistic target of rapamycin (mTOR) inhibitors, together with TCR engagement, initiates the expression of forkhead box P3 (FOXP3). To produce antigen-specific cells, the cells are stimulated with either tolerogenic dendritic cells (DCs) or tolerogenic peptides (instead of using the more common method of TCR stimulation, anti-CD3 monoclonal antibodies (mAbs)). In the next stage, the selected or induced T_{reg} cells are expanded using strong TCR stimulation in the presence of either IL-2 or IL-2–anti-IL-2 immune complexes. Additionally, the proliferating T_{reg} cells can be supplemented with 5-azacytidine and mTOR inhibitors to stabilize FOXP3 expression and increase T_{reg} cell function.

responders. This suggested that T_{reg} 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_{reg} cells, without affecting T_{eff} cells, and in substantial clinical improvement[9]. 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_{reg} cells and clinical improvement[196]. Indeed, very low doses of IL-2 have even been used to prevent the development of GVHD[197]. 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[198]. Importantly, this study documented that the IL-2 administration expanded T_{reg} 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_{reg} cell population in patients with SLE following administration of low-dose IL-2 (REFS[199–201]). 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[81], 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_{reg} cell function, increased IL-17 production, increased T_{FH} cell activity and an expanded population of double-negative T cells[202], controlled studies could reveal clinical usefulness.

As IL-2 can activate T_{eff} cells or T_{reg} 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_{reg} cells is crucial. On the basis of a dose-finding double-blind placebo-controlled study[203], 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_{reg} 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_{reg} 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_{reg} cell numbers. Intense work has therefore been put into generating mutant IL-2 proteins that have increased specificity for T_{reg} cells over T_{eff} cells[204].

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_{reg} 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[205] or fusion of IL-2 with

Table 2 | **Current clinical trials with small molecules to improve T_{reg} cell function**

Drug	Autoimmune disease	Trial phase	Clinical trial identifier
Rapamycin	T1D	II	NCT02803892 and NCT02505893
Metformin	SLE flares	IV	NCT02741960
N-Acetyl cysteine	SLE and MS	I/II and II	NCT00775476 and NCT02804594

MS, multiple sclerosis; SLE, systemic lupus erythematosus; T1D, type 1 diabetes; T_{reg} cell, regulatory T cell.

carrier proteins such as immunoglobulin fragments[206] or albumin[207,208] increases its half-life (FIG. 3). However, these approaches risk rendering the recombinant fusion proteins immunogenic or even able to stimulate T_{eff} 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 explored[209]. Some of these complexes preferentially expand T_{reg} cell populations and improve disease in lupus-prone mice[187]. 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_{reg} cells would allow their use in the clinic. Human antibodies that favour the expansion of T_{eff} cells over T_{reg} cells have been described[210], 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_{reg} cells in vitro and selective expansion of T_{reg} cells in vivo, has been developed[211].

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 DCs[212]. Thus, cognate antigen presentation by immature $LY75^+$ DCs to naive $CD4^+$ T cells can promote the development of functional T_{reg} cells that have the canonical T_{reg} cell signature (including expression of chemokine receptors, CTLA4 and IL-10)[44]. In these T_{reg} cells, the *CNS2* region of the *FOXP3* promoter is hypomethylated and allows for stable expression of FOXP3 and prolonged cell survival[213]. In the preclinical setting, $LY75^+$ DCs promoted tolerance through the generation of T_{reg} cells and protected mice from the development of T1D and experimental autoimmune encephalomyelitis (EAE), which result from pancreas-embedded[214] and myelin oligodendrocyte glycoprotein (MOG)[215] antigens, respectively.

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_{reg} cells and T_H2 cells in culture, both of which produced immunosuppressive cytokines[216]. 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-κB 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_{eff} cells decreased, the ratio of T_{reg} cells to T_{eff} cells increased, and serum levels of inflammatory cytokines and chemokines (IL-15, IL-29, CXCL1 and CXCL11) were reduced[217].

Tolerogenic peptides

Myelin basic protein (MBP) is an antigen derived from myelin sheaths. A polypeptide known as copolymer-I (COP-I), which contains the four immunogenic amino acids found in MBP, was designed and proved clinically efficient in treating patients with multiple sclerosis[218]. In the murine model of EAE, administration of COP-I was associated with induction of T_{reg} cells[219].

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 cells[220,221] that also expressed BCL-X_L (REFS[222,223]). 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 benefit[224].

Both histone peptides derived from a histone autoepitope and the pConsensus peptide, which is based on T cell determinants in the variable chain heavy (V_H) 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_{reg} cells[225,226].

A peptide derived from heat shock protein 60 (HSP60), designated $HSP60_{p216}$, was shown to be tolerogenic in a murine model for rheumatoid arthritis. $HSP60_{p216}$-specific $CD8^+$ T_{reg} cells were induced following the administration of tetramers composed of $HSP60_{p216}$ and the murine MHC class Ib molecule Qa1. These cells used perforin and IL-15 to suppress pathogenic T_{FH} cells and T_H17 cells, reduce the production of collagen-specific autoantibodies and inhibit the development of arthritis in these mice[227].

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_{reg} cells and improved clinical indices in a rat adjuvant-induced arthritis model[228].

Table 3 | **Current clinical trials studying low-dose IL-2**

Target tissue or condition	Treatment groups	Trial phase	Sponsoring institute	Clinical trial identifier
Transplantation				
Liver	s.c. 0.30 MIU per m^2 body surface area for 4 weeks	II	BIDMC, Boston, USA	NCT02739412
	Unknown dose; 4 weeks	IV	King's College London, UK	NCT02949492
Autoimmune diseases				
Rheumatoid arthritis, ankylosing spondylitis, SLE, psoriasis, IBD and autoimmune hepatitis	• Induction: s.c. 1 MIU per day for 5 days • Maintenance: s.c. 1 MIU every 15 days for 6 months • Maintenance (in SLE): s.c. 1 MIU every 7 days for 6 months	II	Assistance Publique — Hôpitaux de Paris, France	NCT01988506
SLE	s.c. 1.5 MIU per day for 5 days every 3 weeks; 4 cycles	II	University of Zurich, Switzerland	NCT03312335
Multiple sclerosis	• Induction period: repeated administration of low-dose IL-2 • Maintenance period: treatment with IL-2	II	Assistance Publique — Hôpitaux de Paris, France	NCT02424396
T1D	s.c. 0.5–1.0 MIU per m^2 per day for 5 days, then 10 days later, every week or every 2 weeks	II	Assistance Publique — Hôpitaux de Paris, France	NCT02411253
Amyotrophic lateral sclerosis	s.c.; unknown dose and regimen	II	CHU de Nimes, France	EudraCT 2015-005347-14

BIDMC, Beth Israel Deaconess Medical Center; CHU, Centre Hospitalier Universitaire; IBD, inflammatory bowel disease; IL, interleukin; MIU, million international

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[229], 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[230]. Also, the T_{eff} cells from patients with SLE are more resistant to suppression by T_{reg} cells in vitro[79,231]. 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} cells 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_{reg} cell recruitment mechanism in numerous tumours — reduces intratumoural accumulation of T_{reg} cells and suppresses tumour growth in mice[238,239]. 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_{reg} cell depletion and reduced tumour growth[114]. Clinical studies conducted with a humanized anti-CCR4 antibody (mogamulizumab, KW-0761), which depletes $CCR4^+$ T_{reg} cells by antibody-dependent cell-mediated cytotoxicity (ADCC), showed intratumoural T_{reg} cell depletion and antitumour activity with minimal to moderate toxicity[240,47]. However, a recent update of two clinical phase I/II trials reported unclear long-term antitumour effects[241]. Occasional serious side effects were reported as well[240,242]. Comparative flow cytometric and RNA sequencing analysis of T_{reg} 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_{reg} cells, is also expressed in T_{reg} cells in the peripheral blood and in activated T_{eff} cells, although in somewhat lesser amounts[123]. It is therefore possible that the CCR4-depleting antibody affects the survival of peripheral T_{reg} cells, which leads to unwanted effects, and affects the survival of T_{eff} cells as well, which limits antitumour responses. Indeed, both T_{reg} cell and T_{eff} cell numbers were decreased in patients who received anti-CCR4 antibody treatment in a clinical study[243]. These data highlight the need to identify molecules that are specific to tumour-dwelling T_{reg} cells to use as therapeutic targets. Notably, two recent studies showed that intratumoural T_{reg} cells from various human tumours express CCR8 at levels that are much higher than those in peripheral T_{reg} cells, or conventional T cells in the periphery of tumours, indicating that CCR8 is a novel promisingly targetable molecule[123,244]. CCR8 is the receptor for the CCL1 and CCL18 chemokines, both of which are differentially upregulated in intratumoural myeloid cells[123].

T_{reg} cell depletion

Numerous specific intratumoural T_{reg} cell depletion strategies have been investigated and reviewed extensively[115,117,130]. Antibodies directed against 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_{reg} cells by ADCC and complement-mediated cytotoxicity. Also, a fusion protein containing IL-2 and diphtheria toxin protein (denileukin diftitox, 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 diftitox had mixed results[115,117,130,250]. Notably, patients with metastatic melanoma treated with denileukin diftitox showed no clinical benefit and severe autoimmune side effects[251]. Depletion of T_{reg} cells from HSCT to treat relapses of leukaemia in patients who did not develop GVHD during the first transplant produced better outcomes[252]. These data suggest that global T_{reg} cell depletion has variable efficacy and the potential to induce systemic complications.

Immune checkpoint inhibitors

Immune checkpoint molecules are upregulated in T_{reg} cells and thus could be targeted to modulate T_{reg} 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[253–255]. Although CTLA4 targeting was initially aimed to reactivate T_{eff} cells, CTLA4 is also highly expressed on T_{reg} cells, and CTLA4 targeting induces T_{reg} cell depletion in the tumour microenvironment by ADCC[256]. Furthermore, CTLA4 antibody binding to T_{reg} cells contributed, independently of its T_{eff} cell binding, to the antitumour activity of this molecule, thus leading to a synergistic maximal antitumour effect in a mouse melanoma model[257]. Intriguingly, however, conditional CTLA4 depletion in T_{reg} cells increased the immunosuppressive functions of T_{reg} cells in adult mice[258], indicating that the role of CTLA4 in T_{reg} cells needs to be further investigated. Undesirable autoimmune manifestations in a subset of patients treated with anti-CTLA4 antibodies indicated systemic loss of T_{reg} cell activity[253,259]. Anti-CTLA4 antibodies have complimentary activity with therapies targeting anti-PD-1 (nivolumab), another checkpoint inhibitor expressed by T_{reg} cells, and their combined use is more beneficial than either agent alone[260]. 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_{reg} 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[261,262]. 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[263].

