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T memory stem cells in health and disease

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Abstract

T memory stem cells (T_{SCM}) 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 subsets. Cumulative evidence in mice, non-human primates and humans indicates that T_{SCM} are minimally differentiated cells at the apex of the hierarchical system of memory T lymphocytes. Here we describe emerging findings demonstrating that T_{SCM}, 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} stemness could be therapeutically leveraged to enhance the efficacy of vaccines and adoptive T-cell therapies for cancer and infectious diseases or, conversely, disrupted to treat T_{SCM}-driven and sustained diseases such as autoimmunity, adult T-cell leukemia, and HIV-1.

Graphical Abstract

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Disclosure of Conflicts of interest:

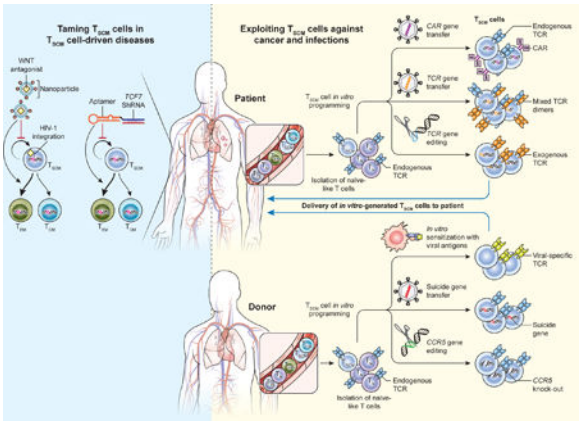
All authors declare that they have no competing interests.

"δὶς γὰρ τὸν αὐτόν, ὥστε καὶ κτείνειν, 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 subsequent encounters with a pathogen – has long been recognized in human history. The first documentation of immunological memory was provided by 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 gain insights into the cellular basis of the immune system and 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 in spite of the high cellular turnover characterizing the immune responses and the lack of replenishment of antigen-specific T cells from hematopoietic stem cells (HSCs) due 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, may be hierarchically organized 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 memory stem cells (T_{SCM}) at center stage in many physiological and pathological human processes. Finally, we highlight ongoing efforts aiming either at harnessing the therapeutic potential of T_{SCM} for adoptive immunotherapies or conversely at destabilizing the T_{SCM} 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 the Timeline.

The discovery of T_{SCM} cells

Advances in multiparameter flow cytometry over the last 20 years have allowed us to dissect the heterogeneity of the T-cell compartment with ever-increasing precision[11]. In a seminal study, van Lier and colleagues identified human naïve, memory, and effector T-cell subsets based on the combinatorial expression of CD27 and CD45RA, with naïve cells expressing both molecules, whereas 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 cells (T_{CM}), which express the lymph node homing molecules CCR7 and CD62L and have limited effector functions, and $CCR7^-CD62L^-$ effector memory T cells (T_{EM}), which preferentially traffic to peripheral tissues and mediate rapid effector functions.

The idea that memory T cells may not solely be confined to the $CD45RA^-$ T-cell compartment, but may also be present within what was considered to be an exclusive naïve T-cell realm, began to take shape following the identification in mice of a novel memory T-cell population characterized by a naïve-like phenotype but expressing high amounts of stem cell antigen-1 (SCA-1) 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} based on the observation that they were capable of sustaining graft-versus-host disease (GVHD) upon serial transplantation in 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}, however, has not been straightforward mainly due to the lack of a human ortholog of SCA-1, the prototypical marker of mouse T_{SCM}. Though it was known that a significant fraction of long-lived antigen-specific $CD8^+$ and $CD4^+$ memory T cells displayed a naïve-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 to pinpoint this elusive memory phenotype in humans was missing. The breakthrough came with the demonstration that mouse T_{SCM} could be successfully generated *in vitro* from naïve precursors by activating the WNT-β-catenin signaling pathway using the WNT ligand, WNT3A, or inhibitors of glycogen synthase kinase-3β[18]. Translated to humans, this strategy has recently allowed the identification of human T_{SCM}[19]. Similar to their murine counterparts, human and non-human primate (NHP) T_{SCM} are clonally expanded cells expressing a largely naïve-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%). Interestingly, the frequency of circulating T_{SCM} does not significantly vary with age[21], but it appears to be heritable and associated with single nucleotide polymorphisms at a genetic locus containing CD95[22], suggesting a potential role of FAS signaling in the regulation of T_{SCM} homeostasis. T_{SCM} exhibit all the defining properties of memory cells, including a diluted content of TCR excision circles, the ability to rapidly proliferate 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 naïve T cells, they share similar recirculation patterns and distribution *in vivo* as evidenced by detailed compartmentalization studies in NHP[24]. For

instance, T_{SCM} are found more abundantly in lymph nodes compared to the spleen and bone marrow and are virtually absent from peripheral mucosae[24]. Thus, T_{SCM} represent a subset of minimally differentiated T cells characterized by phenotypic and functional properties bridging naïve and conventional memory cells (Fig. 1).

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 the existence of a stem cell pool of memory T lymphocytes was initially postulated by Fearon and colleagues[7], the authors pointed to T_{CM} as putative T memory stem cells. This assumption was based on the evidence that T_{CM} 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 in lymph nodes and secondary lymphoid organs, and T_{CM} were, at that time, the only antigen-experienced T cells known to express CCR7 and CD62L. The notion that T_{CM} might function as T memory stem cells was further supported by subsequent findings demonstrating that T_{CM} have superior immune reconstitution capacity and a greater ability to persist *in vivo* than T_{EM}[26]. Recent clonogenic experiments in mice based on single cell serial transfer have formally demonstrated the ability of murine T_{CM} to self-renew and generate T_{EM} and effector progenies *in vivo*[27,28]. Strikingly, T_{EM} were unable to serially reconstitute the host even when transferred at 100-fold higher numbers, showing a limited capacity for self-renewal. Although these experiments did not evaluate T_{SCM}, these results, combined with that of sophisticated experiments tracking T cell fates in mice based on genetic barcoding[29] and on single naïve 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 2 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 naïve lymphocytes upon priming, and further differentiate into shorter-lived effector subsets in a hierarchical differentiation tree, similar to that of other organ systems[31] (Fig. 1). Using hematopoietic stem cell transplantation (HSCT) from haploidentical donors as a model system to study T-cell differentiation, two independent groups have recently showed at polyclonal, antigen-specific, and clonal levels, that human T_{SCM} differentiate directly from naïve precursors, emerging early upon *in vivo* priming[33,34]. By multiparametric flow cytometry and TCR sequencing of individual T lymphocytes it was possible to trace and quantify, at the clonal level, the *in vivo* differentiation landscapes of transferred naïve and antigen-experienced T cells, highlighting T_{SCM} as privileged players in the diversification of naïve cells upon priming[33,34]. Indeed, discrete T-cell subsets traced across HSCT behaved preferentially within a progressive framework of differentiation. Notably, only naïve T cells and T_{SCM} were able to reconstitute the entire heterogeneity of memory T-cell subsets, including T_{SCM}[33]. A fraction of originally T_{EM} reverted to a T_{CM} phenotype[33]. By contrast, only a very limited number of T_{CM} and T_{EM} converted to T_{SCM}[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} – only when T_{SCM} were present within the infused cell product[35]. Altogether these results strengthen earlier *in vitro* observations in human[19] and NHP[24] showing that the potential to form diverse progeny is progressively restricted proceeding from T_{SCM} to T_{CM} and T_{EM}. Thus, granting some level of plasticity to the system, these data point to a progressive model of T-cell differentiation, in which T_{SCM} 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} with antigen-experienced T cells, and places them at a hierarchically superior level than T_{CM}[19,23,36,37].

The concept of stemness also involves self-renewal and implicates long-term persistence[25]. The long-term persisting ability of T_{SCM} and other antigen-experienced T cells cannot be easily addressed in humans, since naïve T cells are continuously generated, 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 allowed the tracking of single T-cell clonotypes over time. In patients affected by ADA-SCID, genetically-engineered T_{SCM} persisted and preserved their precursor potential for decades[38]. In leukemic patients treated with haploidentical HSCT and donor lymphocytes retrovirally transduced to express a suicide gene, engineered lymphocytes were traced for up to 14 years[35]. This study revealed that the extent of expansion and the amount of persisting gene-marked T cells tightly correlate with the number of T_{SCM} infused, indicating that this subset of memory cells is endowed with enhanced proliferative potential, immune reconstitution capacity and longevity[35]. Interestingly, the same observation has been reported in a clinical trial based on the infusion of autologous T cells genetically-engineered to express a chimeric antigen receptor (CAR)[39], underscoring that this phenomenon is not confined to the HSCT model. In patients treated with suicide gene therapy, by combining T-cell sorting with sequencing of integration, TCRα and TCRβ clonal markers, it was possible to show that dominant long-term T-cell clones preferentially originate from infused T_{SCM} and to a lesser degree from T_{CM} clones[35]. Together, these results indicate that human T_{SCM} have an exceptional capacity to persist long-term. Similar conclusions were reached by monitoring T-cell subset dynamics in NHP infection models[24] and HIV-1 patients undergoing antiretroviral therapy (ART)[40], in which antigen load and time of antigen exposure can be precisely controlled. Taking advantage of the peculiar biology of the Tat-specific epitope TL8, which uniformly undergoes escape mutation within 4–5 weeks after Simian Immunodeficiency Virus (SIV) infection, Lugli *et al.*[24] investigated the persistence of different memory T-cell subsets in the virtual absence of any antigen perturbation. In this setting, T_{SCM} were able to persist at unchanged levels for up to 70 days after infection, whereas T_{CM} and T_{EM} contracted 10-fold and 100-fold, repesctively[24]. Likewise, pharmacological antigen withdrawal in ART-treated HIV-1 patients was associated with a decline of HIV-1-specific T_{EM} and terminally differentiated effector cells (T_{TE}), whereas T_{SCM} were restored and even expanded under these conditions[40]. Mirroring these findings, YF-specific T-cell subsets declined after vaccination with attrition rates progressively increasing along with a differentiation from T_{CM} to T_{EM} and T_{TE}[36]. Remarkably, the frequency of YF-specific T_{SCM} was stably maintained even 25 years after vaccination[36]. Taken together, this series of studies provides compelling evidence that

human T_{SCM} are generated directly from naïve lymphocytes and are endowed with long-term self-renewal capacity and multipotency.

T_{SCM} cells in antimicrobial immune defense and after vaccination

Human T_{SCM} have been increasingly identified in acute and chronic infections caused by a variety of pathogens, including viruses, bacteria and parasites[19,35,36,40–42]. These results demonstrate that T_{SCM} 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, making it difficult to study T-cell priming and kinetics. By contrast, active vaccination offers the possibility to induce an immune response in a supervised fashion. Smallpox and YF vaccines are particularly suitable models of human primary acute viral infection as they consist of live attenuated, replication competent viruses capable of inducing strong immune responses with consequent clinical symptoms[43]. Using YF vaccination as model system, the kinetics of T_{SCM} formation and long-term maintenance have recently been studied in great detail[36]. Consistent with findings from studies of SIV infection in NHP[24], YF-specific T_{SCM} were detectable at early time points after vaccination when the immune response was dominated by effector T cells[36]. These T_{SCM} persisted at stable levels, becoming 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} play a central role in the maintenance of long-term T-cell memory.

The presence of a relevant pool of T_{SCM} might also be essential for the control of persisting infections, in which effector T cells undergoing functional exhaustion and replicative senescence need to be continuously replenished by less-differentiated T-cell subsets[44–46]. Interestingly, recent studies in chronic viral[40,47] and parasitic infections[42] have shown the existence of a negative correlation between the severity of disease and the frequency of circulating T_{SCM}. It is unclear whether these observations result from the inability of T_{SCM} to be maintained under conditions of strong inflammation and high antigenic load, or vice versa, the lack of physiological numbers of T_{SCM} impairs the ability of the immune system to keep the infection in check. However, emerging findings suggest that T_{SCM} are a fundamental pillar of immune homeostasis as high levels of infection and subsequent functional perturbation of the T_{SCM} compartment have been linked to the development of symptomatic immune deficiency following SIV and HIV-1 infections[48,49]. Indeed, high quantities of SIV DNA were found in $CD4^+$ T_{SCM} from rhesus macaques, who typically develop an AIDS-like clinical picture when left untreated, but not in $CD4^+$ T_{SCM} from SIV-infected sooty mangabeys, a group of NHP who are refractory to clinical or laboratory signs of immune deficiency even when high levels of virus circulate in the peripheral blood[48,50,51]. Resonating with this observation, viremic non-progressors – a rare group of untreated HIV-1 patients, 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} in comparison to HIV-1 patients with ordinary rates of disease progression, who instead show high amounts of HIV-1 infected T_{SCM} despite a relative depletion of the total $CD4^+$ T_{SCM} compartment[49]. Altogether these results underscore a critical function of T_{SCM} in sustaining long-lasting cellular immunity against acute and chronic microbