REVIEW

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T memory stem cells in health and disease

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T memory stem (T_{SCM}) cells are a rare subset of memory lymphocytes endowed with the stem cell–like ability to self-renew and the multipotent capacity to reconstitute the entire spectrum of memory and effector T cell subsets. Cumulative evidence in mice, nonhuman primates and humans indicates that T_{SCM} cells are minimally differentiated cells at the apex of the hierarchical system of memory T lymphocytes. Here we describe emerging findings demonstrating that T_{SCM} cells, owing to their extreme longevity and robust potential for immune reconstitution, are central players in many physiological and pathological human processes. We also discuss how T_{SCM} cell stemness could be leveraged therapeutically to enhance the efficacy of vaccines and adoptive T cell therapies for cancer and infectious diseases or, conversely, how it could be disrupted to treat T_{SCM} cell driven and sustained diseases, such as autoimmunity, adult T cell leukemia and HIV-1.

> δὶς γὰρ τὸν αὐτον, ὥστε καὶ κτείν, οὐκ ἐπελάμβανεν.
> For this disease never took any man the second time so as to be mortal.
>
> —Thucydides, *The History of the Peloponnesian War* (translation by Thomas Hobbes)

Immunological memory—the ability to remember and respond rapidly and more vigorously to a pathogen upon subsequent encounters—has long been recognized in human history. The first documentation of immunological memory came from the Greek historian Thucydides, who vividly described the plague that struck the city of Athens in 430 BC, recounting that "this disease never took any man the second time"[1]. It took us more than two millennia to understand that immunological memory is a fundamental property of the adaptive immunity conveyed by B and T lymphocytes[2].

Despite the enormous progress in our understanding of basic aspects of T cell immunity, the ontogeny of memory T cells remains a matter of active debate[3,4]. It is clear, however, that immunological memory and protective immunity can last several decades and perhaps a lifetime, even in the absence of re-exposure to the pathogen[5,6]. This astonishing stability of T cell memory, despite the high cellular turnover that characterizes immune responses and the lack of replenishment of antigen-specific T cells from hematopoietic stem cells (HSCs)—owing to constraints imposed by stochastic recombination of the T cell receptor (TCR) and thymic involution—has sparked the idea that T cell immunity could be maintained via stem cell–like memory T cells[7]. Over the past decade, the realization that memory T cells share a core transcriptional signature with HSCs[8] and display functional properties found in stem cells, such as the capacity to divide asymmetrically to generate cellular heterogeneity[9], has further strengthened the view that T cells, akin to all somatic tissues, might be organized hierarchically and sustained by antigen-specific T memory stem cells[10].

In this Review, we outline emerging findings demonstrating that a subset of minimally differentiated memory T cells behave as antigen-specific adult stem cells. We also discuss recent evidence placing these T_{SCM} cells at center stage in many physiological and pathological human processes. Finally, we highlight ongoing efforts aimed at either harnessing the therapeutic potential of T_{SCM} cells for adoptive immunotherapies or, conversely, at destabilizing the T_{SCM} cell compartment to eliminate drug-resistant viral reservoirs or treat adult T cell leukemia and autoimmune diseases. The conceptual work and key discoveries that have shaped this field of investigation are summarized in **Figure 1**.

The discovery of T_{SCM} cells

Advances in multiparameter flow cytometry over the past 20 years have enabled dissection of the heterogeneity of the T cell compartment with ever-increasing precision[11]. In a seminal study, van Lier and colleagues identified human naive, memory and effector T cell subsets on the basis of the combinatorial expression of CD27 and CD45RA, with naive cells expressing both molecules, and memory and effector cells expressing only CD27 or CD45RA, respectively[12]. Subsequent work by Sallusto *et al.*[13] revealed the presence of two major functional subsets within the $CD45RA^-$ memory T cell pool: central memory T (T_{CM}) cells, which express the lymph node homing molecules CCR7 and CD62L and have limited effector functions, and

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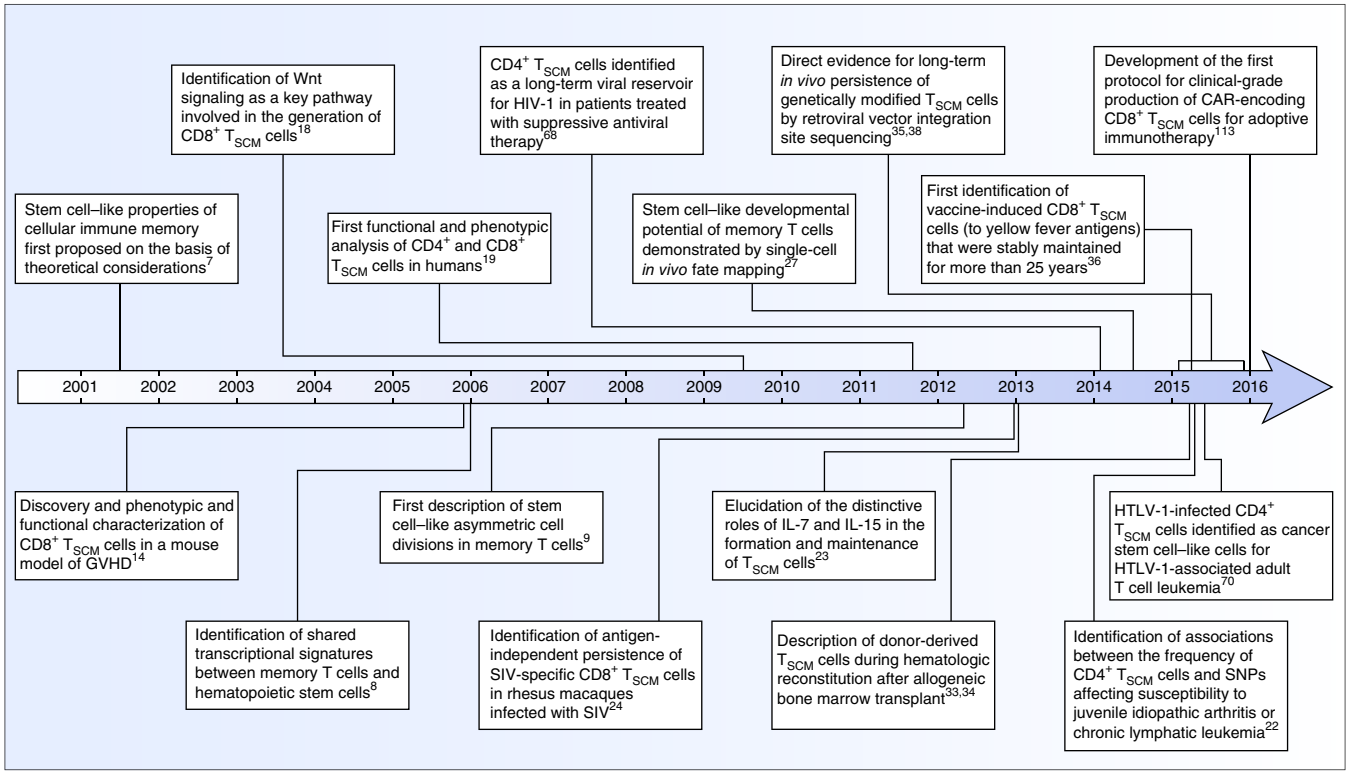

