Regulatory T cells, tumour immunity and immunotherapy

Weiping Zou

Abstract | Tumours express a range of antigens, including self-antigens. Regulatory T cells are crucial for maintaining T-cell tolerance to self-antigens. Regulatory T cells are thought to dampen T-cell immunity to tumour-associated antigens and to be the main obstacle tempering successful immunotherapy and active vaccination. In this Review, I consider the nature and characteristics of regulatory T cells in the tumour microenvironment and their potential multiple suppressive mechanisms. Strategies for therapeutic targeting of regulatory T cells and the effect of regulatory T cells on current immunotherapeutic and vaccine regimens are discussed.

Regulatory T cells
A T-cell population that can functionally suppress an immune response by influencing the activity of another cell type. Several phenotypically distinct regulatory T-cell populations exist. The classic regulatory T cells are $CD4^+CD25^+$ $FOXP3^+$ T cells (T_{Reg} cells).

Tolerance is the 'holy grail' in the field of immunology and it is generally divided into two broad categories: central tolerance and peripheral tolerance. Central tolerance concerns immature lymphocytes as they differentiate in the primary lymphoid organs. The main mechanisms of inducing central tolerance are clonal deletion and inactivation of self-reactive lymphocytes. Peripheral tolerance concerns mature lymphocytes once they have exited primary lymphoid organs and are circulating in the periphery. It is suggested that regulatory T cells are responsible for inducing and maintaining peripheral tolerance. Regulatory T cells can be defined as a T-cell population that functionally suppresses an immune response by influencing the activity of another cell type[1].

Regulatory T cells were initially described by Gershon *et al.* in the early 1970s and were called suppressive T cells[2,3]. Five years later, Sehon and colleagues suggested that these regulatory T cells negatively regulated tumour immunity and contributed to tumour growth in mice[4]. After another five years, North and colleagues published a series of experiments providing evidence that $CD4^+CD25^+$ T cells from tumour-bearing mice inhibited tumour rejection, indicating the existence of tumour-suppressor T cells[5–7]. These pioneering studies established the field of regulatory T cells in tumour immunology. Unfortunately, despite the importance of these studies there was extensive skepticism in the immunological field about the existence of these cells, and suppressive T cells left the centre stage of immunology for decades. However, in 1995, Sakaguchi and colleagues showed that the interleukin-2 (IL-2) receptor α-chain, CD25, could serve as a phenotypic marker for $CD4^+$ suppressor T cells or $CD4^+$ regulatory T cells[8]. More recent studies have shown that the transcription factor forkhead box P3 (FOXP3) is not only a key intracellular marker but is also a crucial developmental and functional factor for $CD4^+CD25^+$ regulatory T cells[9–11]. Thereafter, the notion of regulatory T cells was revived and the field of regulatory T cells has evolved rapidly. Indeed, over the past few years, several phenotypically distinct regulatory T-cell populations have been suggested[8,12–19] (TABLE 1). The classic regulatory T cells are thymus-derived $CD4^+CD25^+FOXP3^+$ T cells (T_{Reg} cells).

Recent evidence has demonstrated that regulatory-T-cell-mediated immunosuppression is one of the crucial tumour immune-evasion mechanisms and the main obstacle of successful tumour immunotherapy[20–23]. By focusing on the tumour microenvironment — the active battlefront between tumours and the host immune system — I summarize and review recent data demonstrating that tumours actively prevent the induction of tumour-associated antigen (TAA)-specific immunity through induction of regulatory T-cell trafficking, differentiation and expansion. Mechanisms to revert regulatory T-cell trafficking, differentiation, function and signalling are discussed as novel therapeutic strategies for cancer.

Regulatory T cells and mouse tumours

T_{Reg} cells constitute 5–10% of peripheral $CD4^+$ T cells in normal mice and humans. The role of T_{Reg} cells in mouse tumour immunopathogenesis has largely been defined using reagents that target T_{Reg} cells *in vivo* in tumour-bearing mice.

Department of Surgery, University of Michigan, 1500 East Medical Center Drive, Ann Arbor, Michigan 48109-0346, USA.
e-mail: wzou@umich.edu
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Table 1 | **Regulatory T-cell populations**

Cell subset	Suggested origin	Original experimental setting	Suggested suppressive mechanism	References
CD4⁺ T-cell subset				
$CD4^+CD25^+FOXP3^+$ (T_{Reg}) cells*	Thymus	Mouse autoimmune diseases	*In vitro*: cell–cell contact. *In vivo*: multiple modes of action	8
$CD4^+IL\text{-}10^+FOXP3^-$ (T_R1) cells*	Periphery	*In vitro* mouse cell-culture system with IL-10	IL-10	12
$CD4^+TGF\beta^+$ (T_H3) cells*	Periphery	*In-vivo*-induced oral tolerance in mice	TGFβ	13
CD8⁺ T-cell subset				
$CD8^+CD25^+$ T cells	Thymus	Human thymus	TGFβ and CTLA4	14
$CD8^+CD28^-$ T cells	Periphery	Human allogeneic organ transplantation	Targeting ILT3 and ILT4 on DCs	15
$CD8^+CD62L^+CD122^+$ T cells	ND	Normal neonatal mice	ND	16
$CD8^+IL\text{-}10^+$ T cells	Periphery	Human ovarian cancer, *in vitro* culture system	IL-10	17–19

*$CD4^+$ regulatory T cells are conceptually divided into three populations. $CD4^+CD25^+FOXP3^+$ T cells are thought to be thymus derived, and are termed naturally occurring regulatory T cells (T_{Reg} cells); $CD4^+IL\text{-}10^+FOXP3^-$ regulatory T cells can be induced *in vitro* with various protocols or *in vivo* in response to exogenous antigen challenge, and are termed adaptive regulatory T cells, induced regulatory T cells or T-regulatory 1 cells (T_R1 cells); $CD4^+TGF\beta^+$ T cells are induced in the context of oral tolerance and are termed T_H3 cells. Notably, in this classification, the cytokine pattern and suppressive modes are not mutually exclusive and they often overlap. For example, human and mouse tumour $CD4^+CD25^+$ T_{Reg} cells express both FOXP3 and IL-10 (as discussed in the text). CTLA4, cytotoxic T-lymphocyte-associated antigen 4; DC, dendritic cell; FOXP3, forkhead box P3; IFN, interferon; IL-10, interleukin-10; ILT, immunoglobulin-like transcript; ND, not determined; TGFβ, transforming growth factor-β; T_H, T helper.

Treatment with CD25-specific antibody. CD25 is expressed by activated effector T cells and T_{Reg} cells. The role of T_{Reg} cells in mouse tumour immunity was initially studied by systemic depletion of $CD25^+$ T cells. Early studies demonstrated that *in vivo* administration of CD25-specific antibody (PC61) suppressed growth of several progressively growing tumours[24,25] (TABLE 2). These studies showed a correlation between reduced T_{Reg}-cell numbers and reduced tumour volume. In support of these findings, depletion of total $CD4^+$ T cells was found to improve tumour immunity and induce effective tumour rejection[26–28]. The antitumour effects of *in vivo* administration of CD25-specific antibody were confirmed in several mouse tumour models[29–39] (TABLE 2). Some of these studies tested several combinatorial treatments, which demonstrated possible additive or synergistic protective effects[27,29,32,33,35] (TABLE 2).

In addition to studies of $CD25^+$ T-cell depletion, experiments with adoptively transferred human[40] and mouse[41,42] T_{Reg} cells provided a direct link between T_{Reg} cells and reduced tumour immunity. Tumour-specific $CD8^+$ T cells were transferred with either T_{Reg} cells or $CD4^+CD25^-$ T cells into mice bearing B16 melanoma. In mice that received T_{Reg} cells, but not in mice that received $CD4^+CD25^-$ T cells, $CD8^+$ T-cell-mediated immunity was abolished[41,42]. These data indicated that T_{Reg} cells inhibit mouse TAA-specific immunity.

Treatment with GITR-specific antibody. In addition to CD25-specific antibody, an agonistic antibody that is specific for the glucocorticoid-induced tumour-necrosis factor (TNF)-receptor related protein (GITR; also known as DTA1) has been used to study the role of T_{Reg} cells in mouse tumour immunity. GITR is expressed on the surface of T_{Reg} cells, but is also expressed to various degrees on $CD4^+CD25^-$ T cells and antigen-presenting cells (APCs)[43]. GITR ligation directly reduced suppressor activity of T_{Reg} cells *in vitro*[43]. Furthermore, administration of GITR-specific antibody protected mice from B16 tumour challenge[41], and it induced tumour regression in mice bearing methylcholanthrene (methA)-induced sarcoma and *N*-nitroso-*N*-methylurethane-induced, undifferentiated colon carcinoma (CT26)[34]. Theoretically, GITR-specific antibody might attenuate the suppressor activity of T_{Reg} cells *in vivo* as reported *in vitro*[43]. However, in addition to T_{Reg} cells, other cell populations express GITR; therefore, it will be interesting to provide a link between the treatment with GITR-specific antibody and the potential deficits in numbers, functional competence or specificity of T_{Reg} cells *in vivo*. For example, it remains to be defined whether purified T_{Reg} cells from mice treated with GITR-specific antibody show reduced suppressor activity *in vitro*. Nonetheless, the mechanism of action and potential synergistic effects of GITR-specific antibody with current immunotherapeutic strategies needs careful investigation.

Treatment with CTLA4-specific antibody. Mouse T_{Reg} cells constitutively express cytotoxic T-lymphocyte-associated antigen 4 (CTLA4)[44,45]. Interest in the role of T_{Reg} cells has been provoked by studies of CTLA4-specific antibody treatment in both mouse tumour models and patients with cancer.

In 1996, Allison and colleagues reported that administration of CTLA4-specific antibody resulted in mouse tumour rejection, even of pre-established tumours. Furthermore, this treatment induced immunity to a second tumour challenge[46]. At the time, this study was not deliberately designed to deplete $CTLA4^+$ T_{Reg} cells or to alter CTLA4 signalling in T_{Reg} cells[46]. Instead, the data were interpreted to mean that CTLA4, a counter-receptor for the B7 family of co-stimulatory molecules,

Antigen-presenting cells (APCs). Cells that uptake, process and present antigen to other immune cells to initiate and activate immune responses. Monocytes, macrophages, dendritic cells and B cells are APCs. Dendritic cells are the most potent APCs.

Cytotoxic T-lymphocyte-associated antigen 4 (CTLA4). Following ligation by B7.1 (CD80) or B7.2 (CD86) on APCs, CTLA4 signalling in activated T cells induces cell-cycle arrest, reduces cytokine production and diminishes T-cell responses. T_{Reg} cells constitutively express CTLA4.

Table 2 | ***In vivo* depletion of CD25⁺ cells and tumour models**

Tumour type*	Treatment	Observation	References
Mouse melanoma (B16)	CD25-specific antibody	Reduced tumour growth and increased survival	25,30
	CD25- and B7-H1-specific antibodies	Improved tumour immunity and reduced tumour growth	W.Z. *et al.*, unpublished observations
	CD25-specific antibody and IFNα	Induced tumour regression	29
Mouse melanoma (B16-F10)	CD25-specific antibody and DCs	Improved DC-mediated immunity and tumour regression	29,33
	CD25-specific antibody and IL-12	Improved tumour immunity against tumour challenge	32
Mouse melanoma (B16-BL6)	CD25- and CTLA4-specific antibodies	Improved tumour immunity and mouse survival	27
Mouse spontaneous leukaemia (ASL1)	CD25-specific antibody	Reduced tumour growth and/or induced tumour regression	24
Mouse radiation-induced leukaemia (RL1)	CD25-specific antibody	Induced tumour regression and immunity against tumour challenge	24,25
Mouse DBA-induced leukaemia (EL4)	CD25-specific antibody	Reduced tumour growth and/or induced tumour regression	24
Mouse spontaneous leukaemia (AKSL2)	CD25-specific antibody	No effects on tumour growth	24
Mouse radiation-induced leukaemia (RL8)	CD25-specific antibody	No effects on tumour growth	24
Mouse C3H-derived plasmacytoma (X5563)	CD25-specific antibody	Reduced tumour growth and/or induced tumour regression	24
Mouse DBA2-derived mastocytoma (P815)	CD25-specific antibody	Reduced tumour growth and/or induced tumour regression	24
Mouse mineral-oil-induced myeloma (MOPC70-A)	CD25-specific antibody	Induced tumour regression and immunity against tumour challenge	24
Mouse methylcholanthrene-induced fibrosarcoma (CMS17)	CD25-specific antibody	Reduced tumour growth and/or induced tumour regression	24,31,34
Mouse colorectal tumour (CT26)	CD25-specific antibody	Improved tumour immunity and reduced tumour growth	36
	CD25-specific antibody and irradiated CT26	Improved tumour immunity against tumour challenge	35
Human cancer of ovary, breast and lung	Denileukin diftitox	T_{Reg}-cell depletion, increased T-cell activation and induced tumour regression	37
Human renal-cell carcinoma	Denileukin diftitox and DCs	T_{Reg}-cell depletion, improved DC-mediated TAA-specific immunity	38
Human melanoma	Denileukin diftitox	Negligible T_{Reg}-cell depletion, no clinical efficacy	39

*The tumour-cell line is given in parentheses. DC, dendritic cell; DBA, dimethylbenzanthracene; IFN, interferon; TAA, tumour-associated antigen; T_{Reg} cell, $CD4^+CD25^+$ regulatory T cell.

FIGO stages
Based on the International Federation of Gynaecology and Obstetrics (FIGO), ovarian cancer is classified into four surgicopathological stages: stage I, the tumour is confined to the ovaries; stage II, the tumour is confined to the pelvis; stage III, the tumour is confined to the abdomen; and stage IV, the tumour extends outside the abdominal cavity.

counteracted the CD28-mediated co-stimulatory signal that is necessary to fully activate T cells[46]. In the following years, this group and others confirmed these observations in various tumour models, and synergistic effects have been documented when CTLA4-specific antibody was used in combination with other treatments[47–54] (TABLE 3).

Current knowledge suggests that *in vivo* administration of CTLA4-specific antibody blocks CTLA4 signalling in $CTLA4^+$ T_{Reg} cells and in turn promotes tumour immunity. However, in patients with stage IV melanoma or renal-cell cancer that are treated with the CTLA4-specific antibody, administration of the antibody did not inhibit the suppressive activity of T_{Reg} cells *in vitro* or *in vivo*. Furthermore, there was neither a decrease in the number of peripheral-blood T_{Reg} cells, nor a decrease in FOXP3 mRNA expression in these cells post-treatment[55]. Therefore, a direct link between attenuated CTLA4 signals in T_{Reg} cells and improved tumour immunity remains to be established. Notably, Melief and colleagues[27] proposed that T_{Reg} cells and CTLA4 signalling represent two independent and synergistic regulatory mechanisms for suppression of T-cell activation *in vivo*. Five lines of experimental evidence exist to support this notion. First, in the absence of $CD25^+$ cells, CTLA4 blockade increased the induction of TAA-specific effector T cells. Second, depletion of $CD25^+$ cells and CTLA4 blockade synergistically improved TAA-specific T-cell immunity[27]. Third, administration of CD25-specific

Table 3 | **Treatment with CTLA4-specific antibody and tumour models**

Tumour type*	Treatment	Observation	Reference
Mouse colon tumour (51BLim10)	CTLA4-specific antibody	Tumour regression	46
Mouse prostate tumour (TRAMP)	CTLA4-specific antibody	Reduced tumour growth	47
	Tumour resection and CTLA4-specific antibody	Reduced metastatic relapse	48
	Irradiated tumour and CTLA4-specific antibody	Reduced tumour incidence and grade	49
	GM-CSF$^+$ tumour and CTLA4-specific antibody	Reduced tumour incidence and grade	49
Mouse mammary carcinoma (SM1)	GM-CSF$^+$ tumour and CTLA4-specific antibody	Reduced tumour growth and/or regression	50
Mouse B6-derived melanoma (B16)	GM-CSF$^+$ tumour and CTLA4-specific antibody	Reduced tumour growth	51
	CD25- and CTLA4-specific antibodies	Reduced tumour growth	27
Mouse methylcholanthrene-induced sarcoma (methA)	rMVAp53 vaccine and CTLA4-specific antibody	Tumour regression and increased survival	52
Human metastatic melanoma	Simultaneous peptide vaccine and CTLA4-specific antibody	Objective response in 3/14 patients, autoimmune disease in 6/14 patients	53
	GM-CSF$^+$ tumour and CTLA4-specific antibody	Tumour necrosis in 3/3 patients	54
	Previous peptide vaccine and CTLA4-specific antibody	No tumour necrosis in 4/4 patients	54
Human metastatic ovarian carcinoma	GM-CSF$^+$ tumour and CTLA4-specific antibody	Stabilization of CA125 in 2/2 patients	54

*The mouse tumour model is given in parentheses. CA125, a protein that is released by ovarian cancer cells and functions as a surrogate marker for ovarian tumour growth; CTLA4, cytotoxic T-lymphocyte-associated antigen 4; GM-CSF, granulocyte/macrophage colony-stimulating factor; methA, methylcholanthrene; rMVAp53, recombinant-modified-vaccinia-virus-Ankara expressing wild-type murine p53; TRAMP, transgenic adenocarcinoma mouse prostate.

antibody did not induce severe autoimmune disease, unlike administration of CTLA4-specific antibody[24,27], indicating that the targets of CD25-specific antibody and CTLA4-specific antibody might not be identical. Fourth, *in vivo* administration of CTLA4-specific antibody increased tumour immunity, but had no detectable effects on peripheral T_{Reg} cells[55]. The fact that T_{Reg} cells showed equivalent suppressive activity in *Ctla4*$^{-/-}$ mice and *Ctla4*$^{+/+}$ mice further supports this notion[56].

Collectively, these studies show that signals from CTLA4 (possibly including signals from CTLA4$^+$ T_{Reg} cells) negatively regulate tumour immunity and limit the efficacy of tumour immunotherapy.

Treatment with cyclophosphamide. Cyclophosphamide is an alkylating agent that mediates DNA crosslinking and is used to treat various tumours. High doses of cyclophosphamide are required for effective tumour chemotherapy that might lead to immunosuppression. Strikingly, low doses of cyclophosphamide induced improved immune responses in various animal tumour models[57–59] and in patients with metastatic melanoma[60], and this might be a result of the depletion of T_{Reg} cells[58]. These observations were initially confirmed when it was shown that cyclophosphamide treatment resulted in immune-mediated regression of the immunogenic, cyclophosphamide-resistant lymphoma (L5178Y cell line) in mice[61,62]. Depletion of T_{Reg} cells by cyclophosphamide was further demonstrated by recent studies in normal mice[63], in mice bearing B16 melanomas[41] or neu-expressing tumours[64], and in rats bearing a chemically induced colon cancer (PROb cell line)[65]. However, as a chemotherapeutic agent, the effects of cyclophosphamide on T_{Reg} cells might not be specific and selective. In addition to depleting T_{Reg} cells, cyclophosphamide-based therapy can deplete CD4$^+$CD25$^-$ T cells and CD4$^+$CD25^{mid} T cells[66]. Nonetheless, although the long-term clinical efficacy of cyclophosphamide as a T_{Reg}-cell-depleting agent remains to be determined, current data support the idea that low doses of cyclophosphamide could be used clinically to reduce T_{Reg}-cell-mediated suppressive activity.

Collectively, studies in mouse models show that depleting T_{Reg} cells or reducing their suppressive activity improves spontaneous or immunotherapy-mediated tumour clearance.

Regulatory T cells and human tumours

Although T_{Reg} cells have been studied extensively in mouse cancer models, the role of regulatory T cells in human tumour immunity is less well studied. T_{Reg} cells mediate peripheral tolerance by suppressing self-antigen-reactive T cells[20,22,67]. As most tumour antigens are self-antigens[68], T_{Reg}-cell-mediated suppression of TAA-reactive lymphocytes has been proposed as a potential mechanism to explain the failure of anti-tumour immunity[23,40]. Both T_{Reg} cells and CD8$^+$ regulatory T cells have been reported in the context of human tumour immunity.

T_{Reg} cells. In 2001, June and colleagues observed increased numbers of T_{Reg} cells in patients with non-small-cell lung cancer and ovarian cancer compared to normal donors[69]. Thereafter, a higher frequency of T_{Reg} cells in peripheral blood was reported in patients with various cancers including breast cancer[70], colorectal cancer[71,72], oesophageal cancer[73], gastric cancer[73,74], hepatocellular carcinoma[75], leukaemia[76], lung cancer[69,72], lymphoma[76,77], melanoma[78,79], ovarian cancer[69] and pancreatic cancer[70]. This list continues to grow following the current interest of studying T_{Reg} cells in human tumours. These studies demonstrated that peripheral T_{Reg} cells showed potent suppressive activity *in vitro* and indicated that a high frequency of T_{Reg} cells would temper TAA-specific immunity in patients with cancer.

T_{Reg} cells have also been extensively studied in human ovarian cancer. T_{Reg} cells within the ovarian-tumour microenvironment expressed FOXP3, inhibited TAA-specific $CD8^+$ T-cell cytotoxicity and contributed to tumour growth *in vivo* in a human severe combined immunodeficiency (SCID) chimeric model[40]. An accumulation of T_{Reg} cells in the tumour predicted a striking reduction of patient survival[40]. Notably, T_{Reg} cells that were isolated from peripheral blood, ascites or solid tumours of patients with ovarian cancer equally suppressed T-cell activation *in vitro*[40]. Therefore, T_{Reg} cells in the tumour microenvironment are not functionally superior to their counterparts in blood. The reduced TAA-specific T-cell immunity is probably a result of the increased accumulation of T_{Reg} cells, rather than the superior suppressive activity in the tumour microenvironment. This is in contrast to some autoimmune diseases, in which onset is related to reduced T_{Reg}-cell suppressive capacity[80–82]. Understanding this striking difference and phenomenon could provide a clue to address the distinct immunopathogenesis of human cancers and autoimmune diseases.

$CD8^+$ regulatory T cells. Plasmacytoid dendritic cells (DCs) from human ovarian tumours induced IL-10^+CD8^+ T cells, which inhibited TAA-specific T-cell immunity *in vitro*[17,18]. These tumour-associated plasmacytoid-DC-induced suppressive T cells had a central memory T-cell phenotype ($CD8^+CD45RO^+$ CC-chemokine receptor 7^+ ($CCR7^+$)) and suppressed TAA-specific T-cell effector functions through IL-10 production. Repetitive stimulation with myeloid DCs could not prevent their suppressive functions. These data indicate that vaccination with myeloid DCs alone might not be sufficient to recover defective T-cell immunity. Furthermore, tumour-associated, but not normal, primary $CD8^+CD45RO^+$ $CCR7^+$ T cells expressed IL-10 and suppressed T-cell activation *in vitro*, which suggests that tumour plasmacytoid DCs might induce $CD8^+CCR7^+$IL-10^+ regulatory T cells *in vivo*[18]. Consistent with these observations, human plasmacytoid DCs can induce allogeneic $CD8^+$ suppressive T cells[19], and mouse $CD8^+$ T cells with a central-memory phenotype also have regulatory T-cell capacity[16]. Therefore, $CD8^+$ regulatory T cells exist and might be functionally operative in patients with cancer (TABLE 1).

Source of regulatory T cells in the tumour

Functional regulatory T cells are found in the tumour microenvironment; these include thymus-derived 'natural' T_{Reg} cells and locally induced T-regulatory 1 (T_R1) cells (see TABLE 1 for definitions of regulatory T cells and see FIG. 1).

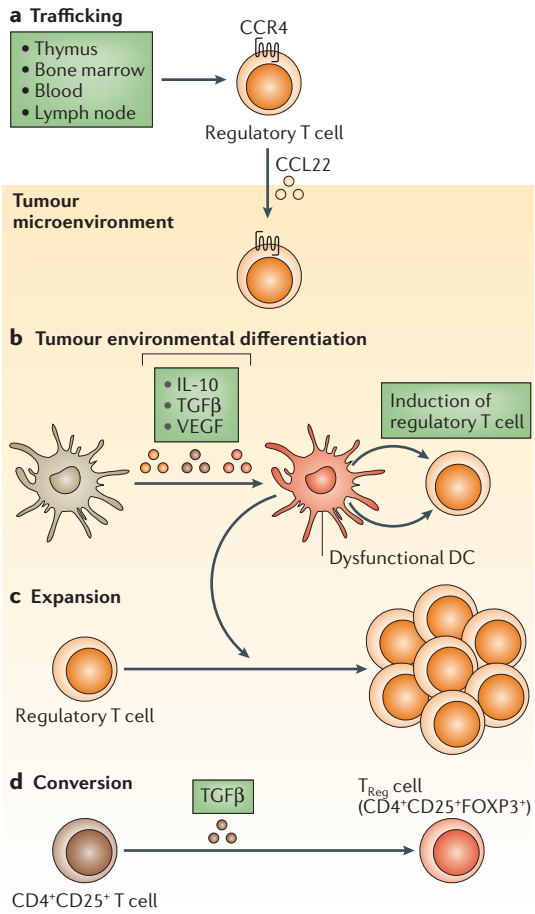

Figure 1 | **Regulatory T cells in the tumour microenvironment.** Regulatory T cells are found in the tumour microenvironment. There are four potential sources for regulatory T cells in the tumour microenvironment. **a** | Trafficking. T_{Reg} cells ($CD4^+CD25^+FOXP3^+$ T cells) from the thymus, lymph nodes, bone marrow and peripheral blood traffic to the tumour. T_{Reg} cells express CC-chemokine receptor 4 (CCR4), and abundant expression of CC-chemokine ligand 22 (CCL22; the ligand for CCR4) in the tumour microenvironment stimulates T_{Reg}-cell tumour infiltration. **b** | Differentiation. The tumour microenvironment contains molecules that can suppress antigen-presenting cell (APC) differentiation and function. These dysfunctional APCs can in turn stimulate regulatory T-cell differentiation. **c** | Expansion. Dendritic cells (DCs) can stimulate regulatory T-cell expansion, and it is predicted that DCs in the tumour microenvironment and draining lymph nodes might also induce regulatory T-cell expansion. **d** | Conversion. Normal T cells can be converted into T_{Reg} cells by transforming growth factor-β (TGFβ), and high levels of TGFβ are often found in the tumour microenvironment. FOXP3, forkhead box P3; IL, interleukin; VEGF, vascular endothelial growth factor.

Severe combined immunodeficiency (SCID). SCID mice do not have B cells or T cells. Tumour cells can be grown in these mice without rejection.

Plasmacytoid dendritic cells
A subset of dendritic cells that is lineage$^-$HLA-DR^+CD11c^- mononuclear cells with a microscopic appearance similar to plasmablasts. Plasmacytoid dendritic cells are the main producers of type I interferon.

Central memory T cells
Antigen-experienced $CD8^+$ T cells that lack immediate effector function but are able to mediate rapid recall responses. They also rapidly develop the phenotype and function of effector-memory cells after restimulation with antigen. Central memory T cells retain the migratory properties of naive cells and therefore circulate through secondary lymphoid organs.

Myeloid dendritic cells
A subset of dendritic cells that is lineage$^-$HLA-DR^+CD11c^+ mononuclear cells with a monocytoid appearance. Human myeloid dendritic cells might develop from myeloid precursors (for example, monocytes, macrophages and $CD11c^+$ precursors).

T_{Reg} cells differentiate in the thymus. The mouse thymus produces T_{Reg} cells as a separate lineage that expresses FOXP3 (REFS 9,83). Because tumour-associated T_{Reg} cells express FOXP3 mRNA and protein[28,34,40], it is possible that these cells traffic to tumours from the thymus, bone marrow[84], lymph nodes and peripheral blood under the influence of tumour microenvironmental CC-chemokine ligand 22 (CCL22)[40] (FIG. 1).

Thymic function is largely reduced after adolescence, whereas T_{Reg} cells persist throughout the human lifespan. Presumably, regulatory T cells can be induced and differentiate in the periphery, such as in the tumour microenvironment. Tumour environmental factors, such as vascular endothelial growth factor (VEGF), IL-10 and transforming growth factor-β (TGFβ) suppressed DC differentiation and function, resulting in immature and/or partially differentiated DCs[22,85]. Plasmacytoid DCs associated with ovarian cancer induced $CD8^+FOXP3^-IL\text{-}10^+$ regulatory T cells[17,18], and dysfunctional myeloid DCs also induced $IL\text{-}10^+$ regulatory T cells *in vitro*[86] and *in vivo* in patients with cancer[87,88]. Tumours convert DCs into TGFβ-expressing immature myeloid DCs that are capable of promoting T_{Reg}-cell proliferation[89]. Normal mature DCs stimulated the *in vivo* expansion of self-antigen-specific T_{Reg} cells in mice[90,91]. Analogously, thymus-derived T_{Reg} cells in the tumour microenvironment might clonally expand following stimulation by tumour-associated DCs (FIG. 1).

In addition to trafficking, differentiation and expansion of regulatory T cells, T_{Reg} cells can also be present in a tumour as a result of conversion from $CD4^+CD25^-$ T cells[92–95]. TGFβ, which is present at high levels in the tumour microenvironment, might mediate this conversion[92,94,96]. Similarly, IL-10 is often found in the tumour microenvironment[22], where it supports the differentiation of $CD4^+IL\text{-}10^+TGF\beta^+$ regulatory T cells[28,97] and induces T_R1 cells[12] (TABLE 1). However, it is unknown whether IL-10 is implicated in the differentiation and expansion of T_{Reg} cells.

The tumour microenvironment might contain thymus-derived natural T_{Reg} cells, expanded and converted natural T_{Reg} cells, and locally differentiated and expanded T_R1 cells. Cell surface CD25, rather than intracellular FOXP3, is used as a marker to isolate and sort viable T_{Reg} cells for functional experiments. As there is no reliable cell-surface marker to distinguish these regulatory T-cell subsets, the statement that "so far, no one has had a pure population of suppressor T cells in a test tube" remains as true today as in 1988 (REF. 98).

Suppressive mechanisms of regulatory T cells

Suppressive mechanisms of regulatory T cells have been addressed using many *in vitro* and *in vivo* mouse models. Multiple suppressive mechanisms including cell–cell contact and soluble factors have been proposed[20,23,67,99]. These mechanisms have not been exclusively investigated in tumour settings. Therefore, I summarize the relevant information and discuss their relevance in the context of tumour immunity (FIG. 2).

It has been suggested that thymus-derived T_{Reg} cells express FOXP3 but not IL-10 and TGFβ, whereas T_R1 cells express IL-10 but not FOXP3 (REFS 20,100) (TABLE 1). However, human T_{Reg}-cell clones specific for the TAA LAGE-1 (REF. 99) expressed both FOXP3 and IL-10. In a T-cell receptor (TCR)-transgenic model, in which both conventional T cells and T_{Reg} cells were specific for influenza virus haemagglutinin, it was found that these haemagglutinin-specific T_{Reg} cells[101,102] expressed both FOXP3 and IL-10. Therefore, TAA-specific T_{Reg} cells seem to share common features with naturally occurring T_{Reg} cells and induced T_R1 cells. Although the role of IL-10 was not investigated in these studies[101,102], the data predict that IL-10 derived from TAA-specific T_{Reg} cells might profoundly suppress APC and T-cell function[100] (FIG. 2). In addition to APCs and T cells, T_{Reg} cells also suppress natural killer (NK)-cell function in a TGFβ-dependent manner in tumour-bearing mice[103]. These data indicate that T_{Reg} cells can dampen adaptive immunity as well as innate immunity.

Notably, TGFβ and IL-10 can be produced by multiple cell types in the tumour microenvironment. T_{Reg} cells might not be the main source of IL-10 and TGFβ *in vivo*. Nonetheless, it is clear that T_{Reg} cells can mediate suppression through the actions of IL-10 and TGFβ *in vivo* in mouse tumour models[103–106]. One possibility is that T_{Reg} cells might condition APCs and enable them to express functional IL-10 (REFS 107,108). Suppression might then be mediated locally by APC-derived IL-10 within the tumour microenvironment (FIG. 2).

Competitive consumption of IL-2. The IL-2 receptor consists of a heterotrimeric receptor complex that is made up of CD25 (α-chain), CD122 (β-chain) and CD132 (γ-chain). T_{Reg} cells express this heterotrimeric receptor complex, which has a 100-fold higher affinity for IL-2 than the dimeric form (made up of CD122 and CD132). Therefore, competition for IL-2 between T_{Reg} cells and conventional T cells was suggested as a suppressive mechanism[20,109,110]. The main cellular source of IL-2 *in vivo* is conventional T cells. Most tumour-infiltrating $CD4^+$ T cells are T_{Reg} cells in established tumours[28,34,40]. Therefore, the supply of IL-2 might be limited in the tumour microenvironment and competitive consumption of IL-2 could be a minor suppressive mechanism for T_{Reg} cells in established tumours (FIG. 2). The role of IL-2 in T_{Reg}-cell function is discussed later.

Perforin and granzyme pathway and T_{Reg}-cell-mediated direct killing. $CD8^+$ T cells and NK cells use the perforin and granzyme pathways to kill infected cells and tumour cells. Activated human T_{Reg} cells express granzyme A and kill T cells and APCs through perforin[111]. A study using granzyme B-deficient and perforin-deficient mice showed that T_{Reg} cells mediated suppression through a granzyme B-dependent but perforin-independent mechanism[112]. Nonetheless, it is unknown whether T_{Reg} cells in tumours express perforin and granzyme B, and if so, whether these pathways are functional for T_{Reg} cells. Another interesting question is whether T_{Reg} cells could kill tumour cells through similar mechanisms (FIG. 2).

LAGE-1 and NY-ESO-1
LAGE-1 and NY-ESO-1 (New York oesophageal squamous-cell carcinoma 1) are two cancer- and testis-specific antigens that show 94% sequence identity. The genes encoding LAGE-1 and NY-ESO-1 are both located on chromosome Xq28 and are frequently co-expressed. LAGE-1 and NY-ESO-1 have been shown to be expressed in 25–50% of various tumour types, such as melanoma, breast carcinoma, prostate and bladder cancers.

Natural killer cell
Cytotoxic lymphocytes that are distinguished from $CD8^+$ T cells by their lack of rearrangement of T-cell receptor genes. They have abundant granule-containing cytoplasm, and their functions are cell killing and cytokine production.

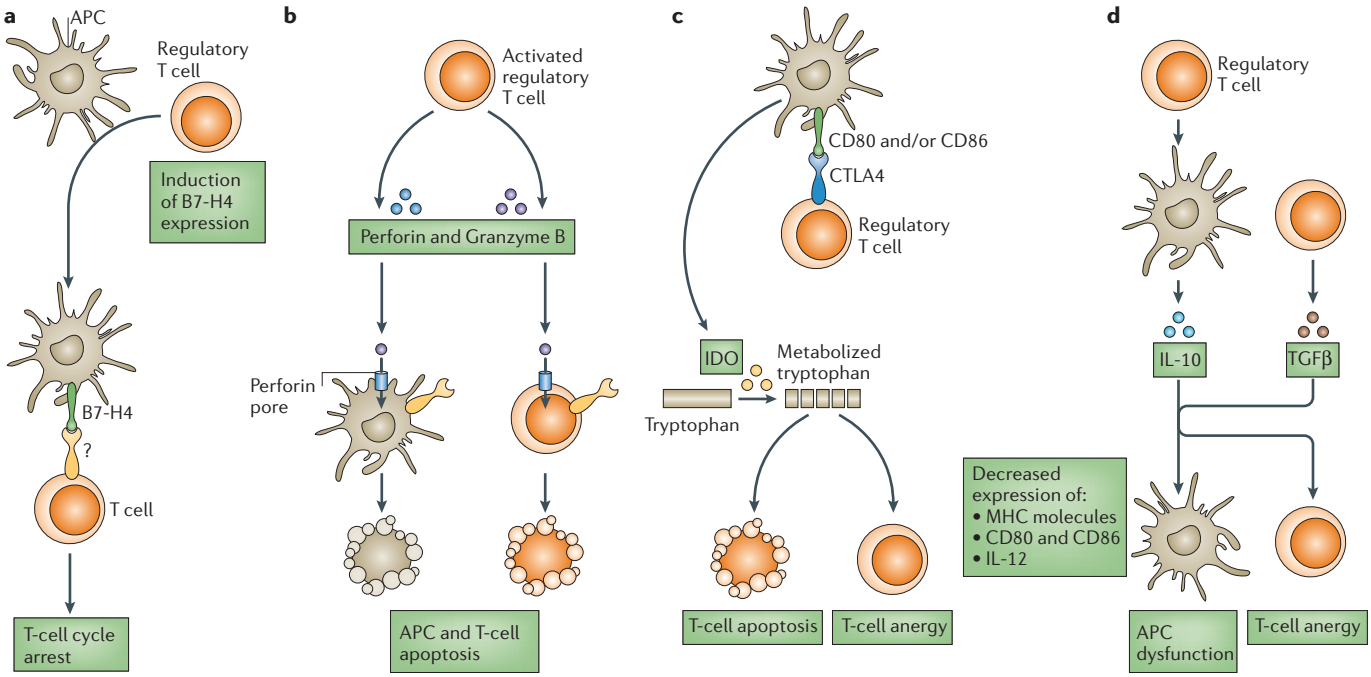

Figure 2 | **Possible suppressive mechanisms of tumour environmental regulatory T cells.** Possible suppressive mechanisms of regulatory T cells (T_{Reg} cells; $CD4^+CD25^+FOXP3^+$) have been addressed in various models *in vitro* and *in vivo*. Multiple suppressive mechanisms rather than a single mode of action are proposed. **a** | T_{Reg} cells induce B7-H4 expression by antigen-presenting cells (APCs), and in turn these B7-H4$^+$ APCs induce T-cell cycle arrest through B7-H4. **b** | Activated human T_{Reg} cells directly kill target cells such as T cells and APCs through perforin- or granzyme B-dependent pathways. **c** | Cytotoxic T-lymphocyte-associated antigen 4 (CTLA4)$^+$ T_{Reg} cells induce indoleamine 2,3-dioxygenase (IDO) expression by APCs, and IDO-expressing APCs in turn suppress T-cell activation by reducing tryptophan. **d** | Regulatory T cells, including T_{Reg} cells, might release interleukin-10 (IL-10) and transforming growth factor (TGFβ) *in vivo*, and directly inhibit T-cell activation and suppress APC function, by inhibiting expression of MHC molecules, CD80, CD86 and IL-12.

CTLA4 induction of indoleamine 2,3-dioxygenase-expressing APCs. Indoleamine 2,3-dioxygenase (IDO) is an enzyme that degrades the essential amino acid tryptophan. IDO$^+$ human APCs reduce tryptophan, and suppress T-cell activation and promote tolerance in the tumour microenvironment[113]. Mouse CTLA4$^+$ T_{Reg} cells mediate their suppressive activity by inducing the expression of IDO in APCs through CTLA4 (REFS 113,114). Because human tumour T_{Reg} cells express CTLA4 (REF. 40) and because IDO$^+$ APCs are found in human tumours and within tumour-draining lymph nodes[113], it seems reasonable to speculate that this mechanism might occur *in vivo* (FIG. 2).

Induction of B7-H4$^+$ APCs. B7-H4 (also known as B7x, B7S1) is a recently discovered member of the B7 family of T-cell co-stimulatory molecules. B7-H4 negatively regulates T-cell responses[115–117]. We observed that ovarian-tumour-associated macrophages, but not normal macrophages, expressed B7-H4 (REF. 107). Tumour B7-H4$^+$ macrophages inhibited TAA-specific T-cell responses, which could be reversed by blocking B7-H4. Interestingly, T_{Reg} cells, but not normal T cells, induced B7-H4 expression by APCs including monocytes, macrophages and myeloid DCs, and rendered APCs immunosuppressive through B7-H4. These data indicate that T_{Reg} cells convey suppressive activity to APCs by stimulating B7-H4 expression. We therefore provide a novel cellular and molecular mechanism for T_{Reg}-cell-mediated immunosuppression at the level of APCs[107,108]. Because high numbers of T_{Reg} cells[40] and B7-H4$^+$ macrophages[107] are found in tumour environments, this suppressive mechanism might also occur in patients with tumours (FIG. 2).

In summary, T_{Reg} cells target not only T cells but also APCs to efficiently temper TAA-specific immunity. It is probable that multiple T_{Reg}-cell-mediated suppressive mechanisms might be operative *in vivo*.

TAA specificity and regulatory T cells

Our current knowledge of the antigen specificity of T_{Reg} cells has come largely from studies with antigen-specific TCR-transgenic mice[20,23]. However, the TAA specificity of T_{Reg} cells is poorly understood. Here I focus on the TAA specificity of T_{Reg} cells in the periphery.

Are T_{Reg} cells TAA specific? Thymus-derived T_{Reg} cells maintain mouse and human T-cell self-tolerance[23] and many TAAs are self-antigens[68]; furthermore, T_{Reg} cells are specific for self-antigens, so it is possible that T_{Reg} cells are specific for at least one subset of TAA[68]. In support of this, human melanoma-infiltrating T_{Reg} cells were cloned, and in one case the target antigen was found to be LAGE-1, which is a cancer- and testis-specific

Indoleamine 2,3-dioxygenase
(IDO). An intracellular haem-containing enzyme that catalyses oxidative catabolism of tryptophan.

B7-H4
A newly defined B7-family member. B7-H4 fusion protein inhibits T-cell-mediated immune responses. The receptor, regulation and detailed function remain to be defined.

antigen[99]. These cloned T_{Reg} cells suppressed LAGE-1-specific T-cell activation. It is important to identify the ligands for T_{Reg} cells, to compare them to the ligands for TAA-specific effector T cells, and to determine whether there are ligands that are exclusively recognized by T_{Reg} cells, and furthermore, whether T_{Reg} cells recognize mutated (novel) tumour antigens.

Is TAA specificity acquired in tumours and draining lymph nodes? Regulatory T cells can be induced in tumour-draining lymph nodes and in the tumour microenvironment. Similar to TAA-specific effector T cells, regulatory T cells can attain TAA specificity in the course of TAA-specific induction. For example, dysfunctional APCs that express TAA might induce regulatory T-cell differentiation. In support of this, T_{Reg} cells isolated from mice that were immunized with self-antigens strongly suppressed peptide-specific T-cell proliferation and activation[118]. Some human vaccinations with TAA-expressing APCs induced TAA-specific regulatory T cells[87,88]. In addition, it is possible that thymus-derived T_{Reg} cells might be activated by crossreactivity between self-antigens and novel tumour antigens and might in turn induce TAA specificity.

Is suppressor activity TAA specific or nonspecific? It is understandable that antigen (including self-antigen)-specific T_{Reg} cells suppress antigen-specific T-cell responses. Theoretically, self-antigen-specific T_{Reg} cells only suppress self-antigen (that is, tumour antigen)-specific T-cell responses. In support of this, NY-ESO-1 (New York oesophageal squamous-cell carcinoma 1)-specific $CD4^+$ T cells were induced from naive populations following $CD4^+CD25^+$ T-cell depletion, indicating that T_{Reg} cells suppressed NY-ESO-1-specific $CD4^+$ T-cell priming[119]. Furthermore, ovarian-tumour T_{Reg} cells suppressed TAA-specific effector T-cell function *in vitro* and *in vivo*[40]. However, T_{Reg} cells isolated from TCR-transgenic mice inhibited responses of $CD4^+CD25^-$ T cells to the same antigen, but also inhibited a distinct antigen-specific T-cell response. Furthermore, T_{Reg} cells suppressed allogeneic T-cell activation *in vitro* and *in vivo*[20]. Therefore, although it remains controversial, it is possible that T_{Reg}-cell differentiation, expansion and activation are driven in an antigen-specific manner[67,101,109,118–121] that determines their antigenicity, whereas activated T_{Reg} cells show antigen-nonspecific suppressor activity[122].

Do T_{Reg} cells suppress TAA-specific T-cell priming or effector function? T_{Reg} cells are homeostatically maintained in lymphoid organs[20,23,67]. Administration of CD25-specific antibody, either 4 days before or 1 day after tumour inoculation, was efficient in promoting tumour regression[24,25,27,30,34,35]. These data indicate that T_{Reg} cells mediate their suppressive effect by inhibiting T-cell priming in lymphoid organs.

By contrast, other evidence indicates that T_{Reg} cells reduce the effector function of TAA-specific cells. Transfer of T_{Reg} cells reduced the therapeutic efficiency of adoptively transferred TAA-specific $CD8^+$ effector T cells in a mouse model of melanoma[42]. Furthermore, depletion of intratumoural T_{Reg} cells induced potent T-cell tumour immunity and resulted in regression of large established tumours[28]. In our studies of advanced ovarian cancer[40], a large number of T_{Reg} cells accumulated in the tumour mass and associated malignant ascites, but there were few in the tumour-draining lymph nodes, indicating that T_{Reg} cells predominantly inhibit extranodal effector-cell function at this stage. However, human tumorigenesis is a long process, and T_{Reg} cells might be present in the draining lymph nodes before clinical manifestation of disease and might block TAA-specific T-cell priming during the priming process. In addition to T_{Reg} cells, other classes of regulatory T cells, such as $CD8^+$ regulatory T cells, might also migrate to draining lymph nodes and block T-cell priming[18]. Nonetheless, T_{Reg} cells are able to hinder both TAA-specific priming and effector function.

Targeting regulatory T cells

Most studies of cancer immunotherapy so far have focused on augmenting tumour immunity through supplementing active immune elements such as dendritic cells, TAA-specific T cells and cytokines. The concept of reversing immunosuppression in cancer has merit as a therapeutic approach[22]. Newer studies targeting suppressive molecules and regulatory T cells point the way to successful application of tumour immunotherapy (FIG. 3).

***Blocking CTLA4* in vivo.** CTLA4 blockade resulted in improved tumour immunity and tumour regression in several mouse models[26,27,46–50,52]. These observations led to interest in CTLA4 blockade for human cancer immunotherapy. Two clinical trials, in which patients with advanced cancer (TABLE 3) were treated with an inhibitory human CTLA4-specific antibody, have been published[53,54]. Treatment with CTLA4-specific antibody induced objective cancer regression in some patients that were vaccinated with HLA-A2-restricted peptides from the melanoma-associated antigen glycoprotein 100 (gp100) or with irradiated, autologous granulocyte/macrophage colony-stimulating factor (GM-CSF)-secreting tumour cells[53,54]. Notably, CTLA4-specific antibody treatment also resulted in severe, but manageable, autoimmune responses in these patients[53] (TABLE 3).

As discussed previously, the mechanistic link between the effects of treatment with CTLA4-specific antibody and T_{Reg}-cell function remains to be determined in these human clinical trials. In fact, CTLA4-specific antibody did not induce depletion of peripheral T_{Reg} cells (or FOXP3 mRNA)[55] or of peripheral $CTLA4^+$ T cells in tumour patients[53]. Nonetheless, *in vivo* administration of CTLA4-specific antibody breaks tumour tolerance in patients with cancer and regardless of the cellular source of CTLA4, these studies provide 'proof of principle' that blocking CTLA4 signals is an option for treating patients with cancer (FIG. 3).

***Depletion of $CD25^+$ T cells* in vivo.** Given that treatment with CD25-specific antibody efficiently depletes T_{Reg} cells, improves tumour immunity and results in mouse tumour regression, it is predicted that depletion

RECIST criteria
A set of criteria, designated the response evaluation criteria in solid tumours.

Objective clinical response
Based on the International Union Against Cancer and World Health Organization, an objective clinical response to treatment is defined as a 50% reduction in the sum of the products of the perpendicular diameters of all lesions without the 25% growth of any lesion or the appearance of new lesions. RECIST defines an objective clinical response as a 30% reduction in the sum of the maximum diameters of lesions, along with the appearance of no new or progressive lesions.

of T_{Reg} cells would be beneficial for cancer patients. Denileukin diftitox (Ontak) is a ligand–toxin fusion protein that consists of full-length IL-2 fused to the enzymatically active and translocating domains of diphtheria toxin[123]. This drug has been approved by the Food and Drug Administration in the United States for treatment of $CD25^+$ cutaneous T-cell leukaemia and lymphoma. This fusion protein is internalized into $CD25^+$ T cells by endocytosis. The ADP-ribosyltransferase activity of diphtheria toxin is cleaved in the endosome and is translocated into the cytosol, where it inhibits protein synthesis, leading to apoptosis[123]. Denileukin diftitox is therefore predicted to deplete T_{Reg} cells in patients with cancer.

We used denileukin diftitox to treat patients with lung, ovarian or breast cancer, where T_{Reg}-cell numbers are greatly increased[40,69]. We demonstrated that a single dose of denileukin diftitox reduced the prevalence and absolute numbers of peripheral T_{Reg} cells and increased effector T-cell activation in all eight patients. This effect was evident for about one month. One patient with ovarian cancer had measurable disease, which permitted the assessment of potential clinical efficacy. CA125 is a protein that is often secreted into the blood by ovarian-tumour cells and the amount of this protein in the blood can provide information on the growth of ovarian cancer. The single denileukin diftitox infusion normalized her blood CA125 levels, and after six additional weekly denileukin diftitox infusions, her bony, visceral and lymphatic metastases were largely resolved. Based on the RECIST (response evaluation criteria in solid tumours) criteria, we observed an objective clinical response. However, our current observations indicate that more than four weekly infusions of denileukin diftitox might also deplete $CD25^+$ effector T cells[37].

Another group has reported similar findings using denileukin diftitox in patients with renal tumours[38]. Patients received a single dose of denileukin diftitox, followed by vaccination with DCs transfected with total tumour RNA. They showed that peripheral blood T_{Reg} cells were eliminated in a dose-dependent manner. Furthermore, denileukin diftitox administration followed by vaccination with tumour-RNA-transfected DCs led to improved stimulation of tumour-specific effector T cells compared with vaccination alone[38].

It was also reported that different doses of denileukin diftitox had variable effects on FOXP3 mRNA expression in $CD4^+$ T cells in patients with metastatic melanoma. Furthermore, there was no significant clinical efficacy observed in this study[39]. Nonetheless, these clinical trials suggest that *in vivo* depletion of functional human T_{Reg} cells is a possible option. Further clinical study with large patient populations is essential to link T_{Reg}-cell depletion with improved immunity and potential clinical efficacy.

Notably, depletion of T_{Reg} cells did not induce meaningful tumour regression in some mouse models[24,25,34] (TABLE 2). To reduce T_{Reg}-cell suppressor activity, when

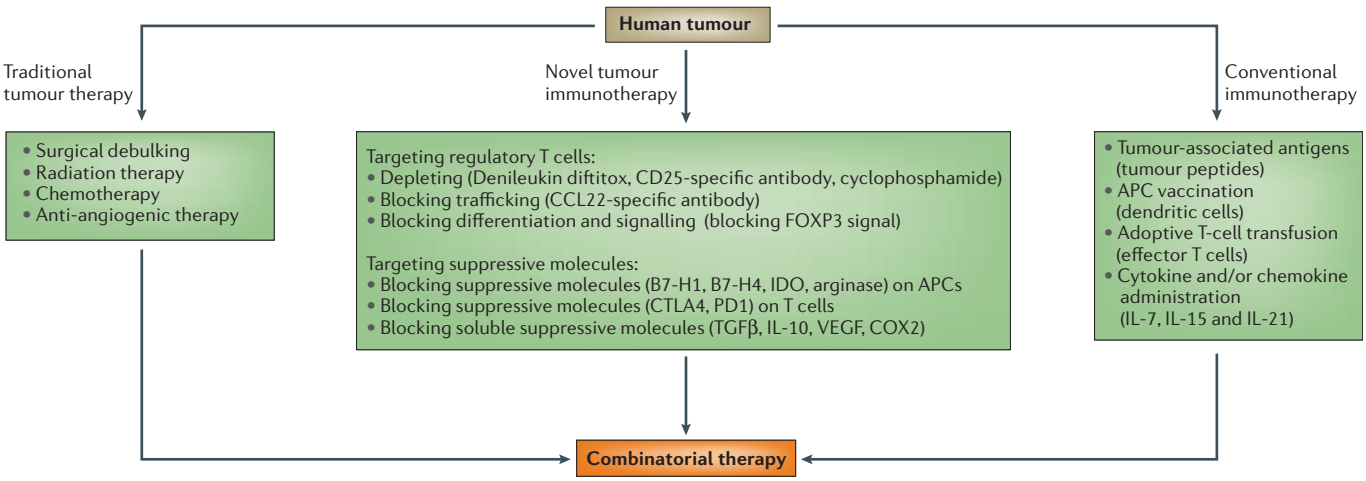

Figure 3 | **Therapeutic targeting of suppressive mechanisms including regulatory T cells.** After clinical and/or pathological diagnosis, patients with cancer might be subjected to traditional tumour therapy, including surgical debulking, radiation therapy, chemotherapy and antitumour angiogenic therapy. Depending on their clinical situation, patients could receive a combination of these strategies. Traditional tumour therapy targets the tumour itself and remains the 'gold standard' therapy. Conventional immunotherapy supplements the immune system and provides essential immune elements, including tumour-associated antigen (TAA), antigen-presenting cells (APCs), effector T cells and cytokines and/or chemokines with the aim of boosting TAA-specific immunity. Early clinical trials with conventional tumour immunotherapy have been encouraging, but improvements in clinical efficacy are needed. Novel immunotherapeutic strategies target the immunosuppressive network of tumours, including regulatory T cells, suppressive molecules and dysfunctional APCs, with the aim of recovering TAA-specific immunity. To attain effective, reliable and consistent clinical efficacy, it might be essential to combine traditional tumour therapy, conventional immunotherapy and novel tumour immunotherapy. COX2, cyclooxygenase 2; CTLA4, cytotoxic T-lymphocyte-associated antigen 4; FOXP3, forkhead box P3; IDO, indoleamine 2,3-dioxygenase; IL, interleukin; PD1, programmed cell death 1; TGFβ, transforming growth factor-β; VEGF, vascular endothelial growth factor.

denileukin diftitox or other agents are considered for clinical trial, several possibilities need to be examined. First, T_{Reg}-cell-mediated suppression might not be the key tolerizing mechanism in certain tumour types or at certain tumour stages, and at these times, other suppressive mechanisms might predominate[22]. Second, it is important to examine the clinical efficacy of the route of administration of T_{Reg}-cell-depletion agents. Systemic administration could result in insufficient concentrations to deplete T_{Reg} cells in the tumour microenvironment. Furthermore, because many human tumours are not suitable for surgery, intratumoural administration of T_{Reg}-cell-depleting agents could be an interesting alternative[28,34]. Third, the treatment might substantially deplete other immune cells, including $CD25^+$ effector T cells. Therefore, although depletion of T_{Reg} cells is an attractive option, careful studies are needed to optimize treatment. These include dose escalation, identification of the window of clinical benefit, the use of combinatorial regimens and identification of which tumour types best respond to such depletion (FIG. 3).

Chemotherapy. As discussed previously, low doses of cyclophosphamide reduces T_{Reg}-cell numbers and suppressive capacity in normal and tumour-bearing mice[41,58,61–63,65]. Other immunosuppressants, including calcineurin inhibitors, cyclosporine (Sandimmune) and tacrolimus (FK506), might also reduce T_{Reg}-cell numbers and function[124,125]. Cyclosporine and tacrolimus inhibit T-cell activation by suppressing IL-2 and are often used to prevent the rejection of transplanted organs. As IL-2 is crucial for T_{Reg}-cell expansion and function[126–131], it is predicted that cyclosporine and tacrolimus abrogate T_{Reg}-cell function by altering IL-2 production and signals. Further pre-clinical studies are needed to assess whether low doses of these agents are an option to promote tumour immunity in patients (FIG. 3).

Other potential strategies to control T_{Reg}-cell function. The first potential strategy is one that could target molecules that are involved in T_{Reg}-cell trafficking. In ovarian cancer, tumour microenvironmental CCL22-mediated trafficking of T_{Reg} cells into the tumour[40] and blockade of CCL22 reduces T_{Reg}-cell tumour trafficking[40]. Therefore, altering T_{Reg}-cell tumour trafficking would be an attractive strategy for blocking T_{Reg}-cell suppressive capacity. A second possible strategy involves altering T_{Reg}-cell differentiation and function. FOXP3 is crucial for T_{Reg}-cell differentiation and function[9–11], and compounds that inhibit the expression, function and signalling of FOXP3 might have therapeutic potential. IL-2, GITR and B7-H4 are also implicated in T_{Reg}-cell differentiation or function and targeting these molecules could also provide additional therapeutic opportunities (FIG. 3).

In summary, depletion of T_{Reg} cells, blockade of T_{Reg}-cell trafficking, differentiation and function represent new ways to augment tumour immunity. This strategy might be used in combination with various current therapeutic approaches, including traditional tumour therapy and conventional immunotherapy (FIG. 3).

Immunotherapy and regulatory T cells

The clinical efficacy of current methods of tumour immunotherapy and vaccination is not satisfactory. One reason for this is that immunosuppressive mechanisms predominate in patients with advanced cancer[21,22,68,132]. Another possibility is that certain immunotherapy and vaccination protocols might promote regulatory T-cell function[121] and tumour trafficking instead of selectively improving the function of antitumour effector cells. Here, I use IL-2 treatment and APC vaccination as examples to discuss this possibility.

IL-2 and T_{Reg} cells. IL-2 has been used to treat patients with HIV and some cancers, such as myelogenous leukaemia, metastatic melanoma and renal-cell carcinoma[127]. Interestingly, in patients with HIV infection, the long-term effect of IL-2 treatment is associated with decreased T-cell proliferation and activation *in vivo*, which is accompanied by an increase of $CD4^+CD25^+$ T-cell numbers[133].

In patients with melanoma, renal cancer[134], Ewing's sarcoma, alveolar rhabdomyosarcoma[66] and ovarian cancer (W.Z. *et al.*, unpublished observations), IL-2 administration increased the number of peripheral T_{Reg} cells. Furthermore, IL-2 stimulated CXC-chemokine receptor 4 (CXCR4) and CCR4 expression on T_{Reg} cells and promoted their migration towards tumour microenvironmental CXCL12 and CCL22 (W.Z. *et al.*, unpublished observations)[40,84,135]. In line with these findings, recent studies have shown that IL-2 contributes to T_{Reg}-cell differentiation, expansion, maintenance and suppressor activity in mice[126–131]. These data indicate that the clinical benefit of IL-2 treatment in patients with cancer and AIDS requires urgent re-evaluation. IL-7, IL-15 and IL-21 share the common cytokine-receptor γ-chain (γ_c) and certain properties with IL-2, and have been shown to stimulate innate immunity and increase $CD8^+$ T cell-mediated antitumour activity[136–138]. Furthermore, addition of IL-7 with IL-15 abrogated the suppressive activity of T_{Reg} cells *in vitro*[139]. Therefore, IL-2 could be therapeutically replaced by other γ_c cytokines, and γ_c cytokines such as IL-7, IL-15 and IL-21, alone or in combination, might be useful as novel immunotherapeutic agents.

APC and TAA-expressing vector vaccination and regulatory T cells. DCs have been used widely as an adjuvant to boost TAA-specific immunity in cancer patients. However, immature and semi-mature DCs induced regulatory T cells in mice[140–142] and humans[87,88,143], and their maturation status might determine their immunogenic versus tolergenic properties[142]. As the maturation status is defined by the cytokine profile[142], it needs careful manipulation to obtain fully matured DCs with therapeutic efficacy. However, recent studies show that mature DCs activate and expand autoantigen-specific T_{Reg} cells, and in a mouse model of diabetes these cells inhibited diabetes mediated by reactivity to multiple antigens in mice[90,91]. Furthermore, mature DCs were shown to induce tumour-specific IL-10-expressing T_R1 cells[144]. These studies indicate the possibility that certain fully matured DCs promote regulatory T-cell function *in vivo*.

Additionally, the tumour antigen LAGE-1 was identified as a ligand for T_{Reg} cells in patients with melanoma[99], and this raises the question of whether vaccination with DCs or vector(s) bearing certain tumour antigens, for example LAGE-1, would promote T_{Reg}-cell clonal expansion and function in patients with cancer. Therefore, additional studies are required to evaluate current and future vaccination trials. The ideal vaccination needs to maximize the potential beneficial effects while minimizing detrimental effects.

Concluding remarks

It is evident that tumours actively develop different mechanisms to escape tumour immunity and defeat conventional tumour immunotherapy[22]. Regulatory T cells have an important role in suppressing TAA-specific immunity. Regulatory T cells inhibit TAA-specific priming in tumour draining lymph nodes, and are further recruited into the tumour microenvironment, where they suppress TAA-specific effector functions. Therefore, manipulation of regulatory T cells, including depletion, blocking trafficking into tumours, or reducing their differentiation and suppressive mechanisms, represent new strategies for cancer treatment. However, although it has been stated that "all anyone is talking about these days is regulatory T cells"[145], regulatory T cells might not be the only or important suppressive mechanism for certain tumour stages and/or certain tumours. Therefore, it is essential to define the molecular basis and nature of regulatory T cells in each individual human tumour microenvironment, including their antigen specificity, induction and mechanisms of action. Therapeutically, agents that can selectively and efficiently target regulatory T cells deserve extensive research. Furthermore, we need to bear in mind that although targeting regulatory T cells is an attractive option in treating human tumours, it is probable that to attain effective, reliable and consistent clinical efficacy, a complicated combinatorial therapeutic regimen of traditional tumour therapy and conventional immunotherapy combined with novel tumour immunotherapy, including targeting regulatory T cell will be warranted (FIG. 3).

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Competing interests statement

The author declares no competing financial interests.

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