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_{reg} cells. Ligation of GITR with an agonistic antibody was shown to suppress T_{reg} cell activity, reduce T_{reg} cell numbers and decrease T_{reg} cell lineage stability selectively in tumours and also limit tumour growth in mice[264–267], particularly when administered together with CTLA4 (REF.[268]) or PD-1 inhibitors[269]. GITR ligation, similar to CTLA4 blockade, also improves T_{eff} cell functions and renders T_{eff} cells resistant to inhibition by T_{reg} cells[270]. Mice treated with an

Antibody-dependent cell-mediated cytotoxicity (ADCC). In this process, targeted cells become coated with antibody, and are then lysed by effector cells that have cytolytic activity and specific immunoglobulin crystallizable fragment (Fc) receptors. Lysis requires direct cell-to-cell contact and does not involve complement.

Complement-mediated cytotoxicity
A process that leads to the lysis of cells coated with immunoglobulin, a marker that is able to activate complement.

Table 4 | **Current clinical approaches to suppress T_{reg} cell activity in cancer**

Treatment	Molecular target or mechanism	Tumour type	Refs
Nonspecific T_{reg} cell depletion			
Cyclophosphamide, low dose (metronomic chemotherapy)	DNA crosslinker	End-stage cancers and breast cancer	232,233,234
Sunitinib or sorafenib	Tyrosine kinase inhibitor	Renal cell carcinoma	235,236
Specific T_{reg} cell depletion			
Daclizumab	Anti-CD25 mAb	Breast cancer	246
LMB-2	Anti-CD25 (scFv)–exotoxin A	Haematological malignancies and melanoma	247–249
Denileukin diftitox	IL-2–diphtheria toxin	Renal cell carcinoma	250
Inhibition of T_{reg} cell trafficking			
Mogamulizumab (KW-0761)	Anti-CCR4 mAb	CCR4-negative solid tumours, T cell lymphoma or leukaemia	47,239, 240,243
Immune checkpoint inhibitors			
Ipilimumab	Anti-CTLA4 mAb	Metastatic melanoma	253–255,260
Nivolumab	Anti-PD-1 mAb	Metastatic melanoma	260
TNFR engagement			
BMS-986156, GWN323 and INCAGN01876	GITR agonist antibody	Advanced solid tumours	276,278
MEDI11873	Hexameric GITR-targeted human IgG1	Advanced solid tumours	276,278
9B12 mouse anti-human mAb	OX40 agonist	Advanced solid tumours	275,276,277
IDO inhibitors			
Indoximod	IDO	Pancreatic, prostate, brain and breast cancers	287
Epacadostat	IDO	Melanoma and myelodysplastic syndrome	287
Navoximod	IDO	Solid tumours	287
IDO1-derived peptide	IDO	Melanoma	287

CCR4, CC-chemokine receptor 4; CTLA4, cytotoxic T lymphocyte antigen 4; IDO, indoleamine 2,3-dioxygenase; IgG1, immunoglobulin G1; IL-2, interleukin 2; mAb, monoclonal antibody; PD-1, programmed cell death 1; scFv, single-chain variable fragment; T_{reg} cell, regulatory T cell.

anti-GITR antibody showed dramatically reduced tumour growth and the intratumoural T_{reg} cells in these mice had decreased expression of HELIOS (also known as IKZF2, a transcription factor that maintains T_{reg} cell stability and function) and increased IFNγ production. These data provide a mechanistic explanation for the reduction of T_{reg} cell lineage stability by GITR ligation and reinforce GITR as a therapeutic target to reverse the immunosuppressive function of intratumoural T_{reg} 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 T_{reg} cell conversion in mice[272]. In addition, the anti-GITR antibody has been reported to increase the number of T_{reg} 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 T_{reg} cell function and improved T_{eff} 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[266,276–278].

Two newly developed antagonistic antibodies that target TNFR2, which is expressed by the most suppressive intratumoural T_{reg} cells and by human cancers, disabled the ability of TNFR2 to bind TNF and activate NF-κB, killed T_{reg} cells and also induced killing of tumour cells[279]. Interestingly, TNFR2 can exert bidirectional control on T_{reg} 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 T_{reg} cell expansion in vitro and to inhibit GVHD in vivo[281–283]. Therefore, TNFR2 is an exciting molecular target for the development of T_{reg} 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 T_{reg} cells[128,284,285]. 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 T_{eff} cell responses and increased T_{reg} 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[286,287]. IDO inhibitors (indoximod (NLG-8189), navoximod (NLG-919), epacadostat (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[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_H17-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[288].

T_{reg} cell-derived cytokines

In another approach, the effects of the anti-inflammatory cytokines (TGFβ, 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 TGFβ expressed on the surface of T_{reg} cells improved the anti-melanoma immune response[282] and suppressed metastasis of pancreatic tumours in mice[113,289–291]. Anti-IL-35 limited tumour growth in multiple mouse models of human cancer[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[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[67,293]. NRP1-deficient intratumoural T_{reg} cells lose their immunosuppressive function and produce IFNγ (a T_{eff} cell cytokine) while retaining FOXP3 expression, thus exhibiting a phenotype that was named T_{reg} cell fragility (REF.[293]). 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, IFNγ 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; IFNγ receptor inactivation abrogates the anti-PD-1 tumour response, as shown in a fibrosarcoma mouse model[293]. 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-κB pathway is required for the maintenance of the active status of T_{reg} cells[294]. *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[294]. 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[295].

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[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 autoinflammatory 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[298]) — 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 T1D.

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[308]. 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[308]. T_{reg} cells that express AREG can also protect lungs from infection-induced damage[309]. AREG controls the immune response by regulating T_{reg} cell function[310]. Involvement of AREG–EGFR signals in T_{reg} cell-mediated tissue regeneration is also observed in skin injury and promotes wound healing[311,312]. Furthermore, skin T_{reg} cells preferentially reside close to hair follicle stem cells (HFSCs) and help HFSC-mediated hair regeneration[313]. Paracrine effects of T_{reg} cells were also shown to promote cardiomyocyte proliferation during pregnancy and after myocardial infarction[314].

T helper 1 (T_H1) cells and T_H17 cells have been shown to promote neuroinflammation, but remyelination is compromised in the absence of lymphocytes[315], 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[316].

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.

