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Paths to stemness: building the ultimate antitumour T cell

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Abstract

Stem cells are defined by the ability to self-renew and to generate differentiated progeny, qualities that are maintained by evolutionarily conserved pathways that can lead to cancer when deregulated. There is now evidence that these stem cell-like attributes and signalling pathways are also shared among subsets of mature memory T lymphocytes. We discuss how using stem cell-like T cells can overcome the limitations of current adoptive T cell therapies, including inefficient T cell engraftment, persistence and ability to mediate prolonged immune attack. Conferring stemness to antitumour T cells might unleash the full potential of cellular therapies.

Be stirring as the time; be fire with fire; Threaten the threatener … seek the lion in his den. W. Shakespeare, *The Life and Death of King John*, c. 1595

Organismal homeostasis requires a precise balance between self-renewal and differentiation. Physiologically, the presence of limited numbers of stem cells in different tissues provides a hierarchical organization of cell types in which a few daughter cells retain a regenerative potential while most enter an irreversible process of differentiation that culminates in the generation of specialized cell types that are destined to die[1]. The existence of stem cells has been documented in multiple tissues, including the haematopoietic[2] and central nervous[3] systems, and the intestine[4], skin[5], cardiac muscle[6] and lung[7]. Similar to their non-transformed counterparts, tumours contain heterogeneous cell populations at various stages of differentiation, suggesting that they might be sustained by relatively undifferentiated transformed progenitors, known as cancer stem cells (CSCs)[8,9].

Recently, the stem cell-like attributes of self-renewal and multipotency have been discovered in subsets of memory T lymphocytes. In this Review, we describe evidence supporting the existence and function of T memory stem cells (T_{SCM}). We further discuss how T helper (T_H) 17 cells and interleukin-17 (IL-17)-producing $CD8^+$ T cells also exhibit stem cell-like behaviours. We high-light how signalling pathways that operate in embryonic stem cells, adult stem cells and CSCs, including WNT–β-catenin, SMAD, signal transducer and activator of transcription 3 (STAT3) and forkhead box O (FOXO) signalling, are active in subsets of T lymphocytes. Finally, we envision how triggering these pathways in tumour-

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reactive T cells or reprogramming terminally differentiated tumour-infiltrating lymphocytes (TILs) to confer stem cell-like properties might be used to augment immunotherapies against cancer and CSCs, which would be akin to fighting ‘fire with fire’.

The heterogeneity of memory T cells

Analogous to other organ systems, mature T cells are comprised of cells that are at various stages of differentiation, which are discernible by the expression of surface molecules, anatomic location and function[10–12] (FIG. 1). T cell subset diversity results from antigenic and environmental stimuli received during T cell priming and subsequent recall responses. In a primary immune response, antigen-specific naive T (T_N) cells encounter professional antigen-presenting cells (APCs) that have processed and presented tumour-associated antigens in the context of major histocompatibility complex molecules. Signals from multiple parameters, including the strength of T cell receptor (TCR), the balance of co-stimulatory and inhibitory molecules, and the quality of the inflammatory milieu, are integrated in responding T cells to initiate a programme of proliferation and differentiation, which culminates in the formation of effector T (T_{EFF}) cells[13] (FIG. 1). Depending on the strength of signalling received[13–15], T cells differentiate into distinct subsets characterized by phenotypic and functional changes that are assessable through the use of polychromatic flow cytometry[16] or, more recently, through mass cytometry[17]. Although the lineage relationship between T cell subsets remains controversial[18] (BOX 1), T cells cluster in populations that can be arranged as a progressive continuum on the basis of phenotypic, functional and transcriptional attributes[17,19] (FIG. 1).

Progressive T cell differentiation.

T_N cells are conventionally defined by the co-expression of the RA isoform of the transmembrane phosphatase CD45, the lymph node homing molecules L-selectin (CD62L) and CCR7, and the co-stimulatory receptors CD27 and CD28 (REF. 20) (FIG. 1). These phenotypic attributes facilitate T cell entry into secondary lymphoid organs to probe APCs for cognate antigen and to respond to activating signals that give rise to more differentiated memory and effector progeny[21]. T cell activation results in the expression of the RNA-binding protein heterogeneous nuclear ribonucleoprotein L-like (HNRPLL), which regulates the alternative splicing of pre-mRNA encoding CD45 to form CD45RO, the prototypical antigen-experienced T cell marker[22,23] (FIG. 1). Among CD45RO-expressing T cells, two major subsets of memory T lymphocytes can be distinguished on the basis of CD62L and CCR7 expression[24]. Similar to T_N cells, CD62L and CCR7 are maintained on central memory T (T_{CM}) cells, whereas these molecules are lost on more differentiated effector memory T (T_{EM}) cells (FIG. 1). Functionally, these phenotypic differences allow antigen-specific T_{CM} and T_{EM} cells to patrol central lymphoid organs and peripheral tissues, respectively[21,24]. The co-stimulatory receptors CD27 and CD28 are also found on the majority of memory T cells; however, expression can be lost as cells become terminally differentiated by progressively acquiring inhibitory signalling molecules, suchas killer cell lectin-like receptor subfamily G, member 1 (KLRG1)[17,25] and through transition into senescence[17,26] (FIG. 1). In contrast to T_N cells, memory T cells are capable of rapidly releasing cytokines on restimulation[27]. Although both subsets are capable of producing

tumour necrosis factor-α (TNFα), T_{CM} cells more efficiently secrete IL-2, and T_{EM} cells have an increased capacity for interferon-γ (IFNγ) release and cytotoxicity[17,24] (FIG. 1). All antigen-experienced T cells upregulate the common IL-2 and IL-15β receptor (IL-2Rβ) — conferring the ability to undergo homeostatic proliferation in response to IL-15 (REFS 28,29) — and also display high amounts of CD95 (also known as FAS)[30], a receptor that provides either co-stimulatory or pro-apoptotic signals depending on the efficiency of CD95 signalling complex formation and on which particular intracellular signalling proteins are part of the complex[31] (FIG. 1).

Recently, CD95 and IL-2Rβ have been found to be expressed in a subset of phenotypically naive-appearing T cells[19]. These cells were observed in viral and tumour-reactive T cell populations and, similar to conventional memory T cells, displayed a diluted content of TCR excision circles, possessed the ability to rapidly release cytokines on activation and proliferated in response to IL-15 (REF. 19). These cells, which are the least differentiated population of antigen-experienced T cells identified to date, were termed stem cell memory T (T_{SCM}) cells by virtue of their enhanced capacity to self-renew and their multipotent ability to generate all memory and effector T cell subsets[19].

A model of T cell differentiation in which cells proceed from T^N cells to T_{SCM}, T_{CM} and T_{EM} cells is supported not only by progressive phenotypic and functional changes, but also by findings using whole-transcriptome analyses. These data revealed that two-thirds of differentially expressed genes are progressively upregulated or downregulated in the order T_N cells to T_{SCM} cells to T_{CM} cells and finally to T_{EM} cells[19]. The expression of genes that encode transcription factors that are associated with T_N cells, including the WNT–β-catenin signalling transducers T cell factor 7 (*TCF7*) and lymphoid enhancer-binding factor 1 (*LEF1*), multiple members of the Kruppel-like factor (*KLF*) family, Forkhead box P1 (*FOXP1*) and the inhibitor of DNA-binding 3 (*ID3*), is progressively lost as cells transition from T_{SCM} to T_{CM} and T_{EM} subsets[19] (FIG. 1). Conversely, *ID2*, eomesodermin (*EOMES*), T-box 21 (*TBX21*; also known as *T–BET*), PR domain-containing 1 with ZNF domain (*PRDM1*; also known as *BLIMP–1*) and zinc finger E-box binding homeobox 2 (*ZEB2*), which each encodes key transcriptional regulators of effector differentiation are acquired in the order T_{SCM} cells to T_{CM} cells to T_{EM} cells[19] (FIG. 1). These findings suggest that $CD8^+$ T cell differentiation proceeds as a function of the graded expression of key naive or effector-associated transcription factors rather than being determined by the selective expression of subset-specific regulators.

Recently, small non-protein-coding RNAs with regulatory properties termed microRNAs (miRNAs) have been found to tune key aspects of both stem cell[32] and mature T cell functions[33–35]. Although a comprehensive profiling of miRNA expression across all naive, memory and effector T cell subsets has yet to be carried out, existing data demonstrate that subsets of miRNAs are reciprocally expressed in T_N cells, T_{EM} and T_{EFF} cells[36,37] (FIG. 1). Although little is known about the function of specific miRNAs in mature $CD8^+$ T cells, miR-29, which is expressed in T_N cells, can limit effector functions through its ability to inhibit *Eomes*, *Tbx21* and *Ifng* [33,34], whereas miR-155, which is upregulated in T_{EM} cells, is linked to the development of inflammatory T_H1 and T_H17 subsets[35] and T cell-mediated graft versus host disease[38]. These findings offer the possibility that graded changes in

miRNA expression might influence T cell differentiation that is analogous to what was observed using gene expression profiling. Thus, although multiple models have been proposed to account for the formation of different T cell subsets[18] (BOX 1), phenotypic, functional and molecular studies seem to be most consistent with a linear progressive model beginning with T_N cells and then proceeding in the order T_{SCM} cells, T_{CM} cells, T_{EM} cells, to ultimately terminate with T_{EFF} cells.

Stem cell-like features in memory T cells.

Similar to organ systems in which terminally differentiated cells are continually replaced by the progeny of less differentiated stem cells, it has been postulated that memory cells represent the stem cell-like cells of the adaptive immune system[39,40]. Several defining attributes of stem cells are indeed also present in memory T and B cells, possibly as a function of a shared core set of genes that regulate stem cell-like behaviour[41]. Like stem cells, memory lymphocytes can self-renew throughout the lifetime of the host[39,40] and they exhibit multipotency, as shown by their ability to differentiate into both effector and memory populations[39,40]. To confer diverse fates among daughter cells, stem cells undergo asymmetric cell division[42]. This process, which is also active in B and T lymphocytes during priming[43,44], has recently been proposed as a mechanism for the simultaneous generation of effector and memory daughter cells by memory T cells on secondary encounter with a pathogen[45]. Commitment to an effector fate might result from the asymmetric segregation of IL-2Rα and T-BET, two crucial drivers of effector differentiation[46,47], in daughter cells[45]. Finally, unlike most somatic cells, both stem cells and memory lymphocytes can activate telomerase to maintain telomere length and replicative potential[48,49].

Among memory T cells, T_{CM} cells were previously thought to represent the stem cell-like memory subset because of their enhanced capacity to undergo self-renewal and asymmetric division, as well as their higher replicative potential relative to T_{EM} cells, which are committed progenitor cells that are prone to terminal differentiation[39]. The identification of T_{SCM} cells repositioned T_{CM} cells as a more committed cell population in the hierarchy of T cell potency and differentiation[19,50]. The existence of T_{SCM} cells has been documented in mice, humans and non-human primates[19,51–53] (TABLE 1). This cell population is identifiable in multiple species by the expression of a core set of markers, including CD62L, CCR7, IL2Rβ, BCL-2 and the chemokine (C-X-C motif) receptor 3 (CXCR3)[19,51,52]. Multiple lines of evidence indicate that T_{SCM} cells possess stem cell-like attributes to a greater extent than any other memory lymphocyte population. Experiments using carboxyfluorescein succinimidyl ester to track cell division have demonstrated that T_{SCM} cells regenerate themselves while giving rise to more differentiated progeny in both mice and humans[19,52]. Although both T_{CM} and T_{EM} cells can also undergo self-renewal, the capacity to form diverse progeny is progressively restricted, so that only T_{SCM} cells are capable of generating all three memory subsets and T_{EFF} cells; T_{CM} cells can give rise to T_{CM}, T_{EM} and T_{EFF} cells; and T_{EM} cells can only produce themselves and T_{EFF} cells[19]. Thus, these data establish T_{SCM} cells at the apex of lineage potential among memory T cells (BOX 2). Consistent with this hierarchy, the proliferative and survival responses of memory T cell subsets to antigenic or homeostatic stimuli progressively decrease from T_{SCM} cells to T_{CM} cells and T_{EM} cells[19,52], possibly as a function of a stepwise loss of telomere

length[24,54]. Moreover, the refractoriness of T_{SCM} cells to undergo attrition in the absence of cognate antigen relative to other memory T cell subsets ensures a long-term reservoir of multipotent antigen-specific memory cells[53]. Perhaps the most compelling evidence for T_{SCM} cell stemness comes from experiments in mice showing the ability of these cells to reconstitute the full diversity of the memory T cell compartment on serial transplantation[52]. Altogether, these findings support the conclusion that stem cell-like cells exist as part of the adaptive immune system in the form of memory T lymphocytes contained in a phenotypically naive-appearing T cell compartment.

Self-renewal pathways in stem cells and T cells

Stem cells are continuously maintained in a poised state between self-renewal and differentiation[55]. What ultimately guides stem cells between these alternative fates are instructive and permissive signals that are provided by growth factors in the stem cell niche[56]. Studies of embryonic stem cells (ESCs), tissue-specific adult stem cells and CSCs have revealed that a common set of cell-surface receptors and intracellular signal transduction pathways contribute to the regulation of this balance[8,57]. Here, using ESCs as a prototypical model, we highlight how many of these same pathways are active in mature T cell subsets, promoting either self-renewal or T cell differentiation.

STAT3 and SMAD signalling.

It has long been recognized that ESCs can be maintained in an undifferentiated state in the presence of leukaemia inhibitory factor (LIF) or related cytokines acting through receptor complexes containing the GP130 signal transducer[58]. The ability of LIF to promote self-renewal depends on the downstream activation of Janus kinase (JAK) and STAT3 (REFS 59,60) (FIG. 2). Recently, LIF–STAT3 signalling has been shown to induce KLF4 and KLF5 to reinforce the pluripotency network and to promote ESC self-renewal[61]. In addition to STAT3 activation, signalling through the LIF receptor–GP130 complex can also recruit the adaptor molecule protein tyrosine phosphatase, non-receptor type 11 (also known as SHP2) to activate the MAPK pathway, which delivers pro-differentiation rather than self-renewal cues[62,63] (FIG. 2). However, bone morphogenetic proteins (BMPs) can limit MAPK-driven differentiation and enhance ESC self-renewal by inducing the expression of both dual specificity phosphatase 9 (DUSP9) [64], a MAPK phosphatase and ID proteins in a SMAD-dependent manner[65] (FIG. 2). ID proteins block ESC differentiation by antagonizing the transcriptional activity of E proteins as oversupply of transcription factor 3 (also known as E2A) abrogates the ability of ID proteins to sustain ESC maintenance[65]. Recently, Yes-associated protein (YAP), a transcriptional coactivator that is negatively regulated by the AKT and Hippo pathways, has been found to promote ESC self-renewal by enhancing ID protein expression in response to BMP–SMAD signalling[66] and by inducing numerous pluripotent genes in response to LIF through its binding to the transcription factor TEA domain (TEAD)[67,68]. These findings indicate that activation of the BMP–SMAD path-way is necessary to direct the response to LIF signalling from differentiation towards self-renewal, and that YAP is capable of potentiating both pathways.

Mature T lymphocytes can also receive environmental signals that trigger STAT3 activity. IL-21 has the unique ability among common γ-chain (γ_C) cytokines to sustain STAT3 activation[69] (FIG. 2). IL-21 has been shown to suppress the differentiation of $CD8^+$ T cells into T_{EFF} cells, maintaining a T_{SCM}-like state that is associated with high proliferative potential and long-term T cell survival[70]. Experiments in IL-21 or IL-21 receptor-deficient mice revealed that $CD8^+$ T cells undergo greater exhaustion and fail to control viral replication compared with wild-type hosts[71,72]. $CD8^+$ T cells were impaired in IL-2 production, a cytokine that is released by less differentiated T cell subsets[73], and the depletion of the STAT3 signalling cytokine IL-10 in IL-21-deficient hosts promoted further accumulation of senescent $KLRG1^+$ T cells[74].

IL-6 receptor-α (IL-6Rα) and the signal transducing chain GP130 are highly expressed in T_N and T_{SCM} cells, and they are progressively lost with T cell activation and differentiation, suggesting that IL-6-mediated activation of STAT3 might be implicated in the maintenance of less differentiated, multipotent T cells[19,75,76]. This hypothesis is supported by the finding that IL-6Rα$^+$ $CD8^+$ T cells that were isolated at the peak of a primary immune response had increased long-term survival compared with IL-6Rα− T cells[77]. IL-6Rα was not merely a marker of memory-forming potential, as activated T cells failed to generate physiological numbers of memory cells following adoptive transfer into IL-6-deficient mice[77]. Taken together, these findings indicate that STAT3 signalling cytokines, including IL-6, IL-10 and IL-21, inhibit effector T cell differentiation and exhaustion while promoting long-term memory.

The role of STAT3 signalling in the formation and maintenance of $CD8^+$ memory T cells has recently been investigated[74]. STAT3-deleted T cells have a shortened lifespan, fail to form less differentiated T_{CM} cells and have reduced expression of the memory-associated transcription factor BCL-6 compared with wild-type cells[74]. Furthermore, STAT3-deficient $CD8^+$ T cells are less able to self-renewal and are impaired in their protective capacity against a secondary infection[74]. These findings extend beyond mice, as human patients with autosomal-dominant hyper-IgE syndrome have a cell-intrinsic defect in T_{CM} formation and an impaired capacity to control intracellular viral infections[78]. As observed in ESCs, STAT3 might limit cell differentiation by inducing KLF transcription factors that promote cell quiescence and the expression of lymphoid-homing molecules in mature T lymphocytes[79,80].

Analogous to ESC biology, ID proteins have now emerged as key regulators of $CD8^+$ T cell memory formation. $CD8^+$ T cells lacking *Id3* failed to form physiological numbers of memory cells and enforced expression was sufficient to rescue long-term survival of terminally differentiated $KLRG1^+$ T cells[81,82]. As observed in ESCs, ID3 was found to mediate its effect by limiting the activity of E proteins. Indeed, deletion of E2A in $CD8^+$ T cells augmented memory T cell formation in response to a viral infection, recapitulating the phenotype caused by enforced expression of ID3 (REFS 81,83).

Similar to ESC biology, YAP has recently been shown to prevent the acquisition of senescence in $CD8^+$ T cells responding toviral infection[84]. Activationof $CD8^+$ T cells in the presence of the pro-differentiating cytokine IL-2 caused the induction of key components of

the Hippo pathways, resulting in the degradation of YAP and in the gain of differentiation-associated molecules. Conversely, ectopic expression of a YAP isoform not susceptible to Hippo-mediated negative regulation suppressed the induction of the master of regulator of terminal differentiation BLIMP1, and favoured the maintenance of IL-7Rα$^+$ and KLRG1− memory precursors. It remains to be determined whether YAP may also augment the expression of transcriptional regulators of stemness, including STAT3 and ID family members, in T cells as it does in ESCs.

Interestingly, STAT3 and SMAD signalling can be triggered by several type 17-polarizing cytokines, including IL-6, IL-21 and transforming growth factor-β (TGFβ), suggesting that these pathways might be activated in T_H 17 cells to regulate stem cell-like behaviour[85–87] (BOX 3). In summary, STAT3 signalling and ID proteins are active in mature T lymphocytes and can promote self-renewal and long-term survival.

WNT–β-catenin signalling pathway.

Numerous studies have shown that activation of WNT–β-catenin signalling (FIG. 2) is causally associated with self-renewal in ESCs[88–94]. The activity of this pathway is centred on β-catenin, which in the absence of WNT signalling is targeted for proteasome-dependent degradation by a destruction complex consisting of adenomatous polyposis coli, axin and the serine/threonine kinases casein kinase 1 and glycogen synthase kinase 3β (GSK3β)[95]. On WNT ligation to the Frizzled receptor and low-density lipoprotein co-receptors, a signalling cascade is initiated that results in the disruption of the destruction complex, leading to the accumulation and nuclear translocation of β-catenin[95] (FIG. 2). Within the nucleus, β-catenin can interact with various DNA-binding partners, notably members of the TCF/LEF family, causing chromatin remodelling and modulation of transcription[95] (FIG. 2).

Analogous to ESCs, WNT reporter systems have shown that the WNT–β-catenin pathway is functionally active in mature T lymphocytes[96]. The WNT signalling transducers *TCF7* and *LEF1* are highly expressed in T_N and T_{SCM} CD8$^+$ T cells and are lost with reiterative stimulations and progressive differentiation, which suggests a possible role in maintaining T cells in a less differentiated state[19,52,75,97]. Additionally, *Tcf7* and β-catenin are abundantly expressed in T_H17 cells (BOX 3) and in IL-17-producing CD8$^+$ T cells[85,87]. Although the role of WNT–β-catenin signalling in IL-17-producing cells has yet to be defined, emerging evidence indicates that this pathway is critically important for the formation and long-term maintenance of memory CD8$^+$ T cells[98,99]. Similar to stem cell biology, WNT3A or inhibitors of GSK3β have been shown to inhibit the differentiation of T_N cells into T_{EFF} cells while promoting the generation of self-renewing T_{SCM} and T_{CM} cells[52,100,101]. Consistent with these findings, enforced expression of a stabilized form of β-catenin inhibited T cell proliferation and the acquisition of effector functions[102]. Moreover, overexpression of TCF1 and stabilized β-catenin reduced the expansion of CD8$^+$ T cells during the effector phase of the immune response and enhanced the generation of memory T cells in several infection models[103]. Conversely, deletion of TCF1 enhanced effector differentiation, as shown by increased numbers of T cells expressing granzyme B and KLRG1 at the peak of the immune response, preventing the establishment of long-term T cell memory[104,105]. *Tcf1*-deficient memory T cells were depleted of T_{CM} cells and were

severely impaired in their ability to respond to pathogen rechallenge[104,105]. Defective T cell memory responses could be rescuedby the p45 isoformof TCF1 but not by the p33 isoform, which lacks the catenin-binding domain, indicating that TCF1 activity was dependent on its ability to bind to β-catenin[104].

Recently, the finding that WNT–β-catenin signalling regulates $CD8^+$ T cell memory has been called into question. GSK3β inhibitors were found to arrest effector differentiation even in T cells in which β-catenin was conditionally knocked out[106]. Additionally, memory formation and function were not impaired in mice with a T cell-specific deletion of β-catenin relative to wild-type controls[107]. Confounding the interpretation of these findings is the observation that WNT reporter activity is not extinguished by conditional deletion of β-catenin, suggesting that additional transducers of WNT signals compensate for β-catenin deficiency[96]. Moreover, it should be noted that a 52 kDa truncated β-catenin protein containing at least three of the seven armadillo repeats that mediate interaction with TCF is generated after cremediated deletion of exon 2 to exon 6 of the *Ctnnb1* locus, and this fragment might retain some functionality[96,108,109]. Furthermore, γ-catenin, an armadillo repeat-containing homologue of β-catenin that is also regulated by the destruction complex, can promote TCF/LEF transcriptional activity in β-catenin-deficient cells[110]. In contrast to that observed in $CD8^+$ T cells that are conditionally deficient in β-catenin[107], T cells lacking both β-catenin and γ-catenin were found to be severely compromised in mediating recall responses[104]. In summary, extensive evidence indicates that WNT–β-catenin signalling inhibits cell differentiation while promoting stemness not only in stem cells but also in mature T lymphocytes.

PI3K–AKT–mTOR signalling pathway.

mTOR is a nutrient-sensitive kinase that regulates cell growth and metabolism. mTOR functions as a central metabolic node that integrates signals from multiple sources, including cytokines and growth factors via the PI3K–AKT pathway, as well as WNT ligands through GSK3β (FIG. 2). Although mTOR activity is essential for the survival and maintenance of pluripotency in ESCs[111], increased signalling through this pathway can also drive ESC differentiation by promoting protein translation via p70 ribosomal protein S6 kinase 1 (p70S6K)[112,113]. Indeed, enhanced p70S6K activity by knockdown of tuberous sclerosis 2 (TSC2), a negative regulator of mTOR, or enforced expression of a constitutively active form of p70S6K impaired ESC self-renewal, indicating that protein synthesis is a major driver of ESC differentiation[100].

mTOR has now emerged as a crucial regulator of $CD8^+$ T cell memory. Similar to ESCs, the activity of mTOR is tightly regulated in T cells to dictate cell fate decisions. For example, high doses of the mTOR inhibitor rapamycin can abrogate $CD8^+$ T cell immune responses, whereas unrestrained mTOR activity by deletion of *Tsc1* abrogates T_N cell quiescence, resulting in T cell effector differentiation and apoptotic cell death[114,115]. Notably, modulation of mTOR activity with low doses of rapamycin has a profound quantitative and qualitative effect on memory responses, resulting in increased numbers of memory T cells, as well as the preferential formation of T_{CM} cells[114,116]. Retroviral transduction of short hairpin RNAs targeting p70S6K and eukaryotic translation initiation factor 4E (eIF4E)

recapitulated the immunostimulatory activity of rapamycin in memory formation[114], demonstrating that restraint of mTOR-mediated protein translation can enhance self-renewal and can limit differentiation not only in ESCs but also in $CD8^+$ T cells.

AKT, a serine/threonine-specific protein kinase the activity of which augments mTOR signalling, has also been found to control $CD8^+$ T cell effector and memory differentiation[117–119]. Sustained AKT function by expression of a constitutively active form of AKT induced terminal differentiation and loss of $CD8^+$ memory T cells[117,119], whereas pharmacological blockade of AKT increased $CD8^+$ memory T cell numbers by rescuing the survival of $KLRG1^+$ short-lived T_{EFF} cells[117]. AKT activity enhanced effector differentiation by promoting mTOR signalling and inhibiting FOXO1 activity through cytosolic sequestration, resulting in augmented T-BET expression and IFNγ production, as well as repression of pro-memory factors, including KLF2, IL-7Rα and BCL-6 (REFS 117,118,120–124). Recently, FOXO1 has been shown to be essential for the maintenance of ESC pluripotency[125], again emphasizing the conserved nature of many of the signalling pathways that balance self-renewal and differentiation in both ESCs and mature T cells.

Conferring stemness to T cells for therapy

Immunotherapies based on the adoptive transfer of naturally occurring or genetically redirected tumour-reactive T cells represent the best evidence of the therapeutic power of T cells. Such approachs can mediate durable complete responses in a minority of patients with advanced haematological[126–129] and solid cancers[130,131]. Why certain patients respond to T cell therapy while others do not remains poorly understood. Undoubtedly, multiple factors can influence the effect of T cell-based therapies, including tumour- and host-associated factors[132]; however, the ability of T cells to engraft and persist long-term seems to be a prerequisite for success[128,130,133,134]. T cell persistence has been highly correlated with tumour responses across multiple clinical trials and has been linked to intrinsic T cell properties that are reflective of their differentiation state and replicative history (FIG. 1). For example, longer telomere lengths[130,135], a short duration of *ex vivo* culture and a rapid expansion rate of T cells[136,137] are each significantly associated with tumour regression in patients. Additionally, the frequency of T_{CM} cells in the infusion product[138] and the expression of the co-stimulatory molecules CD28 and CD27 have been correlated with responses[130,135,139]. Altogether, these data suggest that the transfer of less differentiated T cells conveys superior antitumour efficacy relative to terminally differentiated effector cells. Experiments in mice transferring defined T cell populations at all stages of differentiation have formally proved that infusion of less differentiated T cells results in greater expansion, persistence[140,141] and tumour destruction[19,52,70,120,142–147]. Paralleling their engraftment and proliferative potentials, the ability of memory T cells to mediate tumour regression progressively decreases from T_{SCM} cells to T_{CM} cells and T_{EM} cells[19,52,144,147] (BOX 2). Notably, the robust proliferative potential, long-term survival capacity and the ability to give rise to all memory and effector T cell subsets allows T_{SCM} cells to mediate highly effective tumour regression when limited numbers of cells are transferred, a condition in which other memory T cell subsets have little or no impact[19,52]. Although tumour eradication is likely to involve multiple components of the innate and adaptive immune systems, maintaining a sustained immunological attack against tumour masses by transferring cells with stem cell-

like properties might represent the most efficient approach for directly and indirectly destroying every cancer cell, including CSCs (FIG. 3). Thus, finding strategies that generate and expand T_{SCM}-like cells is pivotal to the development of the next generation of highly effective T cell-based immunotherapies.

Arresting T cell differentiation.

Current methods used to produce T cells for adoptive immunotherapy often rely on variations of a strategy developed more than 20 years ago[148,149], before the implication of T cell differentiation on *in vivo* tumour efficacy was fully appreciated[150]. This approach is dependent on potent activating stimuli, including monoclonal antibodies to CD3, high concentrations of IL-2 and allogeneic feeder cells that allow for the generation of large numbers of tumour-reactive T cells but that inexorably drive T cells towards terminal differentiation and senescence. To limit the detrimental influence of *ex vivo* expansion on T cell differentiation, new methodologies have been explored, including the use of common γ_C cytokines other than IL-2, and small molecules that target key metabolic and developmental pathways[151] (FIG. 4a).

IL-15 can sustain T cell proliferation without the robust pro-differentiating activity that characterizes IL-2. Although IL-2 promotes T cell differentiation into T_{EFF} and T_{EM}-like cells, priming of T cells in the presence of IL-15 results in the generation of T cells with the phenotypic, functional, metabolic and gene expression attributes found in naturally arising T_{CM} cells[116,143,144,152–154]. Accordingly, tumour-reactive T cells mediated greater antitumour responses when generated in the presence of IL-15 than in the presence of IL-2 (REF. 144). More recently, several groups have evaluated the activity of another γ_C cytokine IL-21 on the expansion and differentiation of tumour-specific $CD8^+$ T cells[70,155–157]. IL-21 profoundly inhibits T cell differentiation, allowing for the generation of T_{SCM}-like T cells. In mouse T cells, the use of IL-21, in contrast to IL-2, caused a dose-dependent blockade of the acquisition of the antigen-experience marker CD44 and lytic capacity while preserving the expression of *Tcf7*, *Lef1* and CD62L and while maintaining the ability to secrete IL-2 (REF. 70). Similarly, the expansion of human tumour-reactive $CD45RA^+$ T cells in the presence of IL-21 prevented the loss of CD45RA, CD62L, CD28, CD27 and IL-7Rα and also retained the ability of the cell to release IL-2 (REFS 156,157). Most importantly, T cells primed and expanded in the presence of IL-21 exhibit enhanced antitumour activity compared with cells grown in other γ_C cytokines[70].

Emerging evidence indicates that the commitment of a cell between effector or memory fates is regulated by evolutionarily conserved metabolic and developmental pathways that integrate multiple signal inputs from cell surface receptors, including TCR, cytokine, co-stimulatory and growth factor receptors[98,151,158–160]. Rational modulation of these pathways by small molecules provides an attractive means to alter T cell differentiation and to enhance the fitness of T cells for therapeutic use (FIG. 4a). As many of these molecules have already been approved for other purposes in patients, the use of these drugs to enhance T cell-based therapies can be rapidly incorporated into new clinical trials. For example, the mTOR inhibitor rapamycin, a drug that is currently used to facilitate solid organ and HSC transplantation[160], not only enhances the formation of $CD8^+$ memory T cells but also

augments their antitumour functions[120]. Similarly, metformin, an AMPK agonist used to treat type 2 diabetes, improves T cell survival, recall responses and *in vivo* antitumour treatment[116]. Finally, inhibitors of GSK3β that are under clinical evaluation for Alzheimer's disease and other neurodegenerative diseases[161] can be repurposed to potentiate the WNT–β-catenin signalling pathway in T cells to generate self-renewing multipotent T_{SCM}-like cells[19,52]. Although these reagents are effective at withholding T cell differentiation and potentiating *in vivo* antitumour functions, they also inhibit T cell proliferation. For this reason, the identification of molecules that uncouple the processes of cell expansion and differentiation is desirable. Recently, pharmacological inhibition of the AKT isoforms AKT1 and AKT2 has been shown to inhibit the acquisition of effector molecules and function while preserving a T_{CM}-like phenotype and migratory capacity without a detrimental effect on cell yield[118]. Thus, inhibitors of AKT might allow for the generation of large numbers of minimally differentiated tumour-reactive T cells for therapeutic purposes.

Reprogramming terminally differentiated T cells.

Chronic antigen stimulation in tumour-bearing hosts can drive tumour-specific T cells towards a state of terminal differentiation and exhaustion[162–164]. Although TILs can be reactivated and expanded *in vitro* in the presence of immunostimulatory cytokines, these cells, although sometimes effective, are currently incapable of mediating durable complete responses in most patients[130]. The successful derivation of pluripotent stem cells from mature fibroblasts by the ectopic co-expression of crucial ESC transcription factors[165,166] or miRNAs[167] has powerfully demonstrated how cell fates can be altered by the manipulation of a few key transcriptional regulators, paving the way for the possibility of reprogramming terminally differentiated TILs into highly effective, stem cell-like tumour-reactive T cells (FIG. 4b,c).

Since Yamanaka's breakthrough study, numerous groups have shown that induced pluripotent stem (iPS) cells can be produced from different somatic cells, including T lymphocytes by enforced expression of the *OCT4*, *SOX2*, *KLF4* and *MYC* transcription factors[168–171]. Importantly, T cell-derived iPS cells maintain the rearranged variable (V), diversity (D) and joining regions (J) of the TCR chains, indicating that iPS cells generated from TILs could retain their antitumour reactivity. Recent insights into the nature of instructive signaling required for T cell development during thymopoiesis has led to the development of *ex vivo* methods that support the generation of T cells from ESC[172], HSC[173–175] and iPS cells[176] providing the tools for re-differentiating TIL-derived iPS cells. Although conceptually attractive and theoretically feasible, this two-step reprogramming approach (FIG. 4b) is currently inefficient both in terms of the frequency of cells successfully reprogrammed and the duration necessary to achieve full reprogramming.

To overcome these limitations, a single approach to directly reprogramme terminally differentiated TILs into more naive, stem cell-like T cells might be possible. A number of reports have shown that direct reprogramming can be used to differentiate various mature cell types into alternative differentiated tissues such as neurons[177,178], cardiomyocytes[179], blood progenitors[180] and hepatocytes[181] by enforced expression of tissue-specific transcription factors. Adapting this approach, ectopic expression of key T_N and T_{SCM}-

associated transcription factors or miRNAs might result in the intra-lineage reprogramming of terminally differentiated T_{EFF} cells into less differentiated T cell subsets (FIG. 4c)

Concluding remarks

It is now clear that subsets of mature T cells exist that are endowed with the stem cell-like attributes of self-renewal, multipotency and the ability to undergo asymmetric division. Similar to conventional stem cells, evolutionarily conserved pathways regulating stemness are active in antigen-experienced T cells, especially T_{SCM} cells, T_H17 cells and IL-17-producing $CD8^+$ T cells. As T cells transition through progressive stages of differentiation, they undergo a stepwise loss of stem cell-associated attributes, including proliferative potential, survival fitness and multipotency. Collectively, these functional changes result in cells that are therapeutically less efficacious upon adoptive transfer. Understanding the epigenetic, genetic and metabolic programmes that regulate T cell self-renewal and persistence provides the ability to pharmacologically or genetically confer stemness to tumour-specific T cells.

Building on recent technologies that have allowed the reprogramming of differentiated somatic cells into iPS cells or cell types of alternative lineages, it is now possible to envision dedifferentiating senescent tumour-reactive T cells from a cancer patient to generate antitumour T cells with improved fitness and therapeutic efficacy for adoptive T cell transfer. The application of regenerative medicine technology to T cell therapies for the treatment of cancer patients using iPS-derived or reprogrammed T cells has several advantages. Terminally ill patients with advanced cancer that is refractory to existing therapies have a favourable risk/benefit ratio profile for receiving reprogrammed cells that might have oncogenic potential[182]. Moreover, safety might be ensured by introducing suicide genes in reprogrammed cells to enable the elimination of infused cells if transformation or other adverse events occur[183,184]. In addition, lymphocytes are motile circulating cells that are capable of autonomously finding their targets, so they do not suffer the ‘anatomical’ problems that plague regenerative medicine efforts in other organ systems, in which fine interactions between cell types are required for proper functioning[185]. Finally, the routine use of immune ablation before adoptive immunotherapy[150] might avoid immune-mediated rejection of reprogrammed cells[186]. In summary, preserving and regenerating stem cell-like qualities in T cells may finally enable cancer immunotherapists to fight ‘fire with fire’ with ever increasing effectiveness.

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Glossary

Self-renewal
A biological process by which a cell gives rise to one or two daughter cells that have a developmental potential that is indistinguishable from that of the mother cell.

Multipotency
The potential for a cell to give rise to progeny with the capacity to form multiple, but not all possible, lineages.

Tumour-infiltrating lymphocytes (TILs).
The heterogeneous population of T cells found in a tumour bed. These cells are characterized by a diversity of phenotypes, antigen specificities, avidities and functional characteristics. They can be activated and expanded *ex vivo* and reinfused into a tumour-bearing host to mediate tumour regression.

Mass cytometry
Also known as cytometry by time-of-flight (CyTOF). A platform that couples flow cytometry with mass spectrometry. This technique enables the simultaneous evaluation of at least 45 simultaneous phenotypic and functional parameters on a single cell without the use of fluorescent agents or interference from spectral overlap.

Senescence
A biological process by which cells undergo growth arrest after extensive replication.

Homeostatic proliferation
A process of activation and proliferation of leukocytes in a lymphopaenic environment. T cell homeostatic proliferation is driven by T cell receptor interactions with self-peptide–MHC complexes and responsiveness to homeostatic cytokines such as interleukin-7 (IL-7), IL-15 and possibly IL-21.

TCR excision circles (TRECs).
Circular, stable extra-chromosomal DNA fragments that are generated during recombination of variable (V), diversity (D) and joining regions (J) of the T cell receptor. TRECs do not replicate with cellular proliferation and are thus diluted with every cell division, allowing the assessment of the replicative history of a T cell.

Asymmetric cell division
A conserved mechanism by which a cell divides into daughter cells of unequal size and cytoplasmic content, thus conferring differential developmental fates to progeny cells.

Telomere
The segment at the end of chromosomal arms consisting of a series of repeated DNA sequences (TTAGGG in all vertebrates) that regulates chromosomal replication at each cell division.

Stem cell niche

A specialized microenvironment containing stem cells that supports their maintenance and regulates their function.

Common γ-chain (γ_C).
A signalling subunit common to the receptors for interleukin-2 (IL-2), IL-4, IL-7, IL-9, IL-15 and IL-21.

Exhaustion
A state of T cell dysfunction arising during reiterative antigen stimulations such as chronic infections and cancer. It is defined by poor effector function and proliferative response to antigenic stimuli, expression of inhibitory receptors and a transcriptional state that is distinct from that of functional effector or memory T cells.

Autosomal-dominant hyper-IgE syndrome (AD-HIES).
Also known as Job's syndrome. A rare primary immunodeficiency characterized by recurrent skin abscesses, cyst-forming pneumonias and extreme increases of serum IgE levels. Most AD-HIES cases are caused by dominant-negative mutations in *STAT3*.

Epigenetic modifications
Heritable molecular alterations of the genome that do not involve changes to the nucleotide sequence that regulates gene or microRNA expression. They include DNA methylation, histone modifications and nucleosome positioning.

Induced pluripotent stem (iPS) cells
Pluripotent stem cells artificially derived from non-pluripotent cells, such as an adult somatic cell by forced expression of specific genes or microRNAs.

Suicide genes
Genes capable of selectively eliminating the cells into which they have been transduced following the administration of a drug.

References

1. Weissman IL Stem cells: units of development, units of regeneration, and units in evolution. Cell 100, 157–168 (2000). [PubMed: 10647940]
2. Spangrude GJ, Heimfeld S & Weissman IL Purification and characterization of mouse hematopoietic stem cells. Science 241, 58–62 (1988). [PubMed: 2898810]
3. Reynolds BA & Weiss S Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. Science 255, 1707–1710 (1992). [PubMed: 1553558]
4. Barker N et al. Identification of stem cells in small intestine and colon by marker gene Lgr5. Nature 449, 1003–1007 (2007). [PubMed: 17934449]
5. Sun TT & Green H Differentiation of the epidermal keratinocyte in cell culture: formation of the cornified envelope. Cell 9, 511–521 (1976). [PubMed: 1009573]
6. Beltrami AP et al. Adult cardiac stem cells are multipotent and support myocardial regeneration. Cell 114, 763–776 (2003). [PubMed: 14505575]
7. Rawlins EL et al. The role of Scgb1a1+ Clara cells in the long-term maintenance and repair of lung airway, but not alveolar, epithelium. Cell Stem Cell 4, 525–534 (2009). [PubMed: 19497281]
8. Reya T, Morrison SJ, Clarke MF & Weissman IL Stem cells, cancer, and cancer stem cells. Nature 414, 105–111 (2001). [PubMed: 11689955]

9. Nguyen LV, Vanner R, Dirks P & Eaves CJ Cancer stem cells: an evolving concept. Nature Rev. Cancer 12, 133–143 (2012). [PubMed: 22237392]
10. Kaech SM, Wherry EJ & Ahmed R Effector and memory T-cell differentiation: implications for vaccine development. Nature Rev. Immunol 2, 251–262 (2002). [PubMed: 12001996]
11. Sallusto F, Geginat J & Lanzavecchia A Central memory and effector memory T cell subsets: function, generation, and maintenance. Annu. Rev. Immunol 22, 745–763 (2004). [PubMed: 15032595]
12. Klebanoff CA, Gattinoni L & Restifo NP CD8+ T-cell memory in tumor immunology and immunotherapy. Immunol. Rev 211, 214–224 (2006). [PubMed: 16824130]
13. Gett AV, Sallusto F, Lanzavecchia A & Geginat J T cell fitness determined by signal strength. Nature Immunol 4, 355–360 (2003). [PubMed: 12640450]
14. D'Souza WN & Hedrick SM Cutting edge: latecomer CD8 T cells are imprinted with a unique differentiation program. J. Immunol 177, 777–781 (2006). [PubMed: 16818730]
15. Sarkar S et al. Functional and genomic profiling of effector CD8 T cell subsets with distinct memory fates. J. Exp. Med 205, 625–640 (2008). [PubMed: 18316415]
16. Chattopadhyay PK et al. Quantum dot semiconductor nanocrystals for immunophenotyping by polychromatic flow cytometry. Nature Med 12, 972–977 (2006). [PubMed: 16862156]
17. Newell EW, Sigal N, Bendall SC, Nolan GP & Davis MM Cytometry by time-of-flight shows combinatorial cytokine expression and virus-specific cell niches within a continuum of CD8+ T cell phenotypes. Immunity 36, 142–152 (2012). [PubMed: 22265676]
18. Ahmed R, Bevan MJ, Reiner SL & Fearon DT The precursors of memory: models and controversies. Nature Rev. Immunol 9, 662–668 (2009). [PubMed: 19680250]
19. Gattinoni L et al. A human memory T cell subset with stem cell-like properties. Nature Med 17, 1290–1297 (2011). [PubMed: 21926977] This paper identifies a new subset of memory T cells with enhanced stem cell-like properties in humans and shows that these cells mediate a superior antitumour response in a mouse model of adoptive T cell therapy.
20. De Rosa SC, Herzenberg LA, Herzenberg LA & Roederer M 11-color, 13-parameter flow cytometry: identification of human naive T cells by phenotype, function, and T-cell receptor diversity. Nature Med 7, 245–248 (2001). [PubMed: 11175858]
21. Weninger W, Crowley MA, Manjunath N & von Andrian UH Migratory properties of naive, effector, and memory CD8+ T cells. J. Exp. Med 194, 953–966 (2001). [PubMed: 11581317]
22. Oberdoerffer S et al. Regulation of CD45 alternative splicing by heterogeneous ribonucleoprotein, hnRNPLL. Science 321, 686–691 (2008). [PubMed: 18669861]
23. Wu Z et al. Memory T cell RNA rearrangement programmed by heterogeneous nuclear ribonucleoprotein hnRNPLL. Immunity 29, 863–875 (2008). [PubMed: 19100700]
24. Sallusto F, Lenig D, Forster R, Lipp M & Lanzavecchia A Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. Nature 401, 708–712 (1999). [PubMed: 10537110]
25. Henson SM et al. KLRG1 signaling induces defective Akt (ser473) phosphorylation and proliferative dysfunction of highly differentiated CD8+ T cells. Blood 113, 6619–6628 (2009). [PubMed: 19406987]
26. Romero P et al. Four functionally distinct populations of human effector-memory CD8+ T lymphocytes. J. Immunol 178, 4112–4119 (2007). [PubMed: 17371966]
27. Kambayashi T, Assarsson E, Lukacher AE, Ljunggren HG & Jensen PE Memory CD8+ T cells provide an early source of IFN-γ. J. Immunol 170, 2399–2408 (2003). [PubMed: 12594263]
28. Judge AD, Zhang X, Fujii H, Surh CD & Sprent J Interleukin 15 controls both proliferation and survival of a subset of memory-phenotype CD8+ T cells. J. Exp. Med 196, 935–946 (2002). [PubMed: 12370255]
29. Tan JT et al. Interleukin (IL)-15 and IL-7 jointly regulate homeostatic proliferation of memory phenotype CD8+ cells but are not required for memory phenotype CD4+ cells. J. Exp. Med 195, 1523–1532 (2002). [PubMed: 12070280]
30. Hamann D et al. Phenotypic and functional separation of memory and effector human CD8+ T cells. J. Exp. Med 186, 1407–1418 (1997). [PubMed: 9348298]

31. Siegel RM, Chan FK, Chun HJ & Lenardo MJ The multifaceted role of Fas signaling in immune cell homeostasis and autoimmunity. Nature Immunol 1, 469–474 (2000). [PubMed: 11101867]
32. Stadler BM & Ruohola-Baker H Small RNAs: keeping stem cells in line. Cell 132, 563–566 (2008). [PubMed: 18295575]
33. Ma F et al. The microRNA miR-29 controls innate and adaptive immune responses to intracellular bacterial infection by targeting interferon-γ. Nature Immunol 12, 861–869 (2011). [PubMed: 21785411]
34. Steiner DF et al. MicroRNA-29 regulates T-box transcription factors and interferon-γ production in helper T cells. Immunity 35, 169–181 (2011). [PubMed: 21820330]
35. O'Connell RM et al. MicroRNA-155 promotes autoimmune inflammation by enhancing inflammatory T cell development. Immunity 33, 607–619 (2010). [PubMed: 20888269]
36. Wu H et al. miRNA profiling of naive, effector and memory CD8 T cells. PLoS ONE 2, e1020 (2007). [PubMed: 17925868]
37. Salaun B et al. Differentiation associated regulation of microRNA expression in vivo in human $CD8^+$ T cell subsets. J. Transl. Med 9, 44 (2011). [PubMed: 21507256]
38. Ranganathan P et al. Regulation of acute graft-versus-host disease by microRNA-155. Blood 119, 4786–4797 (2012). [PubMed: 22408260]
39. Fearon DT, Manders P & Wagner SD Arrested differentiation, the self-renewing memory lymphocyte, and vaccination. Science 293, 248–250 (2001). [PubMed: 11452114] This perspective article is the first to draw a parallel between stem cells and memory B and T lymphocytes.
40. Stemberger C et al. Stem cell-like plasticity of naive and distinct memory CD8+ T cell subsets. Semin. Immunol 21, 62–68 (2009). [PubMed: 19269852]
41. Luckey CJ et al. Memory T and memory B cells share a transcriptional program of self-renewal with long-term hematopoietic stem cells. Proc. Natl Acad. Sci. USA 103, 3304–3309 (2006). [PubMed: 16492737] This paper describes a common transcriptional signature shared between memory T cells, memory B cells and long-term haematopoietic stem cells, providing systems biology evidence for Fearon's hypothesis.
42. Knoblich JA Mechanisms of asymmetric stem cell division. Cell 132, 583–597 (2008). [PubMed: 18295577]
43. Chang JT et al. Asymmetric T lymphocyte division in the initiation of adaptive immune responses. Science 315, 1687–1691 (2007). [PubMed: 17332376] This paper provides the first evidence that asymmetric cell division can occur in mature T cells to confer memory and effector cell fates to daughter cells.
44. Barnett BE et al. Asymmetric B cell division in the germinal center reaction. Science 335, 342–344 (2012). [PubMed: 22174128]
45. Ciocca ML, Barnett BE, Burkhardt JK, Chang JT & Reiner SL Cutting edge: asymmetric memory T cell division in response to rechallenge. J. Immunol 188, 4145–4148 (2012). [PubMed: 22467651]
46. Kalia V et al. Prolonged interleukin-2Rα expression on virus-specific CD8+ T cells favors terminal-effector differentiation *in vivo*. Immunity 32, 91–103 (2010). [PubMed: 20096608]
47. Joshi NS et al. Inflammation directs memory precursor and short-lived effector CD8+ T cell fates via the graded expression of T-BET transcription factor. Immunity 27, 281–295 (2007). [PubMed: 17723218]
48. Morrison SJ, Prowse KR, Ho P & Weissman IL Telomerase activity in hematopoietic cells is associated with self-renewal potential. Immunity 5, 207–216 (1996). [PubMed: 8808676]
49. Hodes RJ, Hathcock KS & Weng NP Telomeres in T and B cells. Nature Rev. Immunol 2, 699–706 (2002). [PubMed: 12209138]
50. Sallusto F & Lanzavecchia A Memory in disguise. Nature Med 17, 1182–1183 (2011). [PubMed: 21988989]
51. Zhang Y, Joe G, Hexner E, Zhu J & Emerson SG Host-reactive CD8+ memory stem cells in graft-versus-host disease. Nature Med 11, 1299–1305 (2005). [PubMed: 16288282] This paper describes a memory stem cell-like CD8+ T cell population in a mouse model of graft-versus-host disease.

52. Gattinoni L et al. Wnt signaling arrests effector T cell differentiation and generates $CD8^+$ memory stem cells. Nature Med 15, 808–813 (2009). [PubMed: 19525962]
53. Lugli E, Gattinoni L, Restifo NP & Roederer M The role of T memory stem cells: pathogenesis and vaccines. J. Acquir. Immune Defic. Syndr 59, 59 (2012). [PubMed: 21926635]
54. Papagno L et al. Immune activation and CD8+ T-cell differentiation towards senescence in HIV-1 infection. PLoS. Biol 2, e20 (2004). [PubMed: 14966528]
55. Chang HH, Hemberg M, Barahona M, Ingber DE & Huang S Transcriptome-wide noise controls lineage choice in mammalian progenitor cells. Nature 453, 544–547 (2008). [PubMed: 18497826]
56. Morrison SJ & Spradling AC Stem cells and niches: mechanisms that promote stem cell maintenance throughout life. Cell 132, 598–611 (2008). [PubMed: 18295578]
57. Eckfeldt CE, Mendenhall EM & Verfaillie CM The molecular repertoire of the 'almighty' stem cell. Nature Rev. Mol. Cell. Biol 6, 726–737 (2005). [PubMed: 16103873]
58. Williams RL et al. Myeloid leukaemia inhibitory factor maintains the developmental potential of embryonic stem cells. Nature 336, 684–687 (1988). [PubMed: 3143916]
59. Niwa H, Burdon T, Chambers I & Smith A Self-renewal of pluripotent embryonic stem cells is mediated via activation of STAT3. Genes Dev 12, 2048–2060 (1998). [PubMed: 9649508]
60. Matsuda T et al. STAT3 activation is sufficient to maintain an undifferentiated state of mouse embryonic stem cells. EMBO J 18, 4261–4269 (1999). [PubMed: 10428964]
61. Hall J et al. Oct4 and LIF/Stat3 additively induce Kruppel factors to sustain embryonic stem cell self-renewal. Cell Stem Cell 5, 597–609 (2009). [PubMed: 19951688]
62. Burdon T, Stracey C, Chambers I, Nichols J & Smith A Suppression of SHP-2 and ERK signalling promotes self-renewal of mouse embryonic stem cells. Dev. Biol 210, 30–43 (1999). [PubMed: 10364425]
63. Qu CK & Feng GS Shp-2 has a positive regulatory role in ES cell differentiation and proliferation. Oncogene 17, 433–439 (1998). [PubMed: 9696036]
64. Li Z et al. BMP4 Signaling Acts via dual-specificity phosphatase 9 to control ERK activity in mouse embryonic stem cells. Cell Stem Cell 10, 171–182 (2012). [PubMed: 22305567]
65. Ying QL, Nichols J, Chambers I & Smith A BMP induction of Id proteins suppresses differentiation and sustains embryonic stem cell self-renewal in collaboration with STAT3. Cell 115, 281–292 (2003). [PubMed: 14636556]
66. Alarcon C et al. Nuclear CDKs drive Smad transcriptional activation and turnover in BMP and TGF-β pathways. Cell 139, 757–769 (2009). [PubMed: 19914168]
67. Lian I et al. The role of YAP transcription coactivator in regulating stem cell self-renewal and differentiation. Genes Dev 24, 1106–1118 (2010). [PubMed: 20516196]
68. Tamm C, Bower N & Anneren C Regulation of mouse embryonic stem cell self-renewal by a Yes-YAP-TEAD2 signaling pathway downstream of LIF. J. Cell Sci 124, 1136–1144 (2011). [PubMed: 21385842]
69. Leonard WJ & Spolski R Interleukin-21: a modulator of lymphoid proliferation, apoptosis and differentiation. Nature Rev. Immunol 5, 688–698 (2005). [PubMed: 16138102]
70. Hinrichs CS et al. IL-2 and IL-21 confer opposing differentiation programs to CD8+ T cells for adoptive immunotherapy. Blood 111, 5326–5333 (2008). [PubMed: 18276844]
71. Yi JS, Du M & Zajac AJ A vital role for interleukin-21 in the control of a chronic viral infection. Science 324, 1572–1576 (2009). [PubMed: 19443735]
72. Frohlich A et al. IL-21R on T cells is critical for sustained functionality and control of chronic viral infection. Science 324, 1576–1580 (2009). [PubMed: 19478140]
73. Yi JS, Ingram JT & Zajac AJ IL-21 deficiency influences CD8 T cell quality and recall responses following an acute viral infection. J. Immunol 185, 4835–4845 (2010). [PubMed: 20844201]
74. Cui W, Liu Y, Weinstein JS, Craft J & Kaech SM An interleukin-21-interleukin-10-STAT3 pathway is critical for functional maturation of memory CD8+ T cells. Immunity 35, 792–805 (2011). [PubMed: 22118527]
75. Wirth TC et al. Repetitive antigen stimulation induces stepwise transcriptome diversification but preserves a core signature of memory CD8+ T cell differentiation. Immunity 33, 128–140 (2010). [PubMed: 20619696]

76. Betz UA & Muller W Regulated expression of gp130 and IL-6 receptor α chain in T cell maturation and activation. Int. Immunol 10, 1175–1184 (1998). [PubMed: 9723704]
77. Castellino F & Germain RN Chemokine-guided CD4+ T cell help enhances generation of IL-6RαhighIL-7Rα high prememory CD8+ T cells. J. Immunol 178, 778–787 (2007). [PubMed: 17202339]
78. Siegel AM et al. A critical role for STAT3 transcription factor signaling in the development and maintenance of human T cell memory. Immunity 35, 806–818 (2011). [PubMed: 22118528] Together with reference 74, this paper identifies STAT3 as a key transcription factor required for the formation and maintenance of T cell memory in mice and humans.
79. Yamada T, Park CS, Mamonkin M & Lacorazza HD Transcription factor ELF4 controls the proliferation and homing of CD8+ T cells via the Kruppel-like factors KLF4 and KLF2. Nature Immunol 10, 618–626 (2009). [PubMed: 19412182]
80. Weinreich MA et al. KLF2 transcription-factor deficiency in T cells results in unrestrained cytokine production and upregulation of bystander chemokine receptors. Immunity 31, 122–130 (2009). [PubMed: 19592277]
81. Ji Y et al. Repression of the DNA-binding inhibitor Id3 by Blimp-1 limits the formation of memory CD8+ T cells. Nature Immunol 12, 1230–1237 (2011). [PubMed: 22057288]
82. Yang CY et al. The transcriptional regulators Id2 and Id3 control the formation of distinct memory CD8+ T cell subsets. Nature Immunol 12, 1221–1229 (2011). [PubMed: 22057289] References 81 and 82 show that the DNA-binding inhibitor ID3 is crucial for the formation of long-lived memory.
83. D'Cruz LM, Lind KC, Wu BB, Fujimoto JK & Goldrath AW Loss of E protein transcription factors E2A and HEB delays memory-precursor formation during the CD8+ T-cell immune response. Eur. J Immunol 42, 2031–2041 (2012). [PubMed: 22585759]
84. Thaventhiran JE et al. Activation of the Hippo pathway by CTLA-4 regulates the expression of Blimp-1 in the CD8+ T cell. Proc. Natl Acad. Sci. USA 27 6 2012 (doi:10.1073/pnas. 1209115109).
85. Muranski P et al. Th17 cells are long lived and retain a stem cell-like molecular signature. Immunity 35, 972–985 (2011). [PubMed: 22177921]
86. Luckey CJ & Weaver CT Stem-cell-like qualities of immune memory; CD4+ T cells join the party. Cell Stem Cell 10, 107–108 (2012). [PubMed: 22305557]
87. Kryczek I et al. Human TH17 cells are long-lived effector memory cells. Sci. Transl. Med 3, 104ra100 (2011).This paper, along with reference 85, provides evidence that TH17 cells are a long-lived memory T cell population that retain stem cell-like attributes in mice and humans.
88. Wray J & Hartmann C WNTing embryonic stem cells. Trends Cell Biol 22, 159–168 (2012). [PubMed: 22196214]
89. ten BD et al. Embryonic stem cells require Wnt proteins to prevent differentiation to epiblast stem cells. Nature Cell Biol 13, 1070–1075 (2011). [PubMed: 21841791]
90. Sato N, Meijer L, Skaltsounis L, Greengard P & Brivanlou AH Maintenance of pluripotency in human and mouse embryonic stem cells through activation of Wnt signaling by a pharmacological GSK-3-specific inhibitor. Nature Med 10, 55–63 (2004). [PubMed: 14702635]
91. Kielman MF et al. Apc modulates embryonic stem-cell differentiation by controlling the dosage of β-catenin signaling. Nature Genet 32, 594–605 (2002). [PubMed: 12426568]
92. Doble BW, Patel S, Wood GA, Kockeritz LK & Woodgett JR Functional redundancy of GSK-3α and GSK-3β in Wnt/β-catenin signaling shown by using an allelic series of embryonic stem cell lines. Dev. Cell 12, 957–971 (2007). [PubMed: 17543867]
93. Wray J et al. Inhibition of glycogen synthase kinase-3 alleviates Tcf3 repression of the pluripotency network and increases embryonic stem cell resistance to differentiation. Nature Cell Biol 13, 838–845 (2011). [PubMed: 21685889]
94. Yi F et al. Opposing effects of Tcf3 and Tcf1 control Wnt stimulation of embryonic stem cell self-renewal. Nature Cell Biol 13, 762–770 (2011). [PubMed: 21685894]
95. Clevers H Wnt/β-catenin signaling in development and disease. Cell 127, 469–480 (2006). [PubMed: 17081971]
96. Jeannet G et al. Long-term, multilineage hematopoiesis occurs in the combined absence of β-catenin and γ-catenin. Blood 111, 142–149 (2008). [PubMed: 17906078]

97. Willinger T et al. Human naive CD8 T cells down-regulate expression of the WNT pathway transcription factors lymphoid enhancer binding factor 1 and transcription factor 7 (T cell factor-1) following antigen encounter in vitro and *in vivo*. J. Immunol 176, 1439–1446 (2006). [PubMed: 16424171]

98. Gattinoni L, Ji Y & Restifo NP Wnt/β-catenin signaling in T-cell immunity and cancer immunotherapy. Clin. Cancer Res 16, 4695–4701 (2010). [PubMed: 20688898]

99. Xue HH & Zhao DM Regulation of mature T cell responses by the Wnt signaling pathway. Ann. NY Acad. Sci 1247, 16–33 (2012). [PubMed: 22239649]

100. Gattinoni L Ji, Y. & Restifo, N. P. β-catenin does not regulate memory T cell phenotype Reply. Nature Med 16, 514–515 (2010).

101. Muralidharan S et al. Activation of Wnt signaling arrests effector differentiation in human peripheral and cord blood-derived T lymphocytes. J. Immunol 187, 5221–5232 (2011). [PubMed: 22013128]

102. Driessens G et al. β-catenin inhibits T cell activation by selective interference with linker for activation of T cells-phospholipase C-γ1 phosphorylation. J. Immunol 186, 784–790 (2011). [PubMed: 21149602]

103. Zhao DM et al. Constitutive activation of Wnt signaling favors generation of memory CD8 T cells. J. Immunol 184, 1191–1199 (2010). [PubMed: 20026746]

104. Jeannet G et al. Essential role of the Wnt pathway effector Tcf-1 for the establishment of functional CD8 T cell memory. Proc. Natl Acad. Sci. USA 107, 9777–9782 (2010). [PubMed: 20457902]

105. Zhou X et al. Differentiation and persistence of memory CD8+ T cells depend on T cell factor 1. Immunity 33, 229–240 (2010). [PubMed: 20727791] References 104 and 105 show that TCF1–β-catenin signalling is essential for the establishment and maintenance of functional CD8+ T cell memory.

106. Driessens G, Zheng Y & Gajewski TF β-catenin does not regulate memory T cell phenotype. Nature Med 16, 513–514 (2010). [PubMed: 20448567]

107. Prlic M & Bevan MJ Cutting edge: β-catenin is dispensable for T cell effector differentiation, memory formation, and recall responses. J. Immunol 187, 1542–1546 (2011). [PubMed: 21724993]

108. Huelsken J & Held W Canonical Wnt signalling plays essential roles. Eur. J. Immunol 39, 3582–3583 (2009). [PubMed: 19941311]

109. Staal FJ & Sen JM Canonical Wnt signalling plays essential roles. Author reply. Eur. J. Immunol 39, 3583–3584 (2009).

110. Maeda O et al. Plakoglobin (γ-catenin) has TCF/LEF family-dependent transcriptional activity in β-catenin-deficient cell line. Oncogene 23, 964–972 (2004). [PubMed: 14661054]

111. Murakami M et al. mTOR is essential for growth and proliferation in early mouse embryos and embryonic stem cells. Mol. Cell. Biol 24, 6710–6718 (2004). [PubMed: 15254238]

112. Sampath P et al. A hierarchical network controls protein translation during murine embryonic stem cell self-renewal and differentiation. Cell Stem Cell 2, 448–460 (2008). [PubMed: 18462695]

113. Easley CA et al. mTOR-mediated activation of p70 S6K induces differentiation of pluripotent human embryonic stem cells. Cell Reprogram 12, 263–273 (2010). [PubMed: 20698768]

114. Araki K et al. mTOR regulates memory CD8 T-cell differentiation. Nature 460, 108–112 (2009). [PubMed: 19543266]

115. Yang K, Neale G, Green DR, He W & Chi H The tumor suppressor Tsc1 enforces quiescence of naive T cells to promote immune homeostasis and function. Nature Immunol 12, 888–897 (2011). [PubMed: 21765414]

116. Pearce EL et al. Enhancing CD8 T-cell memory by modulating fatty acid metabolism. Nature 460, 103–107 (2009). [PubMed: 19494812] Together with reference 114, this paper provides evidence that the commitment of a T cell between effector or memory fates is regulated by key, pharmacologically targetable, metabolic pathways.

117. Kim EH et al. Signal integration by Akt regulates CD8 T cell effector and memory differentiation. J. Immunol 188, 4305–4314 (2012). [PubMed: 22467649]

118. Macintyre AN et al. Protein kinase B controls transcriptional programs that direct cytotoxic T cell fate but is dispensable for T cell metabolism. Immunity 34, 224–236 (2011). [PubMed: 21295499]
119. Hand TW et al. Differential effects of STAT5 and PI3K/AKT signaling on effector and memory CD8 T-cell survival. Proc. Natl Acad. Sci. USA 107, 16601–16606 (2010). [PubMed: 20823247]
120. Rao RR, Li Q, Odunsi K & Shrikant PA The mTOR kinase determines effector versus memory CD8+ T cell fate by regulating the expression of transcription factors T-BET and Eomesodermin. Immunity 32, 67–78 (2010). [PubMed: 20060330]
121. Rao RR, Li Q, Gubbels Bupp M. R. & Shrikant PA Transcription factor foxo1 represses t-bet-mediated effector functions and promotes memory CD8+ T cell differentiation. Immunity 36, 374–387 (2012). [PubMed: 22425248]
122. Sinclair LV et al. Phosphatidylinositol-3-OH kinase and nutrient-sensing mTOR pathways control T lymphocyte trafficking. Nature Immunol 9, 513–521 (2008). [PubMed: 18391955]
123. Kerdiles YM et al. Foxo1 links homing and survival of naive T cells by regulating L-selectin, CCR7 and interleukin 7 receptor. Nature Immunol 10, 176–184 (2009). [PubMed: 19136962]
124. Tang TT et al. The forkhead transcription factor AFX activates apoptosis by induction of the BCL-6 transcriptional repressor. J. Biol. Chem 277, 14255–14265 (2002). [PubMed: 11777915]
125. Zhang X et al. FOXO1 is an essential regulator of pluripotency in human embryonic stem cells. Nature Cell Biol 13, 1092–1099 (2011). [PubMed: 21804543]
126. Kolb HJ et al. Graft-versus-leukemia effect of donor lymphocyte transfusions in marrow grafted patients. Blood 86, 2041–2050 (1995). [PubMed: 7655033]
127. Kochenderfer JN et al. B-cell depletion and remissions of malignancy along with cytokine-associated toxicity in a clinical trial of anti-CD19 chimeric-antigen-receptor-transduced T cells. Blood 119, 2709–2720 (2012). [PubMed: 22160384]
128. Kalos M et al. T cells with chimeric antigen receptors have potent antitumor effects and can establish memory in patients with advanced leukemia. Sci. Transl. Med 3, 95ra73 (2011).
129. Bollard CM et al. Complete responses of relapsed lymphoma following genetic modification of tumor-antigen presenting cells and T-lymphocyte transfer. Blood 110, 2838–2845 (2007). [PubMed: 17609424]
130. Rosenberg SA et al. Durable complete responses in heavily pretreated patients with metastatic melanoma using T-cell transfer immunotherapy. Clin. Cancer Res 17, 4550–4557 (2011). [PubMed: 21498393]
131. Robbins PF et al. Tumor regression in patients with metastatic synovial cell sarcoma and melanoma using genetically engineered lymphocytes reactive with NY-ESO-1. J. Clin. Oncol 29, 917–924 (2011). [PubMed: 21282551]
132. Klebanoff CA, Khong HT, Antony PA, Palmer DC & Restifo NP Sinks, suppressors and antigen presenters: how lymphodepletion enhances T cell-mediated tumor immunotherapy. Trends Immunol 26, 111–117 (2005). [PubMed: 15668127]
133. Robbins PF et al. Cutting edge: persistence of transferred lymphocyte clonotypes correlates with cancer regression in patients receiving cell transfer therapy. J. Immunol 173, 7125–7130 (2004). [PubMed: 15585832]
134. Pule MA et al. Virus-specific T cells engineered to coexpress tumor-specific receptors: persistence and antitumor activity in individuals with neuroblastoma. Nature Med 14, 1264–1270 (2008). [PubMed: 18978797]
135. Zhou J et al. Telomere length of transferred lymphocytes correlates with *in vivo* persistence and tumor regression in melanoma patients receiving cell transfer therapy. J. Immunol 175, 7046–7052 (2005). [PubMed: 16272366]
136. Schwartzentruber DJ et al. In vitro predictors of therapeutic response in melanoma patients receiving tumor-infiltrating lymphocytes and interleukin-2. J. Clin. Oncol 12, 1475–1483 (1994). [PubMed: 8021739]
137. Besser MJ et al. Clinical responses in a phase II study using adoptive transfer of short-term cultured tumor infiltration lymphocytes in metastatic melanoma patients. Clin. Cancer Res 16, 2646–2655 (2010). [PubMed: 20406835]

138. Louis CU et al. Antitumor activity and long-term fate of chimeric antigen receptor-positive T cells in patients with neuroblastoma. Blood 118, 6050–6056 (2011). [PubMed: 21984804]
139. Huang J et al. Modulation by IL-2 of CD70 and CD27 expression on CD8+ T cells: importance for the therapeutic effectiveness of cell transfer immunotherapy. J. Immunol 176, 7726–7735 (2006). [PubMed: 16751420]
140. Berger C et al. Adoptive transfer of effector CD8+ T cells derived from central memory cells establishes persistent T cell memory in primates. J. Clin. Invest 118, 294–305 (2008). [PubMed: 18060041]
141. Wang X et al. Engraftment of human central memory-derived effector CD8+ T cells in immunodeficient mice. Blood 117, 1888–1898 (2011). [PubMed: 21123821]
142. Sussman JJ, Parihar R, Winstead K & Finkelman FD Prolonged culture of vaccine-primed lymphocytes results in decreased antitumor killing and change in cytokine secretion. Cancer Res 64, 9124–9130 (2004). [PubMed: 15604282]
143. Gattinoni L et al. Acquisition of full effector function in vitro paradoxically impairs the *in vivo* antitumor efficacy of adoptively transferred CD8+ T cells. J. Clin. Invest 115, 1616–1626 (2005). [PubMed: 15931392] This paper is the first to conceptualize and provide experimental evidence demonstrating the detrimental effect of T cell differentiation on the antitumour activity of CD8+ T lymphocytes used for adoptive T cell-based therapy.
144. Klebanoff CA et al. Central memory self/tumor-reactive CD8+ T cells confer superior antitumor immunity compared with effector memory T cells. Proc. Natl Acad. Sci. USA 102, 9571–9576 (2005). [PubMed: 15980149]
145. Le HK et al. Incubation of antigen-sensitized T lymphocytes activated with bryostatin 1 + ionomycin in IL-7 + IL-15 increases yield of cells capable of inducing regression of melanoma metastases compared to culture in IL-2. Cancer Immunol. Immunother 58, 1565–1576 (2009). [PubMed: 19198835]
146. Stark FC, Sad S & Krishnan L Intracellular bacterial vectors that induce CD8(+) T cells with similar cytolytic abilities but disparate memory phenotypes provide contrasting tumor protection. Cancer Res 69, 4327–4334 (2009). [PubMed: 19435919]
147. Klebanoff CA et al. Determinants of successful CD8+ T-cell adoptive immunotherapy for large established tumors in mice. Clin. Cancer Res 17, 5343–5352 (2011). [PubMed: 21737507]
148. Topalian SL, Muul LM, Solomon D & Rosenberg SA Expansion of human tumor infiltrating lymphocytes for use in immunotherapy trials. J. Immunol. Methods 102, 127–141 (1987). [PubMed: 3305708]
149. Riddell SR et al. Restoration of viral immunity in immunodeficient humans by the adoptive transfer of T cell clones. Science 257, 238–241 (1992). [PubMed: 1352912]
150. Gattinoni L, Powell DJ, Jr, Rosenberg SA & Restifo NP Adoptive immunotherapy for cancer: building on success. Nature Rev. Immunol 6, 383–393 (2006). [PubMed: 16622476]
151. Gattinoni L, Klebanoff CA & Restifo NP Pharmacologic induction of CD8+ T cell memory: better living through chemistry. Sci. Transl. Med 1, 11ps12 (2009).
152. Manjunath N et al. Effector differentiation is not prerequisite for generation of memory cytotoxic T lymphocytes. J. Clin. Invest 108, 871–878 (2001). [PubMed: 11560956]
153. van der Windt GJ et al. Mitochondrial respiratory capacity is a critical regulator of CD8+ T cell memory development. Immunity 36, 68–78 (2012). [PubMed: 22206904]
154. Butler MO et al. Establishment of antitumor memory in humans using in vitro-educated CD8+ T cells. Sci. Transl. Med 3, 80ra34 (2011).
155. Zeng R et al. Synergy of IL-21 and IL-15 in regulating CD8+ T cell expansion and function. J. Exp. Med 201, 139–148 (2005). [PubMed: 15630141]
156. Li Y, Bleakley M & Yee C IL-21 influences the frequency, phenotype, and affinity of the antigen-specific CD8 T cell response. J. Immunol 175, 2261–2269 (2005). [PubMed: 16081794]
157. Albrecht J et al. IL-21-treated naive CD45RA+ CD8+ T cells represent a reliable source for producing leukemia-reactive cytotoxic T lymphocytes with high proliferative potential and early differentiation phenotype. Cancer Immunol. Immunother 60, 235–248 (2011). [PubMed: 21046101]

158. Pearce EL Metabolism in T cell activation and differentiation. Curr. Opin. Immunol 22, 314–320 (2010). [PubMed: 20189791]

159. Aberle H Bauer A, Stappert J, Kispert A & Kemler R β-catenin is a target for the ubiquitin-proteasome pathway. EMBO J 16, 3797–3804 (1997). [PubMed: 9233789]

160. Chi H Regulation and function of mTOR signalling in T cell fate decisions. Nature Rev. Immunol 12, 325–338 (2012). [PubMed: 22517423]

161. Cohen P & Goedert M GSK3 inhibitors: development and therapeutic potential. Nature Rev. Drug Discov 3, 479–487 (2004). [PubMed: 15173837]

162. Zippelius A et al. Effector function of human tumor-specific CD8 T cells in melanoma lesions: a state of local functional tolerance. Cancer Res 64, 2865–2873 (2004). [PubMed: 15087405]

163. Ahmadzadeh M et al. Tumor antigen-specific CD8 T cells infiltrating the tumor express high levels of PD-1 and are functionally impaired. Blood 114, 1537–1544 (2009). [PubMed: 19423728]

164. Baitsch L et al. Exhaustion of tumor-specific CD8 T cells in metastases from melanoma patients. J. Clin. Invest 121, 2350–2360 (2011). [PubMed: 21555851]

165. Takahashi K & Yamanaka S Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126, 663–676 (2006). [PubMed: 16904174]

166. Takahashi K et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 131, 861–872 (2007). [PubMed: 18035408] References 165 and 166 provide evidence in mice and humans that a pluripotent stem cell can be artificially derived from an adult somatic cell by forced expression of a limited number of key transcription factors.

167. Anokye-Danso F et al. Highly efficient miRNA-mediated reprogramming of mouse and human somatic cells to pluripotency. Cell Stem Cell 8, 376–388 (2011). [PubMed: 21474102] This paper shows that a single microRNA cluster (microRNA302–367) can efficiently reprogramme adult somatic cells to acquire pluripotency.

168. Eminli S et al. Differentiation stage determines potential of hematopoietic cells for reprogramming into induced pluripotent stem cells. Nature Genet 41, 968–976 (2009). [PubMed: 19668214]

169. Loh YH et al. Reprogramming of T cells from human peripheral blood. Cell Stem Cell 7, 15–19 (2010). [PubMed: 20621044]

170. Staerk J et al. Reprogramming of human peripheral blood cells to induced pluripotent stem cells. Cell Stem Cell 7, 20–24 (2010). [PubMed: 20621045]

171. Seki T et al. Generation of induced pluripotent stem cells from human terminally differentiated circulating T cells. Cell Stem Cell 7, 11–14 (2010). [PubMed: 20621043]

172. Schmitt TM et al. Induction of T cell development and establishment of T cell competence from embryonic stem cells differentiated in vitro. Nature Immunol 5, 410–417 (2004). [PubMed: 15034575]

173. Schmitt TM & Zuniga-Pflucker JC Induction of T cell development from hematopoietic progenitor cells by delta-like-1 in vitro. Immunity 17, 749–756 (2002). [PubMed: 12479821] This paper describes a simple *in vitro* culture system that allows for the generation of mature T cells from haematopoietic progenitors.

174. Clark RA, Yamanaka K, Bai M, Dowgiert R & Kupper TS Human skin cells support thymus-independent T cell development. J. Clin. Invest 115, 3239–3249 (2005). [PubMed: 16224538]

175. Zhao Y et al. Extrathymic generation of tumor-specific T cells from genetically engineered human hematopoietic stem cells via Notch signaling. Cancer Res 67, 2425–2429 (2007). [PubMed: 17363559]

176. Lei F, Haque R, Weiler L, Vrana KE & Song J T lineage differentiation from induced pluripotent stem cells. Cell. Immunol 260, 1–5 (2009). [PubMed: 19811778]

177. Vierbuchen T et al. Direct conversion of fibroblasts to functional neurons by defined factors. Nature 463, 1035–1041 (2010). [PubMed: 20107439]

178. Marro S et al. Direct lineage conversion of terminally differentiated hepatocytes to functional neurons. Cell Stem Cell 9, 374–382 (2011). [PubMed: 21962918]

179. Ieda M et al. Direct reprogramming of fibroblasts into functional cardiomyocytes by defined factors. Cell 142, 375–386 (2010). [PubMed: 20691899]

180. Szabo E et al. Direct conversion of human fibroblasts to multilineage blood progenitors. Nature 468, 521–526 (2010). [PubMed: 21057492]

181. Sekiya S & Suzuki A Direct conversion of mouse fibroblasts to hepatocyte-like cells by defined factors. Nature 475, 390–393 (2011). [PubMed: 21716291]

182. Miura K et al. Variation in the safety of induced pluripotent stem cell lines. Nature Biotechnol 27, 743–745 (2009). [PubMed: 19590502]

183. Bonini C et al. HSV-TK gene transfer into donor lymphocytes for control of allogeneic graft-versus-leukemia. Science 276, 1719–1724 (1997). [PubMed: 9180086]

184. Di SA et al. Inducible apoptosis as a safety switch for adoptive cell therapy. N. Engl. J. Med 365, 1673–1683 (2011). [PubMed: 22047558]

185. Wu SM & Hochedlinger K Harnessing the potential of induced pluripotent stem cells for regenerative medicine. Nature Cell Biol 13, 497–505 (2011). [PubMed: 21540845]

186. Zhao T, Zhang ZN, Rong Z & Xu Y Immunogenicity of induced pluripotent stem cells. Nature 474, 212–215 (2011). [PubMed: 21572395]

187. Wherry EJ et al. Lineage relationship and protective immunity of memory CD8 T cell subsets. Nature Immunol 4, 225–234 (2003). [PubMed: 12563257]

188. Chang JT et al. Asymmetric proteasome segregation as a mechanism for unequal partitioning of the transcription factor T-bet during T lymphocyte division. Immunity 34, 492–504 (2011). [PubMed: 21497118]

189. Appay V et al. Memory CD8+ T cells vary in differentiation phenotype in different persistent virus infections. Nature Med 8, 379–385 (2002). [PubMed: 11927944]

190. Willinger T, Freeman T, Hasegawa H, McMichael AJ & Callan MF Molecular signatures distinguish human central memory from effector memory CD8 T cell subsets. J. Immunol 175, 5895–5903 (2005). [PubMed: 16237082]

191. Holmes S, He M, Xu T & Lee PP Memory T cells have gene expression patterns intermediate between naive and effector. Proc. Natl Acad. Sci. USA 102, 5519–5523 (2005). [PubMed: 15809420]

192. Waddington CH The Strategy of the Genes (George Allen & Unwin, 1957).

193. Chapuis AG et al. Transferred melanoma-specific CD8+ T cells persist, mediate tumor regression, and acquire central memory phenotype. Proc. Natl Acad. Sci. USA 109, 4592–4597 (2012). [PubMed: 22393002]

194. Swain SL, McKinstry KK & Strutt TM Expanding roles for CD4+ T cells in immunity to viruses. Nature Rev. Immunol 12, 136–148 (2012). [PubMed: 22266691]

195. Pepper M et al. Different routes of bacterial infection induce long-lived TH1 memory cells and short-lived TH17 cells. Nature Immunol 11, 83–89 (2010). [PubMed: 19935657]

196. Luthje K et al. The development and fate of follicular helper T cells defined by an IL-21 reporter mouse. Nature Immunol 13, 491–498 (2012). [PubMed: 22466669]

197. Nurieva RI et al. Generation of T follicular helper cells is mediated by interleukin-21 but independent of T helper 1, 2, or 17 cell lineages. Immunity 29, 138–149 (2008). [PubMed: 18599325]

At a glance

- T lymphocytes transition through progressive stages of differentiation that are characterized by a stepwise loss of functional and therapeutic potential.
- Subsets of mature T cells exhibit the stem cell-like attributes of self-renewal, multipotency and the ability to undergo asymmetric division.
- Evolutionarily conserved pathways regulating stemness are active in T cells, including T memory stem cells, T helper 17 cells and interleukin-17 (IL-17)-producing $CD8^+$ T cells.
- Pharmacological and genetic induction of stem cell pathways can be used to generate tumour-specific T cells with stem cell-like properties.
- Reprogramming terminally differentiated tumour-reactive T cells to display naive or stem cell-like functionalities might be obtained through the expression of transcription factors or microRNAs that are associated with naive or T memory stem cells.
- Stem cell-like T cells possess enhanced capacities to engraft, persist and mediate prolonged immune attack against tumour masses that are sustained by long-lived cancer stem cells.

Box 1 |

Models of effector and memory T cell lineage relationships

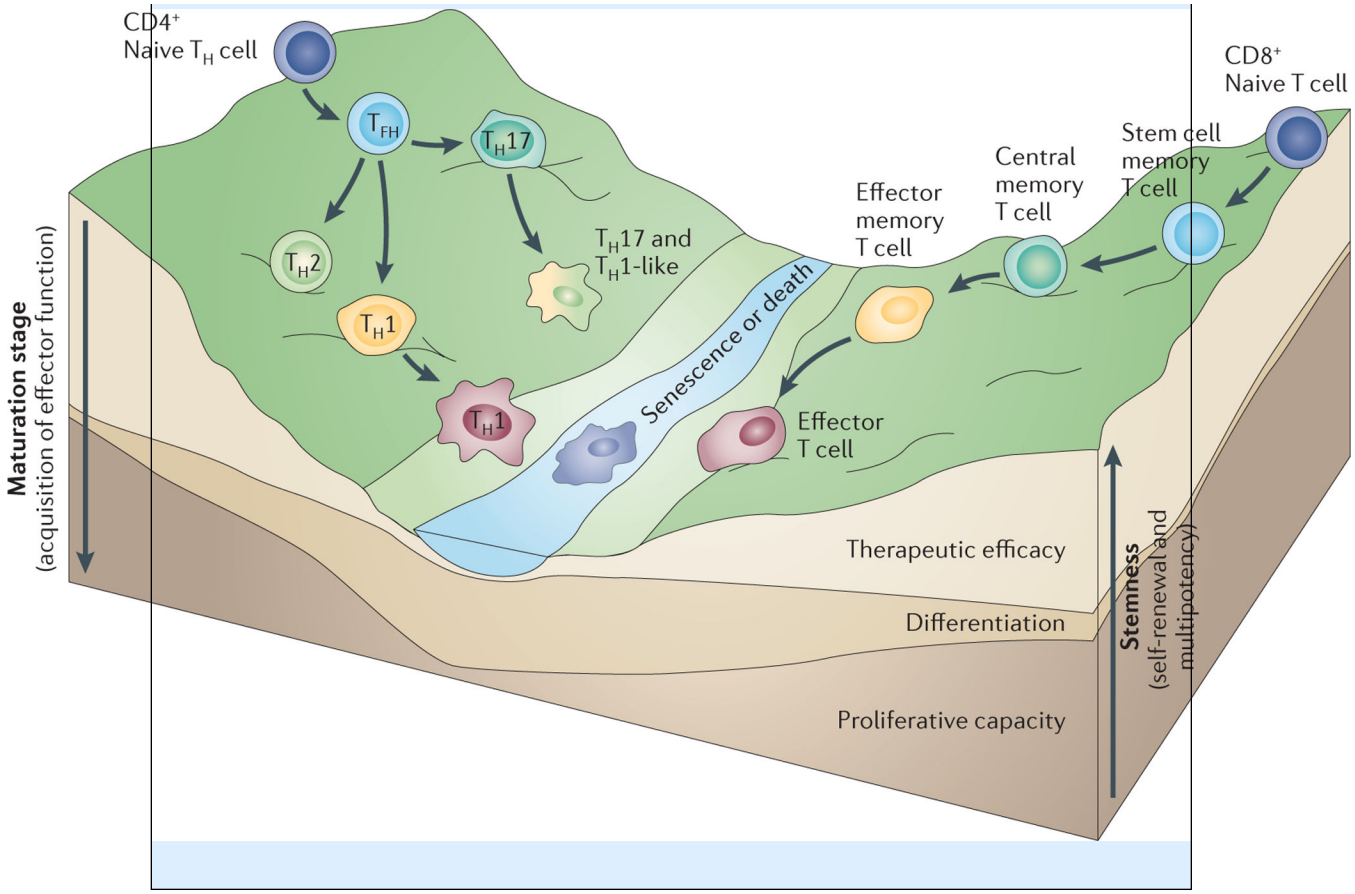
There has been a long-standing controversy regarding how memory T cells form, and about their relationship with effector T cells (T_{EFF}). The precise understanding of this interrelationship is crucially important for developing immune strategies to enhance T cell responses against cancer. Thus far, three main models of memory formation have been proposed[18].

- The linear differentiation model[187]: in this model, the priming of naive T cells results in the generation of T_{EFF} cells that are destined either to die or to enter into the effector memory T (T_{EM}) cell pool. With time, T_{EM} cells can give rise to long-lived central memory T (T_{CM}) cells. Evidence in support of this model is derived from the observation that T_{CM} cells become the predominant persisting memory T cell subset following the transfer of a population highly enriched for T_{EM} cells.
- The bifuractive differentiation model[43,188]: this model proposes that a primed naïve T cell can give rise to two daughter cells with alternative differentiation fates through asymmetric division. Evidence in support of this model is the finding that there is an unequal partitioning of key molecules and transcription factors that regulate effector differentiation at the first cell division following naive T cell priming.
- The progressive differentiation model (also known as the decreasing potential of memory development or the self-renewing effector model)[19]: this model proposes that, depending on the strength and quality of stimulatory signals received, naïve T cells are driven towards progressive stages of differentiation in the order T memory stem cell (T_{SCM}) to T_{CM} cell and to T_{EM} cell, as cells receive progressively greater signal strengths. This model culminates in the generation of short lived T_{EFF} cells, which are terminally differentiated. Evidence in support of this model include *ex vivo* phenotypic analyses of virus-specific T cells[189], measurement of telomere length[24,54], gene-expression profiling[19,190,191] and *in vitro* differentiation studies[19,54].

Box 2 |

A Waddington view of stem cell-like potential of CD4$^+$ and CD8$^+$ T cell subsets

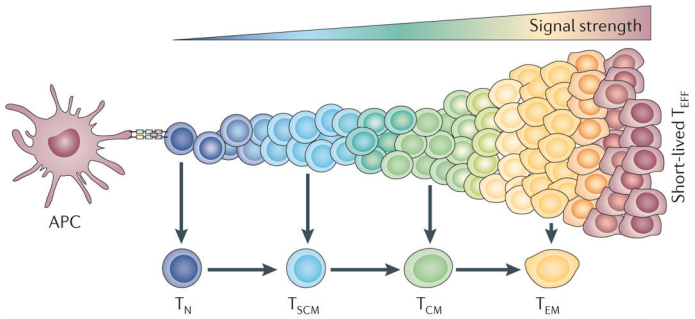
In 1957, Conrad Waddington, conceptualized an epigenetic landscape composed of ‘peaks’ and ‘valleys’ in which an undifferentiated, pluripotent cell residing at the peak of its potential can travel down various pathways of differentiation, like a ball placed precariously atop a hill[192]. Some 50 years later, it is now clear that underlying gene and microRNA expression dynamics are changes to the physical organization of chromatin through epigenetic modifications. Extrapolating from the Waddington model of cellular potential, T cells can be visualized as resting in valleys placed at different altitudes corresponding to T cell subsets with diverse differentiation potentials (see the figure). At the peak of the ‘hill’ a naive CD4$^+$ T helper (T_H) cell or CD8$^+$ T cell exists that is capable of forming all T cell subsets within its respective lineage. As a cell moves down the hill, its potential to differentiate into other subsets becomes progressively restricted, culminating in a terminally differentiated cell that is destined to die. Cellular differentiation in the Waddington model generally proceeds unidirectionally from the least to the most differentiated cell. However, there is evidence to suggest that cells can dedifferentiate, under some circumstances, and reoccupy a vacant niche, such as that seen in the regeneration of diverse phenotypes like the reacquisition of stem cell antigen 1 in pro-erythrocytes[55] or the re-emergence of CD62L$^+$ cells from effector memory T cells[140,187,193], although these processes are highly inefficient under physiological conditions.



Box 3 |

Stem cell-like qualities in CD4+ T cell subsets

$CD4^+$ T cells have crucial roles in coordinating immune responses to infectious diseases and cancer. Despite a wealth of knowledge relevant to $CD4^+$ T cell biology and our increasing understanding and identification of new subsets, relatively little is known about memory $CD4^+$ T cells and the ability of different $CD4^+$ T helper (T_H) cell subsets to persist long term[194]. The functional diversity and, in particular, the developmental plasticity of the T_H subsets has posed unique challenges in defining memory in this lineage and has sometimes led investigators to incorrect conclusions. For example, T_H cells releasing interleukin-17 (IL-17) (T_H17) have been purported as short-lived effector cells, as these cells display some phenotypic traits that are characteristic of terminally differentiated $CD8^+$ T cells (that is, a lack of expression of CD62L and CD27), and IL-17A production by antigen-specific cells is extinguished over time during an infection[195]. However, this interpretation is at odds with recent evidence indicating that congenically marked highly purified T_H17 cells exhibit not only superior recall and persistence relative to interferon-γ (IFNγ)-secreting T_H1 cells, but also mediate enhanced auto-immunity and antitumour immunity[85]. Moreover, T_H17 cells were found to exhibit the stem cell-like attributes of multipotency (these cells could give rise to both IL-17- and IFNγ-secreting progeny), activation of stem cell-associated molecular pathways (these cells highly expressed β-catenin and *Tcf7*) and a shared gene expression signature with early memory $CD8^+$ T cells[85]. Similar findings have been described in human T_H17 cells[87]. These findings place T_H17 cells at a higher cellular potential than T_H1 cells on a Waddington landscape (BOX 2). Recent findings demonstrating that T follicular helper (T_{FH}) cells can be recruited into other helper T subsets and can also give rise to memory cells[196], combined with the observation that T_{FH} cell formation is independent of T_H1, T_H2 and T_H17 development[197], place T_{FH} cells at the apex of cellular potential for antigen-experienced $CD4^+$ T cells, paralleling memory stem cells among the $CD8^+$ T cell lineage (BOX 2).



		T_N	T_{SCM}	T_{CM}	T_{EM}
Phenotype	CCR7	+++	+++	++	–
	CD62L	+++	+++	++	–
	CD27	+++	+++	++	+/–
	CD28	++	+++	+++	+/–
	CD45RA	+	+	–	+/–
	CD45RO	–	–	+	+
	CD122	–	++	+++	+++
	CD95	–	++	+++	+++
	KLRG1	–	–	+/–	++
Transcription factors	TCF7	+++	+++	++	+
	ID3	+++	+++	++	+
	LEF1	+++	++	+	–
	FOXP1	+++	++	+	–
	KLF7	+++	++	+	–
	EOMES	+	+	+	++
	ID2	+	+	++	+++
	PRDM1	–	+	+	+++
	TBX21	–	+	++	+++
	ZEB2	–	+	++	+++
miRNA	let7 family	+++	ND	ND	–
	miR-26a/b	+++	ND	ND	–
	miR-29a/b	+++	ND	ND	–
	miR-30a-5P	+++	ND	ND	–
	miR-142-5P	+++	ND	ND	+++
	miR-21	–	ND	ND	+++
	miR-146	–	ND	ND	+++
	miR-155	–	ND	ND	+++
Function	IFNγ	–	+	++	+++
	IL-2	–	++	+++	+/–
	Cytoxicity	–	+/–	+	+++
	Telomere	+++	+++	++	+
	Self-renewal	+	+++	++	+

Figure 1 |. A model of progressive T cell differentiation.

During an immune response, naïve T (T_N) cells are primed by antigen-presenting cells (APCs). Depending on the strength and quality of stimulatory signals, proliferating T cells progress along a differentiation pathway that culminates in the generation of terminally differentiated short-lived effector T (T_{EFF}) cells. When antigenic and inflammatory stimuli cease, primed T cells become quiescent and enter into the memory stem cell (T_{SCM}), central memory (T_{CM}) cell or effector memory (T_{EM}) cell pools depending on the signal strength received. The phenotypic attributes, expression levels of key transcription factors and

microRNAs (miRNAs), and the functional properties of naive and memory T cell subsets are illustrated as not expressed (–), low expression (+), intermediate expression (++) and high expression (+++). EOMES, eomesodermin; FOXP1, Forkhead box P1; ID, inhibitor of DNA-binding; IFNγ, interferon-γ; IL-2, interleukin-2; KLF, Kruppel-like factor; KLRG1, killer cell lectin-like receptor subfamily G, member 1; LEF1, lymphoid enhancer-binding factor 1; ND, not determined; PRDM1, PR domain-containing 1 with ZNF domain; TBX21, T-box 21; TCF7, T cell factor 7; ZEB2, zinc finger E-box binding homeobox 2.

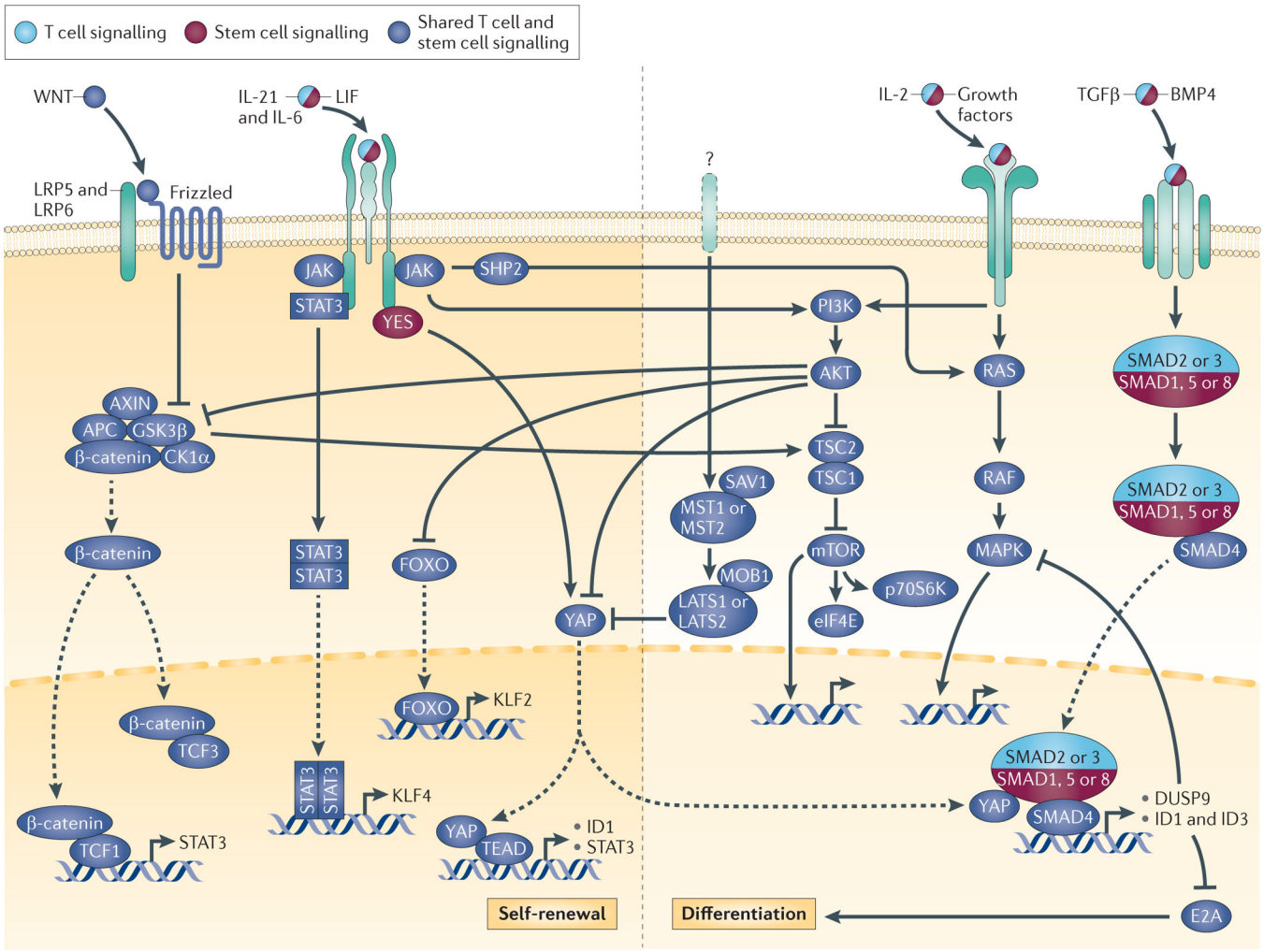


Figure 2 |. Signalling pathways regulating self-renewal and differentiation shared between stem cells and T lymphocytes.

Self-renewal and differentiation are tightly balanced by opposing signals received from cell surface receptors. Self-renewal is promoted by WNT ligand binding to Frizzled–low-density lipoprotein receptor related protein 5 (LRP5) or LRP6 complexes or by ligand engagement of receptor complexes signalling through signal transducer and activator of transcription 3 (STAT3), including receptors containing the GP130 subunit or the interleukin-21 (IL-21) receptor. Activation of these signalling pathways leads to the transcription of target genes that favour self-renewal and that withhold differentiation, including STAT3 and Kruppel-like factor (KLF) family members and inhibitor of DNA binding (ID) proteins. Conversely, pro-mitotic cytokines such as IL-2 and growth factors can drive cellular differentiation by triggering the PI3K–AKT–mTOR pathway, as well as the RAS–RAF–MAPK pathway. The pro-differentiating influence of the RAS–RAF–MAPK pathway can be counteracted by SMAD signalling that is induced by transforming growth factor-β (TGFβ) or bone morphogenetic protein (BMP) family members through the induction of dual specificity phosphatase 9 (DUSP9) and the E protein regulators, ID molecules. Finally, activation of the Hippo pathways through a poorly characterized ligand–receptor interaction causes

inactivation of Yes-associated protein (YAP), resulting in enhanced cellular differentiation. Between these self-renewal and pro-differentiation pathways exists a significant amount of crosstalk such that the net influence of each pathway is finely balanced and tuned. The dashed arrows indicate translocation into the nucleus. APC, adenomatous polyposis coli; CK1α, casein kinase 1, alpha 1; eIF4E, eukaryotic translation initiation factor 4E; FOXO, forkhead box O; GSK3β, glycogen synthase 3β; JAK, janus kinase; LATS, large tumour suppressor; LIF, leukaemia inhibitory factor; MOB, MOB kinase activator 1; MST, mammalian sterile-20-like kinases; p70S6K, p70 ribosomal protein S6 kinase 1; SAV1, salvador homologue 1; SHP2, SH2 domain-containing protein tyrosine phosphatase-2; TCF, T cell factor; TEAD, TEA domain family member; TSC, tuberous sclerosis.

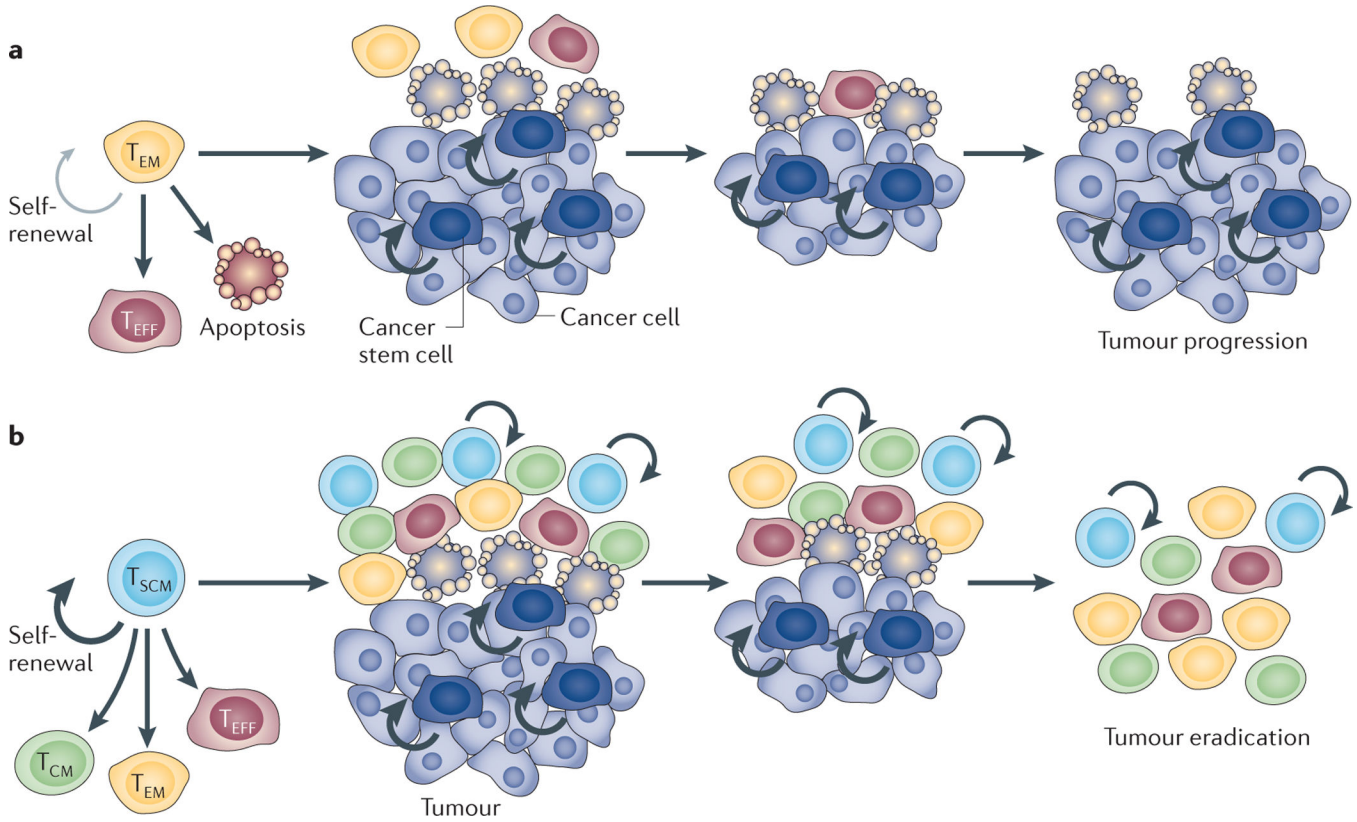


Figure 3 |. Fighting fire with fire.

a | Current T cell-based immunotherapies predominantly transfer cells with effector memory (T_{EM})-like phenotypic and functional characteristics. These cells have limited self-renewal capacity and are oligopotent. These cells can mediate tumour destruction but are handicapped to compete with expanding tumour masses (shown as purple tumour cells) that are sustained by the activity of self-renewing multipotent cancer stem cells (CSCs; shown as dark purple tumour cells). **b** | Future T cell-based immunotherapies might benefit from the transfer of T memory stem cells (T_{SCM}) that have enhanced self-renewal and the multipotent capacity to form all memory and effector subsets. These properties allow T_{SCM} cells to sustain a prolonged immune attack by giving rise to more differentiated, highly lytic effector T (T_{EFF}) and T_{EM} cells while maintaining a continuous supply of less differentiated T_{SCM} and central memory (T_{CM}) cells that can refresh the pool of cytotoxic T cells over time. In this manner, T_{SCM} cells might overtake the last tumour cell, including CSCs, and so cure the host.

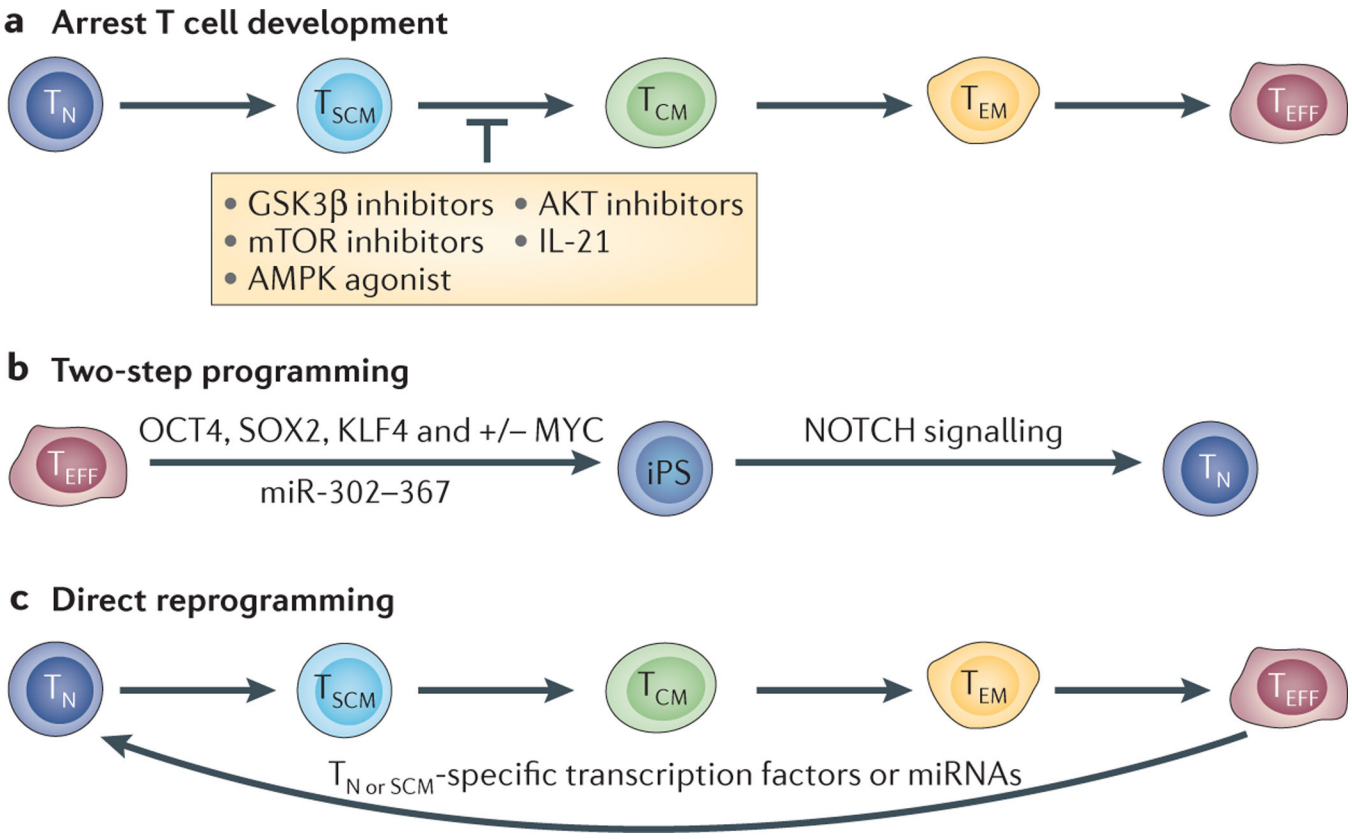


Figure 4 |. Strategies that might be used to preserve or to confer stemness to T cells.

a | The process of arresting T cell development is shown. Differentiation of primed naive T (T_N) cells can be suppressed using cytokines, such as interleukin-21 (IL-21), or by using small molecules targeting key metabolic and developmental pathways. **b** | Two step reprogramming of terminally differentiated effector T (T_{EFF}) cells through an induced pluripotent stem (iPS) cell intermediate is shown. T_{EFF} cells are reprogrammed to generate iPS cells by ectopic co-expression of the Yamanaka factors, and OCT4, sex determining region Y (SRY) BOX 2 (SOX2) and Kruppel-like factor 4 (KLF4) with or without MYC or by forced expression of the microRNA (miRNA) cluster 302–367. iPS cells can be subsequently redifferentiated into T_N cells through the induction of NOTCH signalling. **c** | Direct reprogramming of T_{EFF} into T_N or memory stem (T_{SCM}) cells by enforced expression of T_N or T_{SCM}-associated transcription factors or miRNAs is shown. GSK3β, glycogen synthase 3β; T_{CM}, central memory; T_{EM}, effector memory.

Table 1 |

Phenotypic and functional characteristics of T memory stem cells

Species	Phenotype	Anatomical location	Antigen specificity	Homeostasis	Evidence of stemness
Mouse	$CD44^{-}$, $CD62L^{+}$, $CCR7^{+}$, $SCA1^{+}$, IL-$2RB^{+}$, BCL-2^{+} and $CXCR3^{+}$ (REFS 51,52)	Not extensively characterized. Described in peripheral blood, spleen, lymph nodes and liver[51]	Alloantigens[51]	Enhanced proliferative response in lymphopaenic hosts. MHC class I independence[52]	Self-renewal and multipotency on serial transplantation[52]
Non-human primates	$CD45RA^{+}$, $CD62L^{+}$, $CCR7^{+}$, $CD27^{+}$ $CD28^{+}$, IL-$7RA^{+}$, $CD95^{+}$, IL-$2RB^{+}$, BCL-2^{+} and $CXCR3^{+}$ (REF. 53)	Preferential homing to lymphoid tissues. Almost completely absent in the gut[53]	Simian immunodeficinecy virus[53]	*In vivo* and *in vitro* proliferative responses to IL-15 (REF. 53)	Long-term persistence in the absence of antigenic stimuli[53]
Human	$CD45RA^{+}$, $CD62L^{+}$, $CCR7^{+}$, $CD27^{+}$, $CD28^{+}$, IL-$7RA^{+}$, $CD45RO^{-}$, $CD95^{+}$, IL-$2RB^{+}$, BCL-2^{+} and $CXCR3^{+}$ (REF. 19)	Not extensively characterized. Described in peripheral and cord blood[19]	Influenza, CMV and MART1 (REF. 19)	Enhanced proliferative response in immunodeficient mice. *In vitro* proliferative responses to IL-15 (REF. 19)	Self-renewal and multipotency in *in vitro* assay. Enhanced engraftment in xenograft models[19]

CMV, cytomegalovirus; CXCR3, chemokine (C-X-C motif) receptor 3; IL, interleukin; MART1, melanoma antigen recognized by T-cells 1.