Figure 1 T cell stemness and T_{SCM} cells: milestones and key discoveries. T_{SCM} cells, T memory stem cells; GVHD, graft-versus-host disease; HIV-1, human immunodeficiency virus type 1; SIV, simian immunodeficiency virus; HTLV-1, human T cell lymphotropic virus type 1; CAR, chimeric antigen receptor; SNP, single-nucleotide polymorphism.

$CCR7^{-}CD62L^{-}$ effector memory T (T_{EM}) cells, which preferentially traffic to peripheral tissues and mediate rapid effector functions.

The idea that memory T cells might not be confined solely to the $CD45RA^{-}$ T cell compartment, but might also be present within what was considered to be a naive T cell population, began to take shape following the identification in mice of a memory T cell population that is characterized by a naive-like phenotype, but that expresses high amounts of stem cell antigen 1 (SCA1) and the memory markers interleukin-2 receptor β (IL-2Rβ) and chemokine C-X-C motif receptor 3 (CXCR3)[14]. These cells were termed T_{SCM} cells because it was observed that they were capable of sustaining graft-versus-host disease (GVHD) upon serial transplantation into allogeneic hosts, and that they could reconstitute the full diversity of memory and effector T cell subsets while maintaining their own pool size through self-renewal[14]. Identifying the human counterpart of T_{SCM} cells, however, has not been straightforward, mainly owing to the lack of a human ortholog of SCA1, the prototypical marker of mouse T_{SCM} cells. Although it was known that a substantial fraction of long-lived antigen-specific $CD8^{+}$ and $CD4^{+}$ memory T cells displayed a naive-like phenotype ($CD45RA^{+}CCR7^{+}CD27^{+}$) years after infection with EBV[15] or vaccination with attenuated smallpox or yellow fever (YF) viruses[16,17], a precise set of surface markers with which to pinpoint this elusive memory phenotype in humans was missing. The breakthrough came with the demonstration that mouse T_{SCM} cells could be generated successfully *in vitro* from naive precursors by activating the Wnt-β-catenin signaling pathway using the Wnt ligand, Wnt3a or inhibitors of glycogen synthase kinase-3β (ref. 18). By using this strategy to characterize extensively the phenotype of candidate human T_{SCM} cells generated *in vitro*, it was possible to identify key surface markers that can distinguish naturally occurring human T_{SCM} cells from the naive T cell pool[19]. Similar to their mouse counterparts, human and nonhuman primate (NHP) T_{SCM} cells are clonally expanded cells that express a largely naive-like phenotype in conjunction with a core of memory markers, such as CD95, CXCR3, IL-2Rβ, CD58 and CD11a[19,20]. These cells represent a small fraction of circulating T lymphocytes (≈2–3%). Notably, the frequency of circulating T_{SCM} cells does not vary substantially with age[21], but it seems to be heritable and associated with single-nucleotide polymorphisms (SNPs) at a genetic locus containing *CD95* (ref. 22), which suggests a potential role of FAS signaling in the regulation of T_{SCM} cell homeostasis. T_{SCM} cells exhibit all the defining properties of memory cells, including a diluted content of TCR excision circles, the ability to proliferate rapidly and release inflammatory cytokines in response to antigen re-exposure, and a dependence on IL-15 and IL-7 for homeostatic turnover[19,23]. Despite being functionally distinct from naive T cells, T_{SCM} cells share with them similar recirculation patterns and distribution *in vivo*, as evidenced by detailed compartmentalization studies in NHPs[24]. For instance, T_{SCM} cells are found more abundantly in lymph nodes than in the spleen and bone marrow, and they are virtually absent from peripheral mucosae[24]. Thus, T_{SCM} cells represent a subset of minimally differentiated T cells characterized by phenotypic and functional properties that bridge naive and conventional memory cells (**Fig. 2**).

T_{SCM} cells: evidence of stemness

The concept of stemness embraces the capacity both to self-renew and to generate the entire spectrum of more differentiated cells[25]. When Fearon and colleagues[7] initially postulated the existence of a stem cell pool of memory T lymphocytes, the authors pointed to T_{CM} cells

as putative T memory stem cells. This assumption was based on the evidence that T_{CM} cells are less differentiated than T_{EM} and effector cells, as shown by their longer telomeres and lower expression of perforin, granzymes and other effector molecules[13]. Furthermore, it was intuitive to assume that the pool of T memory stem cells should be confined to lymph nodes and secondary lymphoid organs, and T_{CM} cells were, at that time, the only antigen-experienced T cells known to express CCR7 and CD62L. The notion that T_{CM} cells might function as T memory stem cells received further support from subsequent findings that demonstrated that T_{CM} cells have superior immune-reconstitution capacity and a greater ability to persist *in vivo* than T_{EM} cells[26]. Recent clonogenic experiments in mice based on single-cell serial transfer have formally demonstrated the ability of mouse T_{CM} cells to self-renew and generate T_{EM} and effector progeny *in vivo*[27,28]. By contrast, T_{EM} cells were unable to serially reconstitute the host, even when transferred at 100-fold higher numbers, and so showed a limited capacity for self-renewal. Although these experiments did not evaluate T_{SCM} cells, these results, when combined with those of sophisticated experiments tracking T cell fates in mice through genetic barcoding[29] and on single naive T cell transfer[30], provided strong support for the progressive model of T cell differentiation originally developed by Sallusto and Lanzavecchia[31]. Indeed, three separate models have been proposed to explain memory T cell differentiation[3]: according to the first two models, memory T cells originate from effectors either after[26] or before[32] the peak of T cell expansion. The progressive differentiation model, on the contrary, suggests that memory T cells are derived directly from naive lymphocytes upon priming, and further differentiate into shorter-lived effector subsets in a hierarchical differentiation tree, similarly to that of other organ systems[31] (**Fig. 2**). By using hematopoietic stem cell transplantation (HSCT) from haploidentical donors as a model system to study T cell differentiation, two independent groups have shown recently at polyclonal, antigen-specific and clonal levels that human T_{SCM} cells differentiate directly from naive precursors and emerge early upon *in vivo* priming[33,34]. By multiparametric flow cytometry and TCR sequencing, it was possible to trace and quantify the *in vivo* differentiation landscapes of transferred naive T cells, which showed that more than 30% of naive T cells undergoing priming differentiate into T_{SCM} cells[33,34]. Indeed, discrete T cell subsets traced across HSCT behaved preferentially within a progressive framework of differentiation. Notably, only naive T cells and T_{SCM} cells were able to reconstitute the entire heterogeneity of memory T cell subsets, including T_{SCM} cells[33]. A fraction of cells originally designated as T_{EM} cells reverted to a T_{CM} cell phenotype[33]. By contrast, only a very limited number of T_{CM} and T_{EM} cells converted to the T_{SCM} cell type[33]. Echoing these findings, the transfer of genetically modified virus-specific T cells reconstituted the full diversity of the T cell memory compartment—inclusive of T_{SCM}, T_{CM} and T_{EM} cells—only when T_{SCM} cells were present within the infused cell product[35]. All together, these results strengthen earlier *in vitro* observations in humans[19] and NHPs[24] showing that the potential to form diverse progeny is progressively restricted as the cell type proceeds from T_{SCM} to T_{CM} and T_{EM} cells. Thus, granting some level of plasticity to the system, these data point to a progressive model of T cell differentiation, in which T_{SCM} cells are at the apex of the hierarchical tree. In line with this concept, the gene expression profile of human T cell subsets partitions T_{SCM} cells with antigen-experienced T cells and places them at a hierarchically superior level over the T_{CM} cell type[19,23,36,37].

The concept of stemness also involves self-renewal and implicates long-term persistence[25]. The long-term persisting ability of

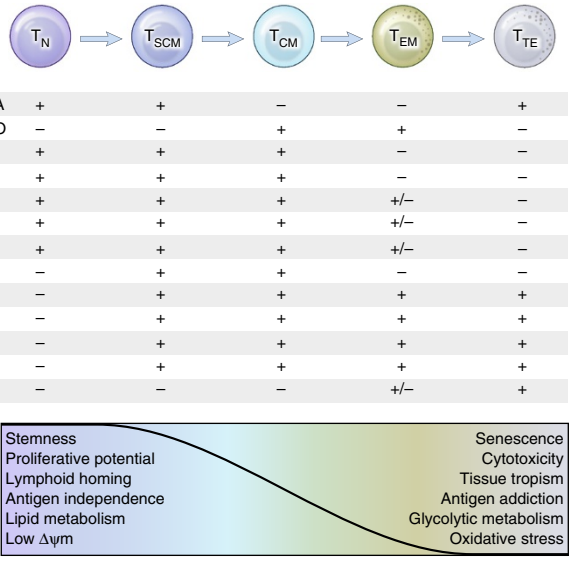

Figure 2 Hierarchical model of human T cell differentiation. After antigen priming, naive T (T_N) cells progressively differentiate into diverse memory T cell subpopulations, and ultimately, into terminally differentiated effector T (T_{TE}) cells. T cell subsets are distinguished by the combinatorial expression of the indicated surface markers. As T_N cells differentiate progressively into the T_{TE} cell type, they lose or acquire specific functional and metabolic attributes. T_{SCM} cell, T memory stem cell; T_{CM} cell, central memory T cell; T_{EM} cell, effector memory T cell; ΔΨm, mitochondrial membrane potential.

T_{SCM} and other antigen-experienced T cells cannot be addressed easily in humans because naive T cells are generated continuously, and several antigenic contacts might occur after the initial encounter. Longitudinal monitoring of genetically engineered lymphocytes infused as antigen-experienced cells, and distinguishable from endogenous lymphocytes thanks to the retroviral integration and transgene expression, has recently enabled the tracking of single T cell clonotypes over time. In patients afflicted with the adenosine deaminase (ADA)-deficient form of severe combined immunodeficiency (SCID), genetically engineered T_{SCM} cells persisted and preserved their precursor potential for decades[38]. Engineered lymphocytes were tracked for up to 14 years in patients with leukemia who were treated with haploidentical HSCT and donor lymphocytes transduced retrovirally to express a suicide gene[35]. This study revealed that the extent of expansion and the amount of persisting gene-marked T cells are tightly correlated with the number of T_{SCM} cells infused, which indicates that this subset of memory cells is endowed with enhanced proliferative potential, immune-reconstitution capacity and longevity[35]. Notably, the same observation has been reported in a clinical trial based on the infusion of autologous T cells that have been genetically engineered to express a chimeric antigen receptor (CAR)[39], which underscores that this phenomenon is not confined to the HSCT model. In patients treated with suicide-gene therapy, it was possible to detect circulating gene-modified T cells from 2 to 14 years after treatment. Viral integration and TCR-α and TCR-β clonal markers were used to trace longitudinally single, gene-modified T cell clones, sorted according to the T cell differentiation phenotype in the infused products and in patients, at long-term follow-up. It was thus possible to show that dominant T cell clones detected long term originate preferentially from infused T_{SCM} cells, and to a lesser degree, from T_{CM} clones[35]. Taken together, these results indicate that human T_{SCM} cells have an exceptional capacity

to persist long term. Similar conclusions were reached by monitoring T cell subset dynamics in NHP models of infection[24] and patients with HIV-1 undergoing antiretroviral therapy (ART)[40]; two experimental settings in which antigen load and time of antigen exposure can be controlled precisely. By taking advantage of the peculiar biology of the Tat-specific epitope TL8, which uniformly undergoes escape mutation within 4–5 weeks after infection with simian immunodeficiency virus (SIV), Lugli *et al.*[24] investigated the persistence of different memory Tat-specific T cell subsets in the virtual absence of any stimulation with antigen. In this setting, T_{SCM} cells were able to persist at unchanged levels for up to 70 d after infection, whereas T_{CM} and T_{EM} cells contracted tenfold and 100-fold, respectively[24]. Similarly, pharmacological antigen withdrawal in ART-treated patients with HIV-1 was associated with a decline of HIV-1-specific T_{EM} cells and terminally differentiated effector (T_{TE}) cells, whereas the T_{SCM} cell type gradually increased in number under these conditions[40]. Mirroring these findings, after YF vaccination virus-specific T_{TE} cells underwent a more pronounced contraction than T_{EM} cells, which in turn declined more steeply than T_{CM} cells[36]. Remarkably, the frequency of YF-specific T_{SCM} cells was maintained stably even 25 years after vaccination[36]. Taken together, this series of studies provides compelling evidence that human T_{SCM} cells are generated directly from naive lymphocytes and are endowed with long-term self-renewal capacity and multipotency.

T_{SCM} cells in host defense

Pathogen-specific T_{SCM} cells have been increasingly identified in human acute and chronic infections caused by viruses, bacteria and parasites[19,35,36,40–42]. These results demonstrate that T_{SCM} cells are commonly generated during natural immune responses against foreign pathogens, but the underlying mechanisms remain poorly understood. Human studies are limited in that the exact time of infection is usually unknown, which makes it difficult to study T cell priming and kinetics. By contrast, active vaccination offers the possibility of inducing an immune response in a supervised fashion. Smallpox and YF vaccines are particularly suitable models of human primary acute viral infection because they consist of live, attenuated, replication-competent viruses capable of inducing strong immune responses with consequent clinical symptoms[43]. By using YF vaccination as a model system, the kinetics of T_{SCM} cell formation and long-term maintenance have recently been studied in great detail[36]. Consistent with findings from studies of SIV infection in NHPs[24], YF-specific T_{SCM} cells were detectable at early time points after vaccination when the immune response was dominated by effector T cells[36]. These T_{SCM} cells persisted at stable levels and became the major YF-specific memory T cell population in the circulation decades after the initial immunization[36]. Considering that YF vaccination provides life-long protection[43], it is reasonable to assume that T_{SCM} cells have a central role in the maintenance of long-term T-cell memory.

The presence of a relevant pool of T_{SCM} cells might also be essential for the control of persisting infections, in which effector T cells undergoing functional exhaustion and replicative senescence need to be replenished continuously by less differentiated T cell subsets[44–47]. Notably, recent studies in chronic viral[40,48] and parasitic infections[42] revealed a negative correlation between the severity of disease and the frequency of circulating T_{SCM} cells. It is unclear whether these observations result from the inability of T_{SCM} cells to be maintained under conditions of strong inflammation and high antigenic load, or vice versa, that the presence of insufficient numbers of T_{SCM} cells impairs the ability of the immune system to control pathogen replication. However, emerging findings suggest that T_{SCM} cells are crucial to the maintenance of immune homeostasis; high levels of infection of the T_{SCM} cell compartment and its subsequent functional perturbation have been linked to the development of symptomatic immune deficiency following SIV and HIV-1 infections[49,50]. Indeed, high quantities of SIV DNA were found in $CD4^+$ T_{SCM} cells from rhesus macaques, who typically develop an AIDS-like clinical picture when left untreated, but they were not found in $CD4^+$ T_{SCM} cells from SIV-infected sooty mangabeys, a group of NHPs that are refractory to clinical or laboratory signs of immune deficiency even when high levels of virus circulate in the peripheral blood[49,51,52]. Resonating with this observation, viremic nonprogressors—a rare group of untreated patients with HIV-1 who develop high levels of HIV-1 replication in the absence of clinical immune deficiency—exhibit reduced levels of HIV-1 DNA in $CD4^+$ T_{SCM} cells in comparison to patients with HIV-1 who show ordinary rates of disease progression[50]. All together, these results underscore a crucial function of T_{SCM} cells in the sustenance of long-lasting cellular immunity against acute and chronic microbial infections.

Given the pivotal role of T_{SCM} cells in the maintenance of life-long immunological memory, it would be desirable to develop vaccines that are capable of inducing substantial numbers of T_{SCM} cells. The majority of clinical vaccine formulations designed to stimulate $CD8^+$ T cell–mediated immunity induce predominantly T_{EM}, and few memory, cells[53,54]. These vaccines are rarely efficacious as compared to those that induce protective antibodies[2,55]. Indeed, current T cell vaccines seem inefficient at triggering mechanisms that are key for the development of memory T cells, including optimal signaling via the TCR and the induction of appropriate metabolic programs, transcription factors and chromatin reorganization[56]. Considering that the activation of $CD8^+$ T cells under conditions of low-level inflammation enhances memory cell formation, one might surmise that vaccines should, ideally, stimulate T cells without triggering the excessive release of proinflammatory cytokines[57]. It is, however, debatable whether optimal generation of memory T cells requires the avoidance of effector cell differentiation. This is illustrated by the fact that natural infections generate sound memory T cell responses, including T_{SCM} cells, despite the initial predominance of effector cells[43]. Indeed, the emergence of T_{SCM} cells was recently observed after the administration of a subunit cancer vaccine capable of inducing a rapid and robust expansion of effector T cells[58]. Much work remains to be done in this area; however, the induction of T_{SCM} cells by novel vaccines should not be at the expense of more differentiated T_{EM} and tissue-resident memory cells, which assure immediate protection at the entry site of re-infection in peripheral tissues[59–61]. Ideally, new vaccines will be able to recreate the full spectrum of memory cell phenotypes that human pathogens and their pathophysiological properties induce *in vivo*[62,63].

T_{SCM} cells can exacerbate human disease

The complex biology of T_{SCM} cells can make it difficult to discriminate between their protective and pathogenic effects because the very characteristics that enable T_{SCM} cells to represent the backbone of life-long cellular immunity under physiologic conditions might empower these cells to drive disease pathogenesis[64]. This seems particularly relevant in the setting of a growing list of immune-mediated diseases associated with aberrant and autoreactive memory T cells. For instance, recent correlative studies have suggested an increased frequency and activation state of $CD8^+$ T_{SCM} cells in individuals with aplastic anemia, a disease mediated by autoreactive cytotoxic T cells targeting hematopoietic progenitors, as compared to healthy individuals[65].

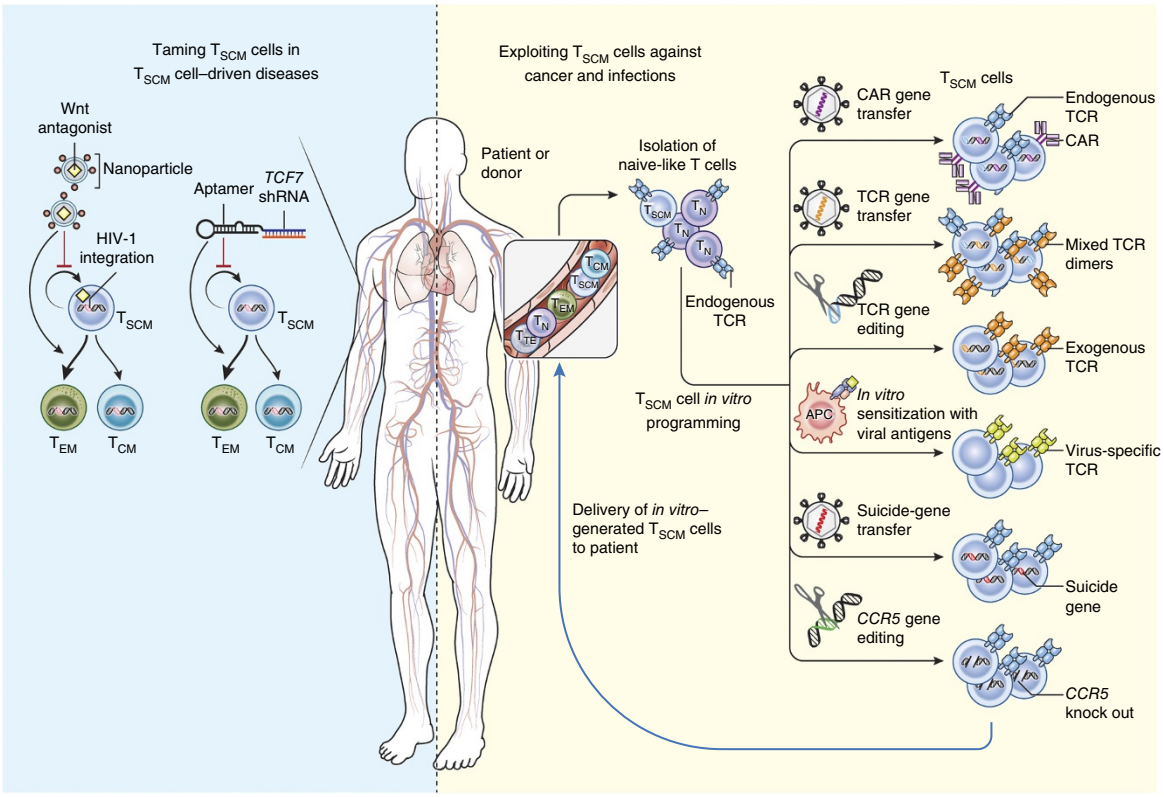

Figure 3 T_{SCM}-cell-based therapeutic interventions for human diseases. T_{SCM} cells can be either disrupted (left) to treat T_{SCM}-driven diseases, such as autoimmunity, T cell leukemia and T cell tropic infections, or exploited (right) to potentiate T cell–based immunotherapies against cancer and infectious diseases. Left, Wnt antagonists or short hairpin RNA (shRNA) targeting key molecules involved in Wnt signaling, such as T cell factor 7 (*TCF7*) could be used to disrupt long-lasting, self-renewing T_{SCM} cell reservoirs by driving them to differentiate into short-lived subsets, such as T_{EM} cells. Nanoparticle or aptamer technology could be employed to target $CD4^+$ T cells or virally infected T cells specifically. Right, patient- or donor-derived naive-like T cells can be used to generate and expand T_{SCM} cells *in vitro* with or without gene engineering. Gene modifications include the insertion of tumor or virus-specific chimeric antigen receptor (CAR) or T cell receptor (TCR) genes, tumor or virus-specific TCR gene editing, suicide-gene transfer for the option to eliminate the transferred T_{SCM} cells and their progeny in case of overwhelming toxicity, and *CCR5* gene editing in the setting of HIV-1 infection. Virus-specific T_{SCM} cells can also be expanded from the naturally occurring antigen-specific TCR repertoire through sensitization protocols *in vitro* favoring the generation of T_{SCM} cells. APC, antigen-presenting cell.

Moreover, an elevated number of $CD8^+$ T_{SCM} cells after immunosuppressive treatment was associated with treatment failure and subsequent aplastic anemia relapse[65]. Elevated numbers of T_{SCM} cells were also noted in patients with uveitis, but not in those with systemic lupus erythematosus, an immune-mediated disease characterized primarily by autoreactive humoral responses[65]. Further pointing toward a role of T_{SCM} cells in the pathogenesis of autoimmune diseases and other illnesses of the lymphatic system, a recent genome-wide association study found a strong association between genetic polymorphisms affecting susceptibility to juvenile idiopathic arthritis or chronic lymphocytic leukemia, and the frequency of $CD4^+$ T_{SCM} cells[22]. How T_{SCM} cells can influence autoimmune diseases will have to be studied in dedicated investigations, but on the basis of current knowledge, it is reasonable to hypothesize that long-lasting autoreactive or abnormally activated T_{SCM} cells might induce self-renewing inflammatory cellular responses that are responsible for the durable, and in most cases life-long, persistence of such diseases[66]. The possible role of T_{SCM} cells in other diseases with profound disturbance of cellular immune responses, such as autoimmune hepatitis, thyroiditis, type 1 diabetes and certain types of glomerulonephritis, are currently unknown but represent a high priority area of future research.

In addition to their role in autoimmunity, T_{SCM} cells might have a distinct role in viral diseases in which T cells represent the predominant targets, such as infections caused by $CD4^+$ T cell tropic retroviruses. Notably, work in the context of HIV-1 infection has shown that $CD4^+$ T_{SCM} cells can effectively support both productive viral replication and a transcriptionally silent form of infection[67]. Moreover, by infecting long-lived $CD4^+$ T_{SCM} cells, HIV-1 is able to exploit their stemness to establish an extremely durable, self-renewing viral reservoir that can persist for decades, despite ART, and continuously replenish virally infected cells, thus perpetuating a disease that they are meant to restrict[68]. Indeed, the half-life of HIV-1-infected T_{SCM} cells in ART-treated individuals has been estimated to be around 277 months, a time period substantially longer than that observed for viral reservoirs established in more short-lived T cell populations[69]. In line with these observations, phylogenetic studies demonstrated close associations between viruses circulating early after HIV-1 infection and viral sequences isolated from $CD4^+$ T_{SCM} cells after almost a decade of suppressive ART[68]. Notably, the ability to use $CD4^+$ T_{SCM} as a long-term viral reservoir also seems to occur in individuals infected with HTLV-1, a retrovirus related to HIV-1 that is the primary cause of adult T cell leukemia (ATL). Emerging data indicate that transformed, HTLV-1 infected $CD4^+$ T_{SCM} cells can act as progenitors for dominant circulating ATL clones and can efficiently propagate ATL upon transplantation in animal models[70]. This suggests that they can serve as a cancer stem

cell population responsible for the propagation and maintenance of HTLV-1-infected malignant cells.

Targeting T_{SCM} cells for therapy

Harnessing T_{SCM} cells for adoptive T cell therapy. The extreme longevity, the robust proliferative potential and the capacity to reconstitute a wide-ranging diversity of the T cell compartment make the T_{SCM} cell type an ideal cell population to employ in adoptive immunotherapy (**Fig. 3**). Driven by the growing success of clinical trials that are based on the transfer of naturally occurring and genetically engineered tumor-reactive T lymphocytes, adoptive immunotherapies are rapidly becoming a real therapeutic option for patients with cancer[71,72]. Although these regimens can induce complete and durable tumor regressions in patients with advanced cancer, current response rates remain mostly inadequate, which underscores the need for further improvements[71,72]. There is now extensive evidence indicating that objective responses are strongly correlated with the level of T cell engraftment and peak of expansion early after transfer[73–79]. T cell persistence, although not strictly indispensable in certain conditions[74–77,80], has also been associated with the likelihood of objective responses in numerous trials[78,79,81–85] and might be required to sustain durable remissions[86]. These parameters are influenced considerably by the composition of the infused T cell product because T cell subsets differ widely in terms of proliferative capacity, immune reconstitution and long-term survival[10,87]. Indeed, the administration of cells with longer telomeres[83,88] or cell products comprising higher fractions of $CD62L^+$, $CD28^+$ or $CD27^+$ T cells has been shown to correlate with objective tumor responses in patients[83,88–90], which suggests that less differentiated T cells are therapeutically superior to T_{TE} cells. Notably, the engraftment and expansion of T cells engineered to express a CD19-specific CAR[39] or a suicide gene[35] was correlated with the frequency of infused $CD8^+CD45RA^+CCR7^+$ T_{SCM} cells. Adoptive transfer experiments in mice, using defined T cell subsets, have demonstrated formally that the infusion of less-differentiated $CD62L^+$ T cell populations results in enhanced T cell engraftment, expansion and persistence, which leads ultimately to more profound and durable tumor regressions[18,19,91–95]. Consistent with the developmental hierarchy, minimally differentiated T_{SCM} cells mediate more potent antitumor responses than T_{CM} cells, which, in turn, are more effective than highly differentiated T_{EM} cells[18,19,96]. Some level of plasticity, however, must be granted to the hierarchical model of memory T cell differentiation. In NHPs, genetically engineered CMV-specific effectors derived from purified T_{CM} cells proved superior to effectors derived from T_{EM} cells in terms of expansion and persistence *in vivo*, which shows that even after manipulation *in vitro* and, apparently, a similar degree of terminal differentiation, T cells maintain some characteristics of the subset of origin, and can possibly, at least in part, revert to that original phenotype and function[97].

Despite overwhelming preclinical data indicating a therapeutic advantage to transferring tumor-reactive $CD62L^+$ T cell subsets[18,19,91–95], clinical trials have largely employed unselected intratumoral or peripheral blood mononuclear cell (PBMC)-derived T cell populations. Tumor-infiltrating lymphocytes are typically in a state of terminal differentiation and functional exhaustion, which makes the isolation of early memory T cell subsets impractical[98,99]. However, the selection of less differentiated T cell subsets becomes realistic and desirable in the context of immunotherapies that are aimed at conferring tumor reactivity to circulating T cells via TCR or CAR gene engineering. The isolation of less differentiated T cell populations also has the advantage of reproducibly generating more defined T cell products. Indeed, PBMC composition can vary substantially between individuals as a consequence of age[100], pathogen exposure[101] and prior systemic treatments[102]. Moreover, unselected populations containing high proportions of T_{EM} and effector cells might fail to generate viable clinical products owing to poor *in vitro* cell expansion[103]. Recently, a few clinical trials in which CD19-specific CAR T cells were generated from isolated T_{CM} cells have been reported[86,104,105]. This strategy led to the generation of infusion products composed of substantially more T_{EM} cells than those originating from unselected PBMCs, which indicates that, in the absence of culture conditions restraining T cell differentiation[18,106–110], the benefit of depleting highly differentiated T cell subsets is outweighed by the concomitant removal of naive and T_{SCM} cells[104]. Notwithstanding the reduction of less differentiated T cell subsets, the rates of objective remissions in patients with acute lymphoblastic leukemia (ALL) were comparable to results of trials that used unselected T cell populations[74,75,78,104,111,112]. Whether differences in manufacturing and T cell product composition will affect the rates and duration of clinical responses in other diseases and settings remains to be shown.

So far, the clinical exploitation of T_{SCM} cells has been hindered by their relative paucity in the circulation[19,20] and the lack—until recently—of robust, clinical-grade manufacturing protocols that are capable of generating and maintaining this cell type *in vitro*. These strategies rely on programming and redirecting T_{SCM} cells from naive-like T cells isolated from PBMCs[23,113] (**Fig. 3**). Although the isolation of naive T cells adds complexity to the manufacturing process, it is a crucial step because the presence of more differentiated T cell subsets during naive T cell stimulation accelerates naive T cell differentiation into T_{EM} and T_{TE} cells[114]. It should also be considered that purifying large numbers of specific cell subsets over multiple parameters under good manufacturing practice conditions is becoming increasingly accessible thanks to recent developments in clinical cell-sorting technologies[87,115]. IL-7 and IL-15 have been used successfully to generate tumor-redirected or suicide-gene-modified T_{SCM} cells from naive cell precursors[23] (**Fig. 3**). IL-7 is essential for the development of these cells[23,116], whereas IL-15 primarily sustains their expansion[23]. IL-7 and IL-15-programmed T_{SCM} cells possess a core gene signature of naturally occurring T_{SCM} cells, display an enhanced proliferative capacity as compared to other T cell subsets and are uniquely capable of expanding and mediating GVHD upon serial transplantation[23]. This cytokine combination could also be employed to generate large numbers of TCR-gene-edited T_{SCM} cells by combining zinc-finger nuclease sets specific for the endogenous TCR gene loci with viral vectors encoding tumor-specific TCRs[117] (**Fig. 3**). Moreover, the ability of IL-7 and IL-15 to support the formation and expansion of T_{SCM} cells makes it an ideal strategy for generating T_{SCM} cells without the need to redirect their specificity. This might be particularly suitable for the generation of virus-specific T_{SCM} cells for the treatment and prevention of life-threatening infections after transplantation (**Fig. 3**), given that infection control can be obtained by transferring relatively small numbers of virus-specific memory cells[118]. A demonstration that IL-7 and IL-15 could be employed successfully to generate and expand virus-specific T_{SCM} cells, starting from isolated naive-like cells, was provided recently by Volk and colleagues[119]. This protocol could also be adapted to generate CAR-modified virus-specific T_{SCM} cells, which might lower the risk of GVHD, given the restricted TCR repertoire, and which may exhibit additional proliferative and survival advantages as a result of the triggering *in vivo* of the native virus-specific TCRs by antigens from

Box 1 T_{SCM} cell biology: outstanding questions

Several questions regarding T_{SCM} cell biology remain unresolved. A major issue is how T_{SCM} cells form during infection. Is T_{SCM} cell fate programmed at the time of naive T cell priming, or is it shaped throughout multiple antigen encounters and the diverse inflammatory environments that their progeny experience? A glimpse into T_{SCM} cell transcriptional and epigenetic landscapes[19,36,37,137,138] and metabolism[113,137] has begun to reveal the molecular and metabolic programs of T_{SCM} cells. Whether asymmetric partitioning of key transcription factors[139,140] and metabolic master regulators[141,142] is programming T_{SCM} cell formation is unknown. Additionally, T_{SCM} cell anatomical niches remain elusive. Progress has been hampered by the rarity of T_{SCM} cells, which limits epigenetic, proteomic and metabolomic studies. The lack of mouse models of infection that are capable of generating robust numbers of T_{SCM} cells has precluded researchers from evaluating specific gene contributions to T_{SCM} cell physiology with genetic tools, and from imaging T_{SCM} cell dynamics within tissues by real-time microscopy.

T_{SCM} cell epigenetic and transcriptional programs. Transcriptomic analyses of whole[19,137] and YF-specific T_{SCM} cells[36,37] have revealed that this cell type is closely related to T_{CM} cells. These findings suggest that the majority of transcriptional pathways shaping T_{CM} cell development and maintenance might also regulate T_{SCM} cells. For instance, Wnt-β-catenin signaling is essential for T_{CM} cell formation and survival[143–146], but is also crucial for the generation of T_{SCM} cells[18,19,113]. Likewise, tempering mTOR signaling enhances the development of both T_{CM}[147,148] and T_{SCM} cells[137]. Whether specific transcriptional networks are uniquely activated to influence T_{SCM} cell fate is unknown. It is also unclear what role CD95–FAS ligand signaling in T_{SCM} cell homeostasis has. Overlying the transcriptome is the undefined T_{SCM} cell epigenetic program. Genome-wide analysis of histone methylation in naive and *in vitro*–generated mouse $CD8^+$ T cell subsets, including T_{SCM} cells, have revealed that chromatin accessibility is mostly regulated in a progressive fashion[138], but that confirmation in *ex vivo*–isolated cells is warranted.

T_{SCM} cell metabolism. Metabolism is intimately linked to T cell activity and fate[149]. Fatty acid oxidation, increased mitochondrial biomass and spare respiratory capacity support the development and function of memory T cells[127,150]. Conversely, aerobic glycolysis favors terminal-effector differentiation, limiting T cell memory formation[128]. Emerging findings indicate that naturally occurring and *in vitro*–generated T_{SCM} cells also exhibit the 'metabolic signature' of conventional memory cells[113,137]. Recently, HSC and T cell stemness have been linked to decreased mitochondrial membrane potential ($\Delta\Psi m$)[151]. Analogously, T_{SCM} cells display lower $\Delta\Psi m$ than other antigen-experienced T cell subsets[137,151]. Whether T_{SCM} cells maintain the fused mitochondrial networks with tight cristae organization seen in conventional memory T cells[152] remains to be determined. Future areas of research include the role of amino acids and a global characterization of the T_{SCM} cell metabolome.

T_{SCM} cell anatomical niches. Stem cell niches are instrumental in the regulation of stem-cell behavior and tissue homeostasis, guiding HSCs to either self-renew or differentiate[153]. Accumulating evidence underscores the crucial role of the bone marrow in sustaining the persistence of memory T cells[154–157]. Whether the bone marrow similarly serves as a T_{SCM} cell niche is a fundamental question. Alternatively, akin to naive T cells[158], T_{SCM} cells might rely on homeostatic cues provided by fibroblastic reticular cell niches within lymph nodes. Finally, the characterization of contact-dependent cross-talk, cytokine networks and metabolite constituents regulating T_{SCM} cells in their niches remains to be addressed.

persistent viruses[82,120]. Another clinical-grade strategy promoting the generation of tumor-reactive T_{SCM} cells is based on the activation of naive-like lymphocytes in the presence of IL-7, IL-21 and the Wnt agonist TWS119 (ref. 113). Although both IL-15 (refs. 121,122) and IL-21 (refs. 123–125) have been implicated in the generation and maintenance of memory T cells, IL-21 is more effective in restraining T cell differentiation[107], owing to its specific ability to activate signal transducer and activator of transcription 3 (STAT3) signaling[126] and to sustain the expression of the Wnt-β-catenin transcription factors *TCF7* and *LEF1* (ref. 107). TWS119 has a synergistic effect with IL-21 to induce maximal expression of *TCF7* and *LEF1* by stabilizing β-catenin[113]. CAR-modified T_{SCM} cells generated under these culture conditions are phenotypically, functionally and transcriptionally equivalent to their naturally occurring counterparts[113]. Moreover, they exhibit metabolic features, such as a high spare respiratory capacity[127] and low glycolytic metabolism[128], that are characteristic of long-lived memory T cells. Although these culture conditions profoundly inhibit T cell proliferation, T_{SCM} cells can be redirected efficiently against a tumor antigen and expanded to clinically relevant numbers[113]. More importantly, CAR-modified $CD8^+$ T_{SCM} cells mediated superior and more durable anti-tumor responses than cells generated with protocols currently employed in clinical trials[113]. CAR-modified T_{SCM} cells might also provide an attractive approach for immunotherapy in the setting of nonmalignant diseases, such as HIV-1 infection or other chronic viral illnesses[129,130] (**Fig. 3**). All together, these studies provide both a strong scientific rationale and practical methodologies for the rapid advancement of T_{SCM} cells in human clinical trials of adoptive immunotherapy[131].

Disrupting T_{SCM} cell reservoirs in retroviral infections and autoimmune diseases. The emerging role of $CD4^+$ T_{SCM} cells in the pathogenesis of chronic viral infections such as HIV-1 and HTLV-1 infection might also offer novel opportunities to prevent, treat or cure these diseases. In the context of HIV-1 infection, specific interventions that eliminate HIV-1-infected $CD4^+$ T_{SCM} cells might allow for the destabilization of HIV-1 reservoirs by reducing the number of HIV-1-infected source cells from which new HIV-1^+ viral and cellular progeny can continuously originate, despite suppressive ART. As the molecular programs that govern the stem cell–like behavior of T_{SCM} cells continue to be understood, new molecules regulating proliferation and self-renewal of T_{SCM} cells might represent attractive targets for reducing viral persistence in $CD4^+$ T_{SCM} cells. For instance, Wnt-β-catenin signaling has been identified as a key driver for the homeostasis of T_{SCM} cells[18], and pharmaceutical inhibition of this pathway might therefore translate into a more limited ability of HIV-1 to use the T_{SCM} compartment for maintaining the survival of virally infected cells (**Fig. 3**). This approach might be facilitated by the availability

of existing pharmacological inhibitors of Wnt-β-catenin designed to target cancer stem cells[132]. Although such a strategy might be not entirely specific to the elimination of HIV-1-specific CD4+ T_{SCM} cells, advances in nanotechnology might enable selective delivery of Wnt-β-catenin antagonists or short hairpin RNAs targeting key mediators of Wnt signaling to CD4+ T cells or virally infected cells via nanoparticles or aptamer-based targeting systems[133,134] (**Fig. 3**). Similar strategies are also conceivable for targeting HTLV-1-infected T_{SCM} cells in the setting of ATL or to disrupt long-lasting reservoirs of autoreactive T_{SCM} cells in autoimmune diseases. Additionally, recent advances in gene editing *ex vivo* might enable the design of CD4+ T_{SCM} cells that are intrinsically resistant to HIV-1, through, for example, targeted deletion of the chemokine receptor *CCR5*, which is necessary for viral entry[135], thus mimicking the *CCR5*Δ32 mutation known to confer resistance to HIV-1 infection[136] (**Fig. 3**). Such a population of long-lasting, HIV-1-resistant CD4+ T cells could be used in adoptive immunotherapy strategies to establish a durable cellular immune system that is no longer able to support HIV-1 infection and that might lead to drug-free remission of HIV-1 infection.

Concluding remarks

T_{SCM} cells are rare, antigen-experienced T cells, probably generated directly from naive lymphocytes and endowed with long-term self-renewal capacity and multipotency. Compelling evidence in mice, NHPs and humans points toward a scenario in which T_{SCM} cells represent the apex of the memory T cell differentiation tree. Their longevity and their capacity to reconstitute the entire heterogeneity of the T cell memory compartment entail a double-edged—protective or pathogenic—role for T_{SCM} cells in human diseases. Their increasingly recognized protective role in acute and chronic infections makes them optimal candidates for therapeutic exploitation in vaccination and adoptive T cell therapy against infectious diseases and cancer. Conversely, their relevance in the pathogenesis of autoimmunity, adult T cell leukemia and HIV-1 makes them an attractive target to tame for these pathological conditions. Several issues regarding T_{SCM} cell biology remain to be addressed: characterization of their metabolic requirements, epigenetic and transcriptional programs and anatomical niches (**Box 1**) will guide innovative T_{SCM} cell-based therapeutic interventions for human diseases.

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