1. Brunkow, M. E. et al. Disruption of a new forkhead/winged-helix protein, scurfin, results in the fatal lymphoproliferative disorder of the scurfy mouse. *Nat. Genet.* **27**, 68–73 (2001).
2. Fontenot, J. D., Gavin, M. A. & Rudensky, A. Y. Foxp3 programs the development and function of CD4 + CD25 + regulatory T cells. *Nat. Immunol.* **4**, 330–336 (2003).
3. Wildin, R. S. et al. X-Linked neonatal diabetes mellitus, enteropathy and endocrinopathy syndrome is the human equivalent of mouse scurfy. *Nat. Genet.* **27**, 18–20 (2001).
4. Bennett, C. L. et al. The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. *Nat. Genet.* **27**, 20–21 (2001).
5. June, C. H. Adoptive T cell therapy for cancer in the clinic. *J. Clin. Invest.* **117**, 1466–1476 (2007).
6. Hoffmann, P. et al. Isolation of CD4 + CD25 + regulatory T cells for clinical trials. *Biol. Blood Marrow Transplant.* **12**, 267–274 (2006).
7. Wright, G. P. et al. Adoptive therapy with redirected primary regulatory T cells results in antigen-specific suppression of arthritis. *Proc. Natl Acad. Sci. USA* **106**, 19078–19083 (2009).
8. Matsuoka, K. et al. Low-dose interleukin-2 therapy restores regulatory T cell homeostasis in patients with chronic graft-versus-host disease. *Sci. Transl Med.* **5**, 179ra43 (2013).
9. Saadoun, D. et al. Regulatory T cell responses to low-dose interleukin-2 in HCV-induced vasculitis. *N. Engl. J. Med.* **365**, 2067–2077 (2011).
This is one of the first studies to show that low-dose IL-2 can produce clinical benefit by expanding T_{reg} cells.
10. Klatzmann, D. & Abbas, A. K. The promise of low-dose interleukin-2 therapy for autoimmune and inflammatory diseases. *Nat. Rev. Immunol.* **15**, 283–294 (2015).
11. Jonuleit, H. et al. Identification and functional characterization of human CD4(+)CD25(+) T cells with regulatory properties isolated from peripheral blood. *J. Exp. Med.* **193**, 1285–1294 (2001).
12. Piccirillo, C. A. & Shevach, E. M. Naturally-occurring CD4 + CD25 + immunoregulatory T cells: central players in the arena of peripheral tolerance. *Semin. Immunol.* **16**, 81–88 (2004).
13. Liu, W. et al. CD127 expression inversely correlates with FoxP3 and suppressive function of human CD4 + T reg cells. *J. Exp. Med.* **203**, 1701–1711 (2006).
14. Morgan, M. E. et al. Expression of FOXP3 mRNA is not confined to CD4 + CD25 + T regulatory cells in humans. *Hum. Immunol.* **66**, 13–20 (2005).
15. Gavin, M. A. et al. Single-cell analysis of normal and FOXP3-mutant human T cells: FOXP3 expression without regulatory T cell development. *Proc. Natl Acad. Sci. USA* **103**, 6659–6664 (2006).
16. Allan, S. E. et al. Activation-induced FOXP3 in human T effector cells does not suppress proliferation or cytokine production. *Int. Immunol.* **19**, 345–354 (2007).
17. Khattri, R., Cox, T., Yasayko, S. A. & Ramsdell, F. An essential role for Scurfin in CD4 + CD25 + T regulatory cells. *Nat. Immunol.* **4**, 337–342 (2003).
18. Hori, S., Nomura, T. & Sakaguchi, S. Control of regulatory T cell development by the transcription factor Foxp3. *Science* **299**, 1057–1061 (2003).
19. Omenetti, S. & Pizarro, T. T. The Treg/Th17 axis: a dynamic balance regulated by the gut microbiome. *Front. Immunol.* **6**, 639 (2015).
20. Lio, C. W. & Hsieh, C. S. A two-step process for thymic regulatory T cell development. *Immunity* **28**, 100–111 (2008).
21. Burchill, M. A. et al. Linked T cell receptor and cytokine signaling govern the development of the regulatory T cell repertoire. *Immunity* **28**, 112–121 (2008).
22. Long, M., Park, S. G., Strickland, I., Hayden, M. S. & Ghosh, S. Nuclear factor-kappaB modulates regulatory T cell development by directly regulating expression of Foxp3 transcription factor. *Immunity* **31**, 921–931 (2009).
23. Luo, C. T. & Li, M. O. Transcriptional control of regulatory T cell development and function. *Trends Immunol.* **34**, 531–539 (2013).
24. Li, X., Liang, Y., LeBlanc, M., Benner, C. & Zheng, Y. Function of a Foxp3 cis-element in protecting regulatory T cell identity. *Cell* **158**, 734–748 (2014).
25. Zheng, Y. et al. Role of conserved non-coding DNA elements in the Foxp3 gene in regulatory T cell fate. *Nature* **463**, 808–812 (2010).
26. Mahmud, S. A. et al. Costimulation via the tumor-necrosis factor receptor superfamily couples TCR signal strength to the thymic differentiation of regulatory T cells. *Nat. Immunol.* **15**, 473–481 (2014).
27. Huynh, A. et al. Control of PI(3) kinase in Treg cells maintains homeostasis and lineage stability. *Nat. Immunol.* **16**, 188–196 (2015).
28. Marie, J. C., Liggitt, D. & Rudensky, A. Y. Cellular mechanisms of fatal early-onset autoimmunity in mice with the T cell-specific targeting of transforming growth factor-beta receptor. *Immunity* **25**, 441–454 (2006).
29. Konkel, J. E., Jin, W., Abbatiello, B., Grainger, J. R. & Chen, W. Thymocyte apoptosis drives the intrathymic generation of regulatory T cells. *Proc. Natl Acad. Sci. USA* **111**, E465–E473 (2014).
30. Chen, W. et al. Conversion of peripheral CD4 + CD25- naive T cells to CD4 + CD25 + regulatory T cells by TGF-beta induction of transcription factor Foxp3. *J. Exp. Med.* **198**, 1875–1886 (2003).
31. Chen, W. & Konkel, J. E. Development of thymic Foxp3(+) regulatory T cells: TGF-β matters. *Eur. J. Immunol.* **45**, 958–965 (2015).
32. Wing, K. et al. CTLA-4 control over Foxp3 + regulatory T cell function. *Science* **322**, 271–275 (2008).
33. Qureshi, O. S. et al. Trans-endocytosis of CD80 and CD86: a molecular basis for the cell-extrinsic function of CTLA-4. *Science* **332**, 600–603 (2011).
34. Liang, B. et al. Regulatory T cells inhibit dendritic cells by lymphocyte activation gene-3 engagement of MHC class II. *J. Immunol.* **180**, 5916–5926 (2008).
35. Sarris, M., Andersen, K. G., Randow, F., Mayr, L. & Betz, A. G. Neuropilin-1 expression on regulatory T cells enhances their interactions with dendritic cells during antigen recognition. *Immunity* **28**, 402–413 (2008).
36. Borsellino, G. et al. Expression of ectonucleotidase CD39 by Foxp3 + Treg cells: hydrolysis of extracellular ATP and immune suppression. *Blood* **110**, 1225–1232 (2007).
37. Kobie, J. J. et al. T regulatory and primed uncommitted CD4 T cells express CD73, which suppresses effector CD4 T cells by converting 5′-adenosine monophosphate to adenosine. *J. Immunol.* **177**, 6780–6786 (2006).
38. Deaglio, S. et al. Adenosine generation catalyzed by CD39 and CD73 expressed on regulatory T cells mediates immune suppression. *J. Exp. Med.* **204**, 1257–1265 (2007).
39. Chinen, T. et al. An essential role for the IL-2 receptor in Treg cell function. *Nat. Immunol.* **17**, 1322–1333 (2016).
40. Malek, T. R. et al. IL-2 family of cytokines in T regulatory cell development and homeostasis. *J. Clin. Immunol.* **28**, 635–639 (2008).
41. Pandiyan, P., Zheng, L., Ishihara, S., Reed, J. & Lenardo, M. J. CD4 + CD25 + Foxp3 + regulatory T cells induce cytokine deprivation-mediated apoptosis of effector CD4 + T cells. *Nat. Immunol.* **8**, 1353–1362 (2007).
42. Gondek, D. C., Lu, L. F., Quezada, S. A., Sakaguchi, S. & Noelle, R. J. Cutting edge: contact-mediated suppression by CD4 + CD25 + regulatory cells involves a granzyme B-dependent, perforin-independent mechanism. *J. Immunol.* **174**, 1783–1786 (2005).
43. Cao, X. et al. Granzyme B and perforin are important for regulatory T cell-mediated suppression of tumor clearance. *Immunity* **27**, 635–646 (2007).
44. Feuerer, M. et al. Genomic definition of multiple ex vivo regulatory T cell subphenotypes. *Proc. Natl Acad. Sci. USA* **107**, 5919–5924 (2010).
45. Smigiel, K. S. et al. CCR7 provides localized access to IL-2 and defines homeostatically distinct regulatory T cell subsets. *J. Exp. Med.* **211**, 121–136 (2014).
46. Bergot, A.-S. et al. TCR sequences and tissue distribution discriminate the subsets of naïve and activated/memory Treg cells in mice. *Eur. J. Immunol.* **45**, 1524–1534 (2015).
47. Sugiyama, D. et al. Anti-CCR4 mAb selectively depletes effector-type FoxP3 + CD4 + regulatory T cells, evoking antitumor immune responses in humans. *Proc. Natl Acad. Sci. USA* **110**, 17945–17950 (2013).
48. Koch, M. A. et al. T-Bet(+) Treg cells undergo abortive Th1 cell differentiation due to impaired expression of IL-12 receptor β2. *Immunity* **37**, 501–510 (2012).
49. Wang, Y., Su, M. A. & Wan, Y. Y. An essential role of the transcription factor GATA-3 for the function of regulatory T cells. *Immunity* **35**, 337–348 (2011).
50. Chaudhry, A. et al. CD4 + regulatory T cells control TH17 responses in a Stat3-dependent manner. *Science* **326**, 986–991 (2009).
This is one of the first studies to introduce the concept that T_{reg} cells express the same transcription factors as the cells they are supposed to suppress.
51. Linterman, M. A. et al. Foxp3 + follicular regulatory T cells control the germinal center response. *Nat. Med.* **17**, 975–982 (2011).
52. Levine, A. G. et al. Stability and function of regulatory T cells expressing the transcription factor T-bet. *Nature* **546**, 421–425 (2017).
53. Levine, A. G., Arvey, A., Jin, W. & Rudensky, A. Y. Continuous requirement for the TCR in regulatory T cell function. *Nat. Immunol.* **15**, 1070–1078 (2014).
54. Smigiel, K. S., Srivastava, S., Stolley, J. M. & Campbell, D. J. Regulatory T cell homeostasis: steady-state maintenance and modulation during inflammation. *Immunol. Rev.* **259**, 40–59 (2014).
55. Cheng, G. et al. IL-2 receptor signaling is essential for the development of Klrg1 + terminally differentiated T regulatory cells. *J. Immunol.* **189**, 1780–1791 (2012).
56. Pierson, W. et al. Antiapoptotic Mcl-1 is critical for the survival and niche-filling capacity of Foxp3(+) regulatory T cells. *Nat. Immunol.* **14**, 959–965 (2013).
57. Yu, A., Zhu, L., Altman, N. H. & Malek, T. R. A low interleukin-2 receptor signaling threshold supports the development and homeostasis of T regulatory cells. *Immunity* **30**, 204–217 (2009).
58. Castro, I., Yu, A., Dee, M. J. & Malek, T. R. The basis of distinctive IL-2- and IL-15-dependent signaling: weak CD122-dependent signaling favors CD8 + T central-memory cell survival but not T effector-memory cell development. *J. Immunol.* **187**, 5170–5182 (2011).
59. Saxton, R. A. & Sabatini, D. M. mTOR signaling in growth, metabolism, and disease. *Cell* **168**, 960–976 (2017).
60. Perl, A. Activation of mTOR (mechanistic target of rapamycin) in rheumatic diseases. *Nat. Rev. Rheumatol.* **12**, 169–182 (2016).
61. Delgoffe, G. M. et al. The kinase mTOR regulates the differentiation of helper T cells through the selective activation of signaling by mTORC1 and mTORC2. *Nat. Immunol.* **12**, 295–303 (2011).
62. Zeng, H. et al. mTORC1 couples immune signals and metabolic programming to establish T(reg)-cell function. *Nature* **499**, 485–490 (2013).
63. Michalek, R. D. et al. Cutting edge: distinct glycolytic and lipid oxidative metabolic programs are essential for effector and regulatory CD4 + T cell subsets. *J. Immunol.* **186**, 3299–3303 (2011).
64. Coe, D. J., Kishore, M. & Marelli-Berg, F. Metabolic regulation of regulatory T cell development and function. *Front. Immunol.* **5**, 590 (2014).
65. Powell, J. D., Pollizzi, K. N., Heikamp, E. B. & Horton, M. R. Regulation of immune responses by mTOR. *Annu. Rev. Immunol.* **30**, 39–68 (2012).
66. Walsh, P. T. et al. PTEN inhibits IL-2 receptor-mediated expansion of CD4 + CD25 + Tregs. *J. Clin. Invest.* **116**, 2521–2531 (2006).
67. Delgoffe, G. M. et al. Stability and function of regulatory T cells is maintained by a neuropilin-1-semaphorin-4a axis. *Nature* **501**, 252–256 (2013).
This paper shows that the immune cell-expressed ligand SEMA4A and the T_{reg} cell-expressed receptor NRP1 interact to potentiate T_{reg} cell function and survival.
68. Apostolidis, S. A. et al. Phosphatase PP2A is requisite for the function of regulatory T cells. *Nat. Immunol.* **17**, 556–564 (2016).
This paper shows that PP2A, a serine/threonine phosphatase, is important for the proper function of T_{reg} cells and that its absence leads to extensive autoimmunity and multiple organ inflammation.
69. Torgerson, T. R. & Ochs, H. D. Immune dysregulation, polyendocrinopathy, enteropathy, X-linked: forkhead box protein 3 mutations and lack of regulatory T cells. *J. Allergy Clin. Immunol.* **120**, 744–750 (2007).
70. Malek, T. R. & Bayer, A. L. Tolerance, not immunity, crucially depends on IL-2. *Nat. Rev. Immunol.* **4**, 665–674 (2004).
71. Malek, T. R. The biology of interleukin-2. *Annu. Rev. Immunol.* **26**, 453–479 (2008).
72. Bernasconi, A. et al. Characterization of immunodeficiency in a patient with growth hormone insensitivity secondary to a novel STAT5b gene mutation. *Pediatrics* **118**, e1584–e1592 (2006).

73. Nadeau, K., Hwa, V. & Rosenfeld, R. G. STAT5b deficiency: an unsuspected cause of growth failure, immunodeficiency, and severe pulmonary disease. *J. Pediatr.* **158**, 701–708 (2011).
74. Schubert, D. et al. Autosomal dominant immune dysregulation syndrome in humans with CTLA4 mutations. *Nat. Med.* **20**, 1410–1416 (2014).
75. Charbonnier, L. M. et al. Regulatory T cell deficiency and immune dysregulation, polyendocrinopathy, enteropathy, X-linked-like disorder caused by loss-of-function mutations in LRBA. *J. Allergy Clin. Immunol.* **135**, 217–227 (2015).
76. Ohl, K. & Tenbrock, K. Regulatory T cells in systemic lupus erythematosus. *Eur. J. Immunol.* **45**, 344–355 (2015).
77. Lyssuk, E. Y., Torgashina, A. V., Soloviev, S. K., Nassonov, E. L. & Bykovskaia, S. N. Reduced number and function of CD4 + CD25highFoxP3 + regulatory T cells in patients with systemic lupus erythematosus. *Adv. Exp. Med. Biol.* **601**, 113–119 (2007).
78. Bonelli, M. et al. Quantitative and qualitative deficiencies of regulatory T cells in patients with systemic lupus erythematosus (SLE). *Int. Immunol.* **20**, 861–868 (2008).
79. Venigalla, R. K. C. et al. Reduced CD4 + ,CD25- T cell sensitivity to the suppressive function of CD4 + ,CD25high, CD127 – /low regulatory T cells in patients with active systemic lupus erythematosus. *Arthritis Rheum.* **58**, 2120–2130 (2008).
80. Miyara, M. et al. Functional delineation and differentiation dynamics of human CD4 + T cells expressing the FoxP3 transcription factor. *Immunity* **30**, 899–911 (2009).
81. Comte, D. et al. Brief report: CD4 + T cells from patients with systemic lupus erythematosus respond poorly to exogenous interleukin-2. *Arthritis Rheumatol.* **69**, 808–813 (2017).
82. Afeltra, A. et al. The involvement of T regulatory lymphocytes in a cohort of lupus nephritis patients: a pilot study. *Intern. Emerg. Med.* **10**, 677–683 (2015).
83. Marwaha, A. K. et al. Cutting edge: increased IL-17-secreting T cells in children with new-onset type 1 diabetes. *J. Immunol.* **185**, 3814–3818 (2010).
84. Long, S. A. et al. Defects in IL-2R signaling contribute to diminished maintenance of FOXP3 expression in CD4(+)CD25(+) regulatory T cells of type 1 diabetic subjects. *Diabetes* **59**, 407–415 (2010).
85. Schneider, A. et al. The effector T cells of diabetic subjects are resistant to regulation via CD4 + FOXP3 + regulatory T cells. *J. Immunol.* **181**, 7350–7355 (2008).
86. Harden, J. L., Krueger, J. G. & Bowcock, A. M. The immunogenetics of psoriasis: a comprehensive review. *J. Autoimmun.* **64**, 66–73 (2015).
87. Soler, D. C. et al. Psoriasis patients exhibit impairment of the high potency CCR5(+) T regulatory cell subset. *Clin. Immunol.* **149**, 111–118 (2013).
88. Sugiyama, H. et al. Dysfunctional blood and target tissue CD4 + CD25high regulatory T cells in psoriasis: mechanism underlying unrestrained pathogenic effector T cell proliferation. *J. Immunol.* **174**, 164–173 (2005).
89. Zhang, K. et al. Functional characterization of CD4 + CD25 + regulatory T cells differentiated in vitro from bone marrow-derived haematopoietic cells of psoriasis patients with a family history of the disorder. *Br. J. Dermatol.* **158**, 298–305 (2008).
90. Bovenschen, H. J. et al. Foxp3 + regulatory T cells of psoriasis patients easily differentiate into IL-17A-producing cells and are found in lesional skin. *J. Invest. Dermatol.* **131**, 1853–1860 (2011).
91. Balandina, A., Lecart, S., Dartevelle, P., Saoudi, A. & Berrih-Aknin, S. Functional defect of regulatory CD4(+)CD25 + T cells in the thymus of patients with autoimmune myasthenia gravis. *Blood* **105**, 735–741 (2005).
92. Thiruppathi, M. et al. Impaired regulatory function in circulating CD4(+)CD25(high)CD127(low/-) T cells in patients with myasthenia gravis. *Clin. Immunol.* **145**, 209–223 (2012).
93. Alahgholi-Hajibehzad, M. et al. Regulatory function of CD4 + CD25 + + T cells in patients with myasthenia gravis is associated with phenotypic changes and STAT5 signaling: 1,25-Dihydroxyvitamin D3 modulates the suppressor activity. *J. Neuroimmunol.* **281**, 51–60 (2015).
94. Renton, A. E. et al. A genome-wide association study of myasthenia gravis. *JAMA Neurol.* **72**, 396–404 (2015).
95. Masuda, M. et al. Clinical implication of peripheral CD4 + CD25 + regulatory T cells and Th17 cells in myasthenia gravis patients. *J. Neuroimmunol.* **225**, 123–131 (2010).
96. Makita, S. et al. CD4 + CD25bright T cells in human intestinal lamina propria as regulatory cells. *J. Immunol.* **173**, 3119–3130 (2004).
97. Maul, J. et al. Peripheral and intestinal regulatory CD4 + CD25(high) T cells in inflammatory bowel disease. *Gastroenterology* **128**, 1868–1878 (2005).
98. Uhlig, H. H. et al. Characterization of Foxp3 + CD4 + CD25 + and IL-10-secreting CD4 + CD25 + T cells during cure of colitis. *J. Immunol.* **177**, 5852–5860 (2006).
99. Monteleone, G. et al. Blocking Smad7 restores TGF-beta1 signaling in chronic inflammatory bowel disease. *J. Clin. Invest.* **108**, 601–609 (2001).
100. Geremia, A., Biancheri, P., Allan, P., Corazza, G. R. & Di Sabatino, A. Innate and adaptive immunity in inflammatory bowel disease. *Autoimmun. Rev.* **13**, 3–10 (2014).
101. Agus, A., Planchais, J. & Sokol, H. Gut microbiota regulation of tryptophan metabolism in health and disease. *Cell Host Microbe* **23**, 716–724 (2018).
102. McFarland, H. F. & Martin, R. Multiple sclerosis: a complicated picture of autoimmunity. *Nat. Immunol.* **8**, 913–919 (2007).
103. Liu, Y., Teige, I., Birnir, B. & Issazadeh-Navikas, S. Neuron-mediated generation of regulatory T cells from encephalitogenic T cells suppresses EAE. *Nat. Med.* **12**, 518–525 (2006).
104. Korn, T. et al. Myelin-specific regulatory T cells accumulate in the CNS but fail to control autoimmune inflammation. *Nat. Med.* **13**, 423–431 (2007).
105. Noori-Zadeh, A. et al. Regulatory T cell number in multiple sclerosis patients: a meta-analysis. *Mult. Scler. Relat. Disord.* **5**, 73–76 (2016).
106. Viglietta, V., Baecher-Allan, C., Weiner, H. L. & Hafler, D. A. Loss of functional suppression by CD4 + CD25 + regulatory T cells in patients with multiple sclerosis. *J. Exp. Med.* **199**, 971–979 (2004).
107. Lechler, R. I., Garden, O. A. & Turka, L. A. The complementary roles of deletion and regulation in transplantation tolerance. *Nat. Rev. Immunol.* **3**, 147–158 (2003).
108. Wood, K. J. & Sakaguchi, S. Regulatory T cells in transplantation tolerance. *Nat. Rev. Immunol.* **3**, 199–210 (2003).
109. Jiang, S., Herrera, O. & Lechler, R. I. New spectrum of allorecognition pathways: implications for graft rejection and transplantation tolerance. *Curr. Opin. Immunol.* **16**, 550–557 (2004).
110. Lee, K., Nguyen, V., Lee, K. M., Kang, S. M. & Tang, Q. Attenuation of donor-reactive T cells allows effective control of allograft rejection using regulatory T cell therapy. *Am. J. Transplant.* **14**, 27–38 (2014).
111. Koga, T. et al. CaMK4-dependent activation of AKT/mTOR and CREM-α underlies autoimmunity-associated Th17 imbalance. *J. Clin. Invest.* **124**, 2234–2245 (2014).
112. Koga, T., Ichinose, K., Mizui, M., Crispin, J. C. & Tsokos, G. C. Calcium/calmodulin-dependent protein kinase IV suppresses IL-2 production and regulatory T cell activity in lupus. *J. Immunol.* **189**, 3490–3496 (2012).
113. Budhu, S. et al. Blockade of surface-bound TGF-beta on regulatory T cells abrogates suppression of effector T cell function in the tumor microenvironment. *Sci. Signal.* **10**, eaak9702 (2017).
114. Facciabene, A. et al. Tumour hypoxia promotes tolerance and angiogenesis via CCL28 and T(reg) cells. *Nature* **475**, 226–230 (2011).
This study shows that tumour hypoxia promotes the recruitment of T_{reg} cells through the induction of expression of the chemokine CCL28, which, in turn, promotes tumour tolerance and angiogenesis.
115. Facciabene, A., Motz, G. T. & Coukos, G. T-Regulatory cells: key players in tumor immune escape and angiogenesis. *Cancer Res.* **72**, 2162–2171 (2012).
116. van der Stegen, S. J. C., Hamieh, M. & Sadelain, M. The pharmacology of second-generation chimeric antigen receptors. *Nat. Rev. Drug Discov.* **14**, 499–509 (2015).
117. Whiteside, T. L. The role of regulatory T cells in cancer immunology. *Immunotargets Ther.* **4**, 159–171 (2015).
118. Halvorsen, E. C., Mahmoud, S. M. & Bennewith, K. L. Emerging roles of regulatory T cells in tumour progression and metastasis. *Cancer Metastasis Rev.* **33**, 1025–1041 (2014).
119. Valzasina, B., Piconese, S., Guiducci, C. & Colombo, M. P. Tumor-induced expansion of regulatory T cells by conversion of CD4 + CD25- lymphocytes is thymus and proliferation independent. *Cancer Res.* **66**, 4488–4495 (2006).
120. Curti, A. et al. Modulation of tryptophan catabolism by human leukemic cells results in the conversion of CD25- into CD25 + T regulatory cells. *Blood* **109**, 2871–2877 (2007).
121. Zhou, G. & Levitsky, H. I. Natural regulatory T cells and de novo-induced regulatory T cells contribute independently to tumor-specific tolerance. *J. Immunol.* **178**, 2155–2162 (2007).
122. Hindley, J. P. et al. Analysis of the T cell receptor repertoires of tumor-infiltrating conventional and regulatory T cells reveals no evidence for conversion in carcinogen-induced tumors. *Cancer Res.* **71**, 736–746 (2011).
123. Plitas, G. et al. Regulatory T cells exhibit distinct features in human breast cancer. *Immunity* **45**, 1122–1134 (2016).
124. Malchow, S. et al. Aire-dependent thymic development of tumor-associated regulatory T cells. *Science* **339**, 1219–1224 (2013).
125. Darrasse-Jeze, G. et al. Tumor emergence is sensed by self-specific CD44hi memory Tregs that create a dominant tolerogenic environment for tumors in mice. *J. Clin. Invest.* **119**, 2648–2662 (2009).
126. Angelin, A. et al. Foxp3 reprograms T cell metabolism to function in low-glucose, high-lactate environments. *Cell Metab.* **25**, 1282–1293 (2017).
This study presents the metabolic cascades that characterize T_{reg} cells.
127. Wang, H., Franco, F. & Ho, P. C. Metabolic regulation of Tregs in cancer: opportunities for immunotherapy. *Trends Cancer* **3**, 583–592 (2017).
128. Mezrich, J. D. et al. An interaction between kynurenine and the aryl hydrocarbon receptor can generate regulatory T cells. *J. Immunol.* **185**, 3190–3198 (2010).
129. Naganuma, M. et al. Cutting edge: critical role for A2A adenosine receptors in the T cell-mediated regulation of colitis. *J. Immunol.* **177**, 2765–2769 (2006).
130. Facciabene, A., Santoro, S. & Coukos, G. Know thy enemy: why are tumor-infiltrating regulatory T cells so deleterious? *Oncoimmunology* **1**, 575–577 (2012).
131. Shang, B., Liu, Y., Jiang, S. J. & Liu, Y. Prognostic value of tumor-infiltrating FoxP3 + regulatory T cells in cancers: a systematic review and meta-analysis. *Sci. Rep.* **5**, 15179 (2015).
132. Bates, G. J. et al. Quantification of regulatory T cells enables the identification of high-risk breast cancer patients and those at risk of late relapse. *J. Clin. Oncol.* **24**, 5373–5380 (2006).
133. Tan, W. et al. Tumour-infiltrating regulatory T cells stimulate mammary cancer metastasis through RANKL-RANK signalling. *Nature* **470**, 548–553 (2011).
134. Ward-Hartstonge, K. A. & Kemp, R. A. Regulatory T cell heterogeneity and the cancer immune response. *Clin. Transl Immunol.* **6**, e154 (2017).
135. Saito, T. et al. Two FOXP3(+)CD4(+) T cell subpopulations distinctly control the prognosis of colorectal cancers. *Nat. Med.* **22**, 679–684 (2016).
This study shows that the presence of $FOXP3^{low}$ T cells in colorectal cancer tissues indicates a significantly better prognosis than the presence of predominantly $FOXP3^{high}$ T_{reg} cells. This study has brought attention to the subpopulation of T_{reg} cells with low FOXP3 expression, which should not be deleted when applying immunotherapeutic regimens.
136. Delacher, M. et al. Genome-wide DNA-methylation landscape defines specialization of regulatory T cells in tissues. *Nat. Immunol.* **18**, 1160–1172 (2017).
137. Trzonkowski, P. et al. Hurdles in therapy with regulatory T cells. *Sci. Transl Med.* **7**, 304ps18 (2015).
138. Taylor, P. A., Lees, C. J. & Blazar, B. R. The infusion of ex vivo activated and expanded CD4(+)CD25(+) immune regulatory cells inhibits graft-versus-host disease lethality. *Blood* **99**, 3493–3499 (2002).
139. Cohen, J. L., Trenado, A., Vasey, D., Klatzmann, D. & Salomon, B. L. CD4(+)CD25(+) immunoregulatory T cells: new therapeutics for graft-versus-host disease. *J. Exp. Med.* **196**, 401–406 (2002).
140. Trzonkowski, P. et al. First-in-man clinical results of the treatment of patients with graft versus host disease with human ex vivo expanded CD4 + CD25 + CD127-T regulatory cells. *Clin. Immunol.* **133**, 22–26 (2009).

141. Di Ianni, M. et al. Tregs prevent GVHD and promote immune reconstitution in HLA-haploidentical transplantation. *Blood* **117**, 3921–3928 (2011).
142. Brunstein, C. G. et al. Infusion of ex vivo expanded T regulatory cells in adults transplanted with umbilical cord blood: safety profile and detection kinetics. *Blood* **117**, 1061–1070 (2011).
143. Juvet, S. C., Whatcott, A. G., Bushell, A. R. & Wood, K. J. Harnessing regulatory T cells for clinical use in transplantation: the end of the beginning. *Am. J. Transplant.* **14**, 750–763 (2014).
144. Stiller, C. et al. Cyclosporine for treatment of early type I diabetes: preliminary results. *N. Engl. J. Med.* **308**, 1226–1227 (1983).
145. Bougneres, P. et al. Factors associated with early remission of type I diabetes in children treated with cyclosporine. *N. Engl. J. Med.* **318**, 663–670 (1988).
146. Marek-Trzonkowska, N. et al. Administration of CD4+CD25highCD127- regulatory T cells preserves beta-cell function in type 1 diabetes in children. *Diabetes Care* **35**, 1817–1820 (2012).
147. Marek-Trzonkowska, N. et al. Therapy of type 1 diabetes with CD4(+)CD25(high)CD127-regulatory T cells prolongs survival of pancreatic islets - results of one year follow-up. *Clin. Immunol.* **153**, 23–30 (2014).
148. Bluestone, J. A. et al. Type 1 diabetes immunotherapy using polyclonal regulatory T cells. *Sci. Transl Med.* **7**, 315ra189 (2015).
149. Kim, H. P. & Leonard, W. J. CREB/ATF-dependent T cell receptor-induced FoxP3 gene expression: a role for DNA methylation. *J. Exp. Med.* **204**, 1543–1551 (2007).
150. Ohkura, N. et al. T cell receptor stimulation-induced epigenetic changes and Foxp3 expression are independent and complementary events required for Treg cell development. *Immunity* **37**, 785–799 (2012).
151. Ohkura, N., Kitagawa, Y. & Sakaguchi, S. Development and maintenance of regulatory T cells. *Immunity* **38**, 414–423 (2013).
152. Yang, R. et al. Hydrogen sulfide promotes Tet1- and Tet2-mediated Foxp3 demethylation to drive regulatory T Cell differentiation and maintain immune homeostasis. *Immunity* **43**, 251–263 (2015).
153. Gerriets, V. A. et al. Foxp3 and Toll-like receptor signaling balance Treg cell anabolic metabolism for suppression. *Nat. Immunol.* **17**, 1459–1466 (2016).
154. Shrestha, S. et al. Treg cells require the phosphatase PTEN to restrain TH1 and TFH cell responses. *Nat. Immunol.* **16**, 178–187 (2015).
155. Strauss, L., Czystowska, M., Szajnik, M., Mandapathil, M. & Whiteside, T. L. Differential responses of human regulatory T cells (Treg) and effector T cells to rapamycin. *PLOS ONE* **4**, e5994 (2009).
156. Fernandez, D. R. et al. Activation of mammalian target of rapamycin controls the loss of TCRzeta in lupus T cells through HRES-1/Rab4-regulated lysosomal degradation. *J. Immunol.* **182**, 2063–2073 (2009).
157. Kato, H. & Perl, A. Mechanistic target of rapamycin complex 1 expands Th17 and IL-4+ CD4-CD8- double-negative T cells and contracts regulatory T cells in systemic lupus erythematosus. *J. Immunol.* **192**, 4134–4144 (2014).
158. Warner, L. M., Adams, L. M. & Sehgal, S. N. Rapamycin prolongs survival and arrests pathophysiologic changes in murine systemic lupus erythematosus. *Arthritis Rheum.* **37**, 289–297 (1994).
159. Fernandez, D., Bonilla, E., Mirza, N., Niland, B. & Perl, A. Rapamycin reduces disease activity and normalizes T cell activation-induced calcium fluxing in patients with systemic lupus erythematosus. *Arthritis Rheum.* **54**, 2983–2988 (2006).
160. Oaks, Z. et al. Mitochondrial dysfunction in the liver and antiphospholipid antibody production precede disease onset and respond to rapamycin in lupus-prone mice. *Arthritis Rheumatol.* **68**, 2728–2739 (2016).
161. Canaud, G. et al. Inhibition of the mTORC pathway in the antiphospholipid syndrome. *N. Engl. J. Med.* **371**, 303–312 (2014).
162. Lai, Z. W. et al. Sirolimus in patients with clinically active systemic lupus erythematosus resistant to, or intolerant of, conventional medications: a single-arm, open-label, phase 1/2 trial. *Lancet* **391**, 1186–1196 (2018).
163. Tkachev, V. et al. Combined OX40L and mTOR blockade controls effector T cell activation while preserving Treg reconstitution after transplant. *Sci. Transl Med.* **9**, eaan3085 (2017).
164. Taylor, P. A. et al. Insights into the mechanism of FTY720 and compatibility with regulatory T cells for the inhibition of graft-versus-host disease (GVHD). *Blood* **110**, 3480–3488 (2007).
165. Chen, Y. B. et al. Increased Foxp3(+)Helios(+) regulatory T cells and decreased acute graft-versus-host disease after allogeneic bone marrow transplantation in patients receiving sirolimus and RGI-2001, an activator of invariant natural killer T cells. *Biol. Blood Marrow Transplant.* **23**, 625–634 (2017).
166. Link, W. et al. Chemical interrogation of FOXO3a nuclear translocation identifies potent and selective inhibitors of phosphoinositide 3-kinases. *J. Biol. Chem.* **284**, 28392–28400 (2009).
167. Liu, G. et al. The receptor S1P1 overrides regulatory T cell-mediated immune suppression through Akt-mTOR. *Nat. Immunol.* **10**, 769–777 (2009).
168. Liu, G., Yang, K., Burns, S., Shrestha, S. & Chi, H. The S1P1-mTOR axis directs the reciprocal differentiation of TH1 and Treg cells. *Nat. Immunol.* **11**, 1047–1056 (2010).
169. Lai, Z. W. et al. N-Acetylcysteine reduces disease activity by blocking mammalian target of rapamycin in T cells from systemic lupus erythematosus patients: a randomized, double-blind, placebo-controlled trial. *Arthritis Rheum.* **64**, 2937–2946 (2012).
170. Yin, Y. et al. Normalization of CD4+ T cell metabolism reverses lupus. *Sci. Transl Med.* **7**, 274ra18 (2015). **This is one of the first studies to suggest that autoimmunity can be controlled through metabolism.**
171. Hancock, W. W., Akimova, T., Beier, U. H., Liu, Y. & Wang, L. HDAC inhibitor therapy in autoimmunity and transplantation. *Ann. Rheum. Dis.* **71** (Suppl. 2), i46–i54 (2012).
172. Regna, N. L. et al. Specific HDAC6 inhibition by ACY-738 reduces SLE pathogenesis in NZB/W mice. *Clin. Immunol.* **162**, 58–73 (2016).
173. Zhang, Y. et al. Mice lacking histone deacetylase 6 have hyperacetylated tubulin but are viable and develop normally. *Mol. Cell. Biol.* **28**, 1688–1701 (2008).
174. de Zoeten, E. F. et al. Histone deacetylase 6 and heat shock protein 90 control the functions of Foxp3(+) T-regulatory cells. *Mol. Cell. Biol.* **31**, 2066–2078 (2011).
175. Fisson, S. et al. Continuous activation of autoreactive CD4+ CD25+ regulatory T cells in the steady state. *J. Exp. Med.* **198**, 737–746 (2003).
176. Dawson, N. A. J. & Levings, M. K. Antigen-specific regulatory T cells: are police CARs the answer? *Transl Res.* **187**, 53–58 (2017).
177. Sadelain, M. CD19 CAR T cells. *Cell* **171**, 1471 (2017).
178. Blat, D., Zigmond, E., Alteber, Z., Waks, T. & Eshhar, Z. Suppression of murine colitis and its associated cancer by carcinoembryonic antigen-specific regulatory T cells. *Mol. Ther.* **22**, 1018–1028 (2014).
179. MacDonald, K. G. et al. Alloantigen-specific regulatory T cells generated with a chimeric antigen receptor. *J. Clin. Invest.* **126**, 1413–1424 (2016).
180. Boardman, D. A. et al. Expression of a chimeric antigen receptor specific for donor HLA class i enhances the potency of human regulatory T cells in preventing human skin transplant rejection. *Am. J. Transplant.* **17**, 931–943 (2017).
181. Adair, P. R., Kim, Y. C., Zhang, A.-H., Yoon, J. & Scott, D. W. Human Tregs made antigen specific by gene modification: the power to treat autoimmunity and antidrug antibodies with precision. *Front. Immunol.* **8**, 1117 (2017).
182. Rosenberg, S. A. et al. A progress report on the treatment of 157 patients with advanced cancer using lymphokine-activated killer cells and interleukin-2 or high-dose interleukin-2 alone. *N. Engl. J. Med.* **316**, 889–897 (1987).
183. Yu, A. et al. Selective IL-2 responsiveness of regulatory T cells through multiple intrinsic mechanisms support the use of low-dose IL-2 therapy in type-1 diabetes. *Diabetes* **64**, 2172–2183 (2015).
184. Laurence, A. et al. Interleukin-2 signaling via STAT5 constrains T helper 17 cell generation. *Immunity* **26**, 371–381 (2007).
185. Ballesteros-Tato, A. et al. Interleukin-2 inhibits germinal center formation by limiting T follicular helper cell differentiation. *Immunity* **36**, 847–856 (2012).
186. Gutierrez-Ramos, J. C., Andreu, J. L., Revilla, Y., Vinuela, E. & Martinez, C. Recovery from autoimmunity of MRL/lpr mice after infection with an interleukin-2/vaccinia recombinant virus. *Nature* **346**, 271–274 (1990).
187. Mizui, M. et al. IL-2 protects lupus-prone mice from multiple end-organ damage by limiting CD4-CD8- IL-17-producing T cells. *J. Immunol.* **193**, 2168–2177 (2014).
188. Sadlack, B. et al. Ulcerative colitis-like disease in mice with a disrupted interleukin-2 gene. *Cell* **75**, 253–261 (1993).
189. Suzuki, H. et al. Deregulated T cell activation and autoimmunity in mice lacking interleukin-2 receptor beta. *Science* **268**, 1472–1476 (1995).
190. Willerford, D. M. et al. Interleukin-2 receptor alpha chain regulates the size and content of the peripheral lymphoid compartment. *Immunity* **3**, 521–530 (1995).
191. Malek, T. R., Yu, A., Vincek, V., Scibelli, P. & Kong, L. CD4 regulatory T cells prevent lethal autoimmunity in IL-2Rβ-deficient mice: implications for the nonredundant function of IL-2. *Immunity* **17**, 167–178 (2002).
192. Lemoine, F. M. et al. Massive expansion of regulatory T cells following interleukin 2 treatment during a phase I-II dendritic cell-based immunotherapy of metastatic renal cancer. *Int. J. Oncol.* **35**, 569–581 (2009).
193. Rosenberg, S. A. IL-2: the first effective immunotherapy for human cancer. *J. Immunol.* **192**, 5451–5458 (2014).
194. Saadoun, D. et al. Restoration of peripheral immune homeostasis after rituximab in mixed cryoglobulinemia vasculitis. *Blood* **111**, 5334–5341 (2008).
195. Landau, D.-A. et al. Correlation of clinical and virologic responses to antiviral treatment and regulatory T cell evolution in patients with hepatitis C virus-induced mixed cryoglobulinemia vasculitis. *Arthritis Rheum.* **58**, 2897–2907 (2008).
196. Koreth, J. et al. Interleukin-2 and regulatory T cells in graft-versus-host disease. *N. Engl. J. Med.* **365**, 2055–2066 (2011). **This is the first study to show that low-dose IL-2 has clinical benefit in patients with GVHD linked to the expansion of T_{reg} cells.**
197. Kennedy-Nasser, A. A. et al. Ultra low-dose IL-2 for GVHD prophylaxis after allogeneic hematopoietic stem cell transplantation mediates expansion of regulatory T cells without diminishing antiviral and antileukemic activity. *Clin. Cancer Res.* **20**, 2215–2225 (2014).
198. Castela, E. et al. Effects of low-dose recombinant interleukin 2 to promote T-regulatory cells in alopecia areata. *JAMA Dermatol.* **150**, 748–751 (2014).
199. Humrich, J. Y. et al. A3.11 Induction of remission by low-dose IL-2-therapy in one SLE patient with increased disease activity refractory to standard therapies: a case report. *Ann. Rheum. Dis.* **73**, A46 (2014).
200. von Spee-Mayer, C. et al. Low-dose interleukin-2 selectively corrects regulatory T cell defects in patients with systemic lupus erythematosus. *Ann. Rheum. Dis.* **75**, 1407–1415 (2015).
201. He, J. et al. Low-dose interleukin-2 treatment selectively modulates CD4(+) T cell subsets in patients with systemic lupus erythematosus. *Nat. Med.* **22**, 991–993 (2016).
202. Moulton, V. R. et al. Pathogenesis of human systemic lupus erythematosus: a cellular perspective. *Trends Mol. Med.* **23**, 615–635 (2017).
203. Hartemann, A. et al. Low-dose interleukin 2 in patients with type 1 diabetes: a phase 1/2 randomised, double-blind, placebo-controlled trial. *Lancet Diabetes Endocrinol.* **1**, 295–305 (2013).
204. Mitra, S. et al. Interleukin-2 activity can be fine tuned with engineered receptor signaling clamps. *Immunity* **42**, 826–838 (2015).
205. Goodson, R. J. & Katre, N. V. Site-directed pegylation of recombinant interleukin-2 at its glycosylation site. *Biotechnology* **8**, 343–346 (1990).
206. Bell, C. J. M. et al. Sustained in vivo signaling by long-lived IL-2 induces prolonged increases of regulatory T cells. *J. Autoimmun.* **56**, 66–80 (2015).
207. Yeh, P. et al. Design of yeast-secreted albumin derivatives for human therapy: biological and antiviral properties of a serum albumin-CD4 genetic conjugate. *Proc. Natl Acad. Sci. USA* **89**, 1904–1908 (1992).
208. Yao, Z., Dai, W., Perry, J., Brechbiel, M. W. & Sung, C. Effect of albumin fusion on the biodistribution of interleukin-2. *Cancer Immunol. Immunother.* **53**, 404–410 (2004).
209. Boyman, O., Kovar, M., Rubinstein, M. P., Surh, C. D. & Sprent, J. Selective stimulation of T cell subsets with antibody-cytokine immune complexes. *Science* **311**, 1924–1927 (2006).

210. Arenas-Ramirez, N. et al. Improved cancer immunotherapy by a CD25-mimobody conferring selectivity to human interleukin-2. *Sci. Transl Med.* **8**, 367ra166 (2016).
211. Trotta, E. et al. A human anti-IL-2 antibody that potentiates regulatory T cells by a structure-based mechanism. *Nat. Med.* **24**, 1005–1014 (2018). **This study shows that a fully human anti-IL-2 antibody stabilizes IL-2 in a conformation that results in the preferential STAT5 phosphorylation of T_{reg} cells in vitro and their selective expansion in vivo to mitigate experimental diabetes and multiple sclerosis.**
212. Mahnke, K. et al. The dendritic cell receptor for endocytosis, DEC-205, can recycle and enhance antigen presentation via major histocompatibility complex class II-positive lysosomal compartments. *J. Cell Biol.* **151**, 673–684 (2000).
213. Polansky, J. K. et al. DNA methylation controls Foxp3 gene expression. *Eur. J. Immunol.* **38**, 1654–1663 (2008).
214. Bruder, D. et al. On the edge of autoimmunity: T cell stimulation by steady-state dendritic cells prevents autoimmune diabetes. *Diabetes* **54**, 3395–3401 (2005).
215. Hawiger, D., Masilamani, R. F., Bettelli, E., Kuchroo, V. K. & Nussenzweig, M. C. Immunological unresponsiveness characterized by increased expression of CD5 on peripheral T cells induced by dendritic cells in vivo. *Immunity* **20**, 695–705 (2004).
216. Raker, V. K., Domogalla, M. P. & Steinbrink, K. Tolerogenic dendritic cells for regulatory T cell induction in man. *Front. Immunol.* **6**, 569 (2015).
217. Benham, H. et al. Citrullinated peptide dendritic cell immunotherapy in HLA risk genotype-positive rheumatoid arthritis patients. *Sci. Transl Med.* **7**, 290ra87 (2015).
218. Johnson, K. P. et al. Copolymer 1 reduces relapse rate and improves disability in relapsing-remitting multiple sclerosis: results of a phase III multicenter, double-blind, placebo-controlled trial. 1995. *Neurology* **57**, S16–S24 (2001).
219. Jee, Y. et al. CD4(+)CD25(+) regulatory T cells contribute to the therapeutic effects of glatiramer acetate in experimental autoimmune encephalomyelitis. *Clin. Immunol.* **125**, 34–42 (2007).
220. Sharabi, A., Zinger, H., Zborowsky, M., Sthoeger, Z. M. & Mozes, E. A peptide based on the complementarity-determining region 1 of an autoantibody ameliorates lupus by up-regulating CD4 + CD25 + cells and TGF-beta. *Proc. Natl Acad. Sci. USA* **103**, 8810–8815 (2006).
221. Sthoeger, Z. M. et al. Treatment of lupus patients with a tolerogenic peptide, hCDR1 (Edratide): immunomodulation of gene expression. *J. Autoimmun* **33**, 77–82 (2009).
222. Sharabi, A., Lapter, S. & Mozes, E. Bcl-xL is required for the development of functional regulatory CD4 cells in lupus-afflicted mice following treatment with a tolerogenic peptide. *J. Autoimmun.* **34**, 87–95 (2010).
223. Sharabi, A. & Mozes, E. The suppression of murine lupus by a tolerogenic peptide involves foxp3-expressing CD8 cells that are required for the optimal induction and function of foxp3-expressing CD4 cells. *J. Immunol.* **181**, 3243–3251 (2008).
224. Urowitz, M. B., Isenberg, D. A. & Wallace, D. J. Safety and efficacy of hCDR1 (Edratide) in patients with active systemic lupus erythematosus: results of phase II study. *Lupus Sci. Med.* **2**, e000104 (2015).
225. Kang, H. K., Michaels, M. A., Berner, B. R. & Datta, S. K. Very low-dose tolerance with nucleosomal peptides controls lupus and induces potent regulatory T cell subsets. *J. Immunol.* **174**, 3247–3255 (2005).
226. Hahn, B. H., Singh, R. P., La Cava, A. & Ebling, F. M. Tolerogenic treatment of lupus mice with consensus peptide induces Foxp3-expressing, apoptosis-resistant, TGFbeta-secreting CD8 + T cell suppressors. *J. Immunol.* **175**, 7728–7737 (2005).
227. Leavenworth, J. W., Wang, X., Wenander, C. S., Spee, P. & Cantor, H. Mobilization of natural killer cells inhibits development of collagen-induced arthritis. *Proc. Natl Acad. Sci. USA* **108**, 14584–14589 (2011).
228. Gertel, S., Serre, G., Shoenfeld, Y. & Amital, H. Immune tolerance induction with multiepitope peptide derived from citrullinated autoantigens attenuates arthritis manifestations in adjuvant arthritis rats. *J. Immunol.* **194**, 5674–5680 (2015).
229. Deshmukh, U. S., Bagavant, H., Lewis, J., Gaskin, F. & Fu, S. M. Epitope spreading within lupus-associated ribonucleoprotein antigens. *Clin. Immunol.* **117**, 112–120 (2005).
230. Herrath, J. et al. The inflammatory milieu in the rheumatic joint reduces regulatory T cell function. *Eur. J. Immunol.* **41**, 2279–2290 (2011).
231. Vargas-Rojas, M. I., Crispin, J. C., Richaud-Patin, Y. & Alcocer-Varela, J. Quantitative and qualitative normal regulatory T cells are not capable of inducing suppression in SLE patients due to T cell resistance. *Lupus* **17**, 289–294 (2008).
232. Ghiringhelli, F. et al. Metronomic cyclophosphamide regimen selectively depletes CD4 + CD25 + regulatory T cells and restores T and NK effector functions in end stage cancer patients. *Cancer Immunol. Immunother.* **56**, 641–648 (2007).
233. Lutsiak, M. E. et al. Inhibition of CD4(+)25 + T regulatory cell function implicated in enhanced immune response by low-dose cyclophosphamide. *Blood* **105**, 2862–2868 (2005).
234. Ge, Y. et al. Metronomic cyclophosphamide treatment in metastasized breast cancer patients: immunological effects and clinical outcome. *Cancer Immunol. Immunother.* **61**, 353–362 (2012).
235. Adotevi, O. et al. A decrease of regulatory T cells correlates with overall survival after sunitinib-based antiangiogenic therapy in metastatic renal cancer patients. *J. Immunother.* **33**, 991–998 (2010).
236. Desar, I. M. et al. Sorafenib reduces the percentage of tumour infiltrating regulatory T cells in renal cell carcinoma patients. *Int. J. Cancer* **129**, 507–512 (2011).
237. Coussens, L. M. & Werb, Z. Inflammation and cancer. *Nature* **420**, 860–867 (2002).
238. Curiel, T. J. et al. Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. *Nat. Med.* **10**, 942–949 (2004).
239. Chang, D. K. et al. Anti-CCR4 monoclonal antibody enhances antitumor immunity by modulating tumor-infiltrating Tregs in an ovarian cancer xenograft humanized mouse model. *Oncoimmunology* **5**, e1090075 (2016).
240. Ogura, M. et al. Multicenter phase II study of mogamulizumab (KW-0761), a defucosylated anti-cc chemokine receptor 4 antibody, in patients with relapsed peripheral T cell lymphoma and cutaneous T cell lymphoma. *J. Clin. Oncol.* **32**, 1157–1163 (2014).
241. Ishida, T. et al. Mogamulizumab for relapsed adult T cell leukemia-lymphoma: updated follow-up analysis of phase I and II studies. *Cancer Sci.* **108**, 2022–2029 (2017).
242. Ifuku, H. et al. Fatal reactivation of hepatitis B virus infection in a patient with adult T cell leukemia-lymphoma receiving the anti-CC chemokine receptor 4 antibody mogamulizumab. *Hepatol. Res.* **45**, 1363–1367 (2015).
243. Kurose, K. et al. Phase Ia study of FoxP3 + CD4 Treg depletion by infusion of a humanized anti-CCR4 antibody, KW-0761, in cancer patients. *Clin. Cancer Res.* **21**, 4327–4336 (2015).
244. De Simone, M. et al. Transcriptional landscape of human tissue lymphocytes unveils uniqueness of tumor-infiltrating T regulatory cells. *Immunity* **45**, 1135–1147 (2016).
245. Attia, P. et al. Selective elimination of human regulatory T lymphocytes in vitro with the recombinant immunotoxin LMB-2. *J. Immunother.* **29**, 208–214 (2006).
246. Rech, A. J. et al. CD25 blockade depletes and selectively reprograms regulatory T cells in concert with immunotherapy in cancer patients. *Sci. Transl Med.* **4**, 134ra62 (2012).
247. Kreitman, R. J. et al. Phase I trial of recombinant immunotoxin anti-Tac(Fv)-PE38 (LMB-2) in patients with hematologic malignancies. *J. Clin. Oncol.* **18**, 1622–1636 (2000).
248. Kreitman, R. J. et al. Complete remissions of adult T cell leukemia with anti-CD25 recombinant immunotoxin LMB-2 and chemotherapy to block immunogenicity. *Clin. Cancer Res.* **22**, 310–318 (2016).
249. Powell, D. J. Jr. et al. Administration of a CD25-directed immunotoxin, LMB-2, to patients with metastatic melanoma induces a selective partial reduction in regulatory T cells in vivo. *J. Immunol.* **179**, 4919–4928 (2007).
250. Dannull, J. et al. Enhancement of vaccine-mediated antitumor immunity in cancer patients after depletion of regulatory T cells. *J. Clin. Invest.* **115**, 3623–3633 (2005).
251. Attia, P., Maker, A. V., Haworth, L. R., Rogers-Freezer, L. & Rosenberg, S. A. Inability of a fusion protein of IL-2 and diphtheria toxin (Denileukin Diftitox, DAB389IL-2, ONTAK) to eliminate regulatory T lymphocytes in patients with melanoma. *J. Immunother.* **28**, 582–592 (2005).
252. Maury, S. et al. Lymphodepletion followed by infusion of suicide gene-transduced donor lymphocytes to safely enhance their antitumor effect: a phase I/II study. *Leukemia* **28**, 2406–2410 (2014).
253. Attia, P. et al. Autoimmunity correlates with tumor regression in patients with metastatic melanoma treated with anti-cytotoxic T-lymphocyte antigen-4. *J. Clin. Oncol.* **23**, 6043–6053 (2005).
254. Hodi, F. S. et al. Improved survival with ipilimumab in patients with metastatic melanoma. *N. Engl. J. Med.* **363**, 711–723 (2010).
255. Robert, C. et al. Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. *N. Engl. J. Med.* **364**, 2517–2526 (2011).
256. Simpson, T. R. et al. Fc-dependent depletion of tumor-infiltrating regulatory T cells co-defines the efficacy of anti-CTLA-4 therapy against melanoma. *J. Exp. Med.* **210**, 1695–1710 (2013).
257. Peggs, K. S., Quezada, S. A., Chambers, C. A., Korman, A. J. & Allison, J. P. Blockade of CTLA-4 on both effector and regulatory T cell compartments contributes to the antitumor activity of anti-CTLA-4 antibodies. *J. Exp. Med.* **206**, 1717–1725 (2009).
258. Paterson, A. M. et al. Deletion of CTLA-4 on regulatory T cells during adulthood leads to resistance to autoimmunity. *J. Exp. Med.* **212**, 1603–1621 (2015).
259. Phan, G. Q. et al. Cancer regression and autoimmunity induced by cytotoxic T lymphocyte-associated antigen 4 blockade in patients with metastatic melanoma. *Proc. Natl Acad. Sci. USA* **100**, 8372–8377 (2003).
260. Hodi, F. S. et al. Combined nivolumab and ipilimumab versus ipilimumab alone in patients with advanced melanoma: 2-year overall survival outcomes in a multicentre, randomised, controlled, phase 2 trial. *Lancet Oncol.* **17**, 1558–1568 (2016).
261. Zhao, H., Liao, X. & Kang, Y. Tregs: where we are and what comes next? *Front. Immunol.* **8**, 1578 (2017).
262. Sasidharan Nair, V. & Elkord, E. Immune checkpoint inhibitors in cancer therapy: a focus on T-regulatory cells. *Immunol. Cell Biol.* **96**, 21–33 (2018).
263. Sabatos, C. A. et al. Interaction of Tim-3 and Tim-3 ligand regulates T helper type 1 responses and induction of peripheral tolerance. *Nat. Immunol.* **4**, 1102–1110 (2003).
264. Coe, D. et al. Depletion of regulatory T cells by anti-GITR mAb as a novel mechanism for cancer immunotherapy. *Cancer Immunol. Immunother.* **59**, 1367–1377 (2010).
265. Cohen, A. D. et al. Agonist anti-GITR monoclonal antibody induces melanoma tumor immunity in mice by altering regulatory T cell stability and intra-tumor accumulation. *PLOS ONE* **5**, e10436 (2010).
266. Schaer, D. A., Murphy, J. T. & Wolchok, J. D. Modulation of GITR for cancer immunotherapy. *Curr. Opin. Immunol.* **24**, 217–224 (2012).
267. Schaer, D. A. et al. GITR pathway activation abrogates tumor immune suppression through loss of regulatory T cell lineage stability. *Cancer Immunol. Res.* **1**, 320–331 (2013).
268. Ko, K. et al. Treatment of advanced tumors with agonistic anti-GITR mAb and its effects on tumor-infiltrating Foxp3 + CD25 + CD4 + regulatory T cells. *J. Exp. Med.* **202**, 885–891 (2005).
269. Lu, L. et al. Combined PD-1 blockade and GITR triggering induce a potent antitumor immunity in murine cancer models and synergizes with chemotherapeutic drugs. *J. Transl Med.* **12**, 36 (2014).
270. Shimizu, J., Yamazaki, S., Takahashi, T., Ishida, Y. & Sakaguchi, S. Stimulation of CD25(+)CD4(+) regulatory T cells through GITR breaks immunological self-tolerance. *Nat. Immunol.* **3**, 135–142 (2002).
271. Murphy, J. T. et al. Anaphylaxis caused by repetitive doses of a GITR agonist monoclonal antibody in mice. *Blood* **123**, 2172–2180 (2014).
272. Nakagawa, H. et al. Instability of Helios-deficient Tregs is associated with conversion to a T-effector phenotype and enhanced antitumor immunity. *Proc. Natl Acad. Sci. USA* **113**, 6248–6253 (2016).
273. Ephrem, A. et al. Modulation of Treg cells/T effector function by GITR signaling is context-dependent. *Eur. J. Immunol.* **43**, 2421–2429 (2013).
274. Liao, G. et al. GITR engagement preferentially enhances proliferation of functionally competent CD4 + CD25 + FoxP3 + regulatory T cells. *Int. Immunol.* **22**, 259–270 (2010).

275. Curti, B. D. et al. OX40 is a potent immune-stimulating target in late-stage cancer patients. *Cancer Res.* **73**, 7189–7198 (2013).
276. Sturgill, E. R. & Redmond, W. L. TNFR agonists: a review of current biologics targeting OX40, 4-1BB, CD27, and GITR. *Am. J. Hematol. Oncol.* **13**, 4–15 (2017).
277. Lesokhin, A. M., Callahan, M. K., Postow, M. A. & Wolchok, J. D. On being less tolerant: enhanced cancer immunosurveillance enabled by targeting checkpoints and agonists of T cell activation. *Sci. Transl Med.* **7**, 280sr1 (2015).
278. Knee, D. A., Hewes, B. & Brogdon, J. L. Rationale for anti-GITR cancer immunotherapy. *Eur. J. Cancer* **67**, 1–10 (2016).
279. Torrey, H. et al. Targeting TNFR2 with antagonistic antibodies inhibits proliferation of ovarian cancer cells and tumor-associated Tregs. *Sci. Signal.* **10**, eaaf8608 (2017).
280. Faustman, D. & Davis, M. TNF receptor 2 pathway: drug target for autoimmune diseases. *Nat. Rev. Drug Discov.* **9**, 482–493 (2010).
281. He, X. et al. A TNFR2-agonist facilitates high purity expansion of human low purity Treg cells. *PLOS ONE* **11**, e0156311 (2016).
282. Okubo, Y., Mera, T., Wang, L. & Faustman, D. L. Homogeneous expansion of human T-regulatory cells via tumor necrosis factor receptor 2. *Sci. Rep.* **3**, 3153 (2013).
283. Chopra, M. et al. Exogenous TNFR2 activation protects from acute GvHD via host T reg cell expansion. *J. Exp. Med.* **213**, 1881–1900 (2016).
284. Fallarino, F. et al. Modulation of tryptophan catabolism by regulatory T cells. *Nat. Immunol.* **4**, 1206–1212 (2003).
285. Fallarino, F. et al. The combined effects of tryptophan starvation and tryptophan catabolites down-regulate T cell receptor zeta-chain and induce a regulatory phenotype in naive T cells. *J. Immunol.* **176**, 6752–6761 (2006).
286. Prendergast, G. C., Malachowski, W. P., DuHadaway, J. B. & Muller, A. J. Discovery of IDO1 inhibitors: from bench to bedside. *Cancer Res.* **77**, 6795–6811 (2017).
287. Vacchelli, E. et al. Trial watch: IDO inhibitors in cancer therapy. *Oncoimmunology* **3**, e957994 (2014).
288. Sharma, M. D. et al. Indoleamine 2,3-dioxygenase controls conversion of Foxp3 + Tregs to TH17-like cells in tumor-draining lymph nodes. *Blood* **113**, 6102–6111 (2009).
289. Soares, K. C. et al. TGF-β blockade depletes T regulatory cells from metastatic pancreatic tumors in a vaccine dependent manner. *Oncotarget* **6**, 43005–43015 (2015).
290. Turnis, M. E. et al. Interleukin-35 limits anti-tumor immunity. *Immunity* **44**, 316–329 (2016).
291. Yu, P. et al. Intratumor depletion of CD4 + cells unmasks tumor immunogenicity leading to the rejection of late-stage tumors. *J. Exp. Med.* **201**, 779–791 (2005).
292. Lu, J. et al. Increased expression of neuropilin 1 in melanoma progression and its prognostic significance in patients with melanoma. *Mol. Med. Rep.* **12**, 2668–2676 (2015).
293. Overacre-Delgoffe, A. E. et al. Interferon-γ drives Treg fragility to promote anti-tumor immunity. *Cell* **169**, 1130–1141 (2017).
This paper shows that NRP1 is required to maintain intratumoural T_{reg} stability and function and that NRP1-deficient T_{reg} cells produce IFNγ, which promotes T_{reg} cell fragility and boosts antitumour activity.
294. Grinberg-Bleyer, Y. et al. IL-2 reverses established type 1 diabetes in NOD mice by a local effect on pancreatic regulatory T cells. *J. Exp. Med.* **207**, 1871–1878 (2010).
295. Clever, D. et al. Oxygen sensing by T cells establishes an immunologically tolerant metastatic niche. *Cell* **166**, 1117–1131 (2016).
This is a novel study that explains the abundance of lung metastases by various tumours. Low oxygen pressure promotes the development of T_{reg} cells, which promote tumour growth.
296. Chen, P. L. et al. Analysis of immune signatures in longitudinal tumor samples yields insight into biomarkers of response and mechanisms of resistance to immune checkpoint blockade. *Cancer Discov.* **6**, 827–837 (2016).
297. Linsley, P. S., Chaussabel, D. & Speake, C. The relationship of immune cell signatures to patient survival varies within and between tumor types. *PLOS ONE* **10**, e0138726 (2015).
298. Kitagawa, Y. et al. Guidance of regulatory T cell development by Satb1-dependent super-enhancer establishment. *Nat. Immunol.* **18**, 173–183 (2017).
This study introduces the concept that super-enhancers can be modulated to dictate cell lineage differentiation.
299. Zhou, X., Tang, J., Cao, H., Fan, H. & Li, B. Tissue resident regulatory T cells: novel therapeutic targets for human disease. *Cell. Mol. Immunol.* **12**, 543–552 (2015).
300. Hovhannisyan, Z., Treatman, J., Littman, D. R. & Mayer, L. Characterization of interleukin-17-producing regulatory T cells in inflamed intestinal mucosa from patients with inflammatory bowel diseases. *Gastroenterology* **140**, 957–965 (2011).
301. Cipolletta, D. et al. PPAR-gamma is a major driver of the accumulation and phenotype of adipose tissue Treg cells. *Nature* **486**, 549–553 (2012).
This study shows that T_{reg} cells that infiltrate tissues and become tissue-resident T cells express transcription factors that are master regulators of the specific tissue. PPARγ is a master regulator of adipose tissue.
302. Feuerer, M. et al. Lean, but not obese, fat is enriched for a unique population of regulatory T cells that affect metabolic parameters. *Nat. Med.* **15**, 930–939 (2009).
303. Vasanthakumar, A. et al. The transcriptional regulators IRF4, BATF and IL-33 orchestrate development and maintenance of adipose tissue-resident regulatory T cells. *Nat. Immunol.* **16**, 276–285 (2015).
304. Atarashi, K. et al. Treg induction by a rationally selected mixture of Clostridia strains from the human microbiota. *Nature* **500**, 232–236 (2013).
305. Malhotra, N. et al. RORα-expressing T regulatory cells restrain allergic skin inflammation. *Sci. Immunol.* **3**, eaao6923 (2018).
This study shows that expression of retinoid-related orphan receptor-α (RORα) in skin-resident T_{reg} cells is important for restraining allergic skin inflammation.
306. Pesenacker, A. M., Broady, R. & Levings, M. K. Control of tissue-localized immune responses by human regulatory T cells. *Eur. J. Immunol.* **45**, 333–343 (2015).
307. Akimova, T. et al. Human lung tumor FOXP3 + Tregs upregulate four "Treg-locking" transcription factors. *JCI Insight* **2**, 94075 (2017).
308. Burzyn, D. et al. A special population of regulatory T cells potentiates muscle repair. *Cell* **155**, 1282–1295 (2013).
This study introduces the concept that T_{reg} cells can participate in the repair of injured tissue through the factors they produce. T_{reg} cells repair injured muscle through the production of amphiregulin.
309. Arpaia, N. et al. A distinct function of regulatory T cells in tissue protection. *Cell* **162**, 1078–1089 (2015).
310. Zaiss, D. M. et al. Amphiregulin enhances regulatory T cell-suppressive function via the epidermal growth factor receptor. *Immunity* **38**, 275–284 (2013).
311. Sanchez Rodriguez, R. et al. Memory regulatory T cells reside in human skin. *J. Clin. Invest.* **124**, 1027–1036 (2014).
312. Nosbaum, A. et al. Cutting edge: regulatory T cells facilitate cutaneous wound healing. *J. Immunol.* **196**, 2010–2014 (2016).
313. Ali, N. et al. Regulatory T cells in skin facilitate epithelial stem cell differentiation. *Cell* **169**, 1119–1129 (2017).
This study provides another example of T_{reg} cells being involved in tissue repair and/or regeneration. Skin-resident T_{reg} cells were found to express preferentially high levels of the NOTCH ligand family member jagged 1, which promoted the function of skin stem cells.
314. Zacchigna, S. et al. Paracrine effect of regulatory T cells promotes cardiomyocyte proliferation during pregnancy and after myocardial infarction. *Nat. Commun.* **9**, 2432 (2018).
315. Bieber, A. J., Kerr, S. & Rodriguez, M. Efficient central nervous system remyelination requires T cells. *Ann. Neurol.* **53**, 680–684 (2003).
316. Dombrowski, Y. et al. Regulatory T cells promote myelin regeneration in the central nervous system. *Nat. Neurosci.* **20**, 674–680 (2017).

Acknowledgements
The authors' work was supported by US National Institutes of Health (NIH) grants AI42269, R37AI49954, AI068787, AI085567 and AR064350 (G.C.T.) and R21-CA195334, R01-AI131648 and the Sylvester Comprehensive Cancer Center at the University of Miami (T.R.M.).

Competing interests
G.C.T. is a consultant for Johnson & Johnson and a science advisory board member for Abpro 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.

Supplementary information
Supplementary information is available for this paper at https://doi.org/10.1038/nrd.2108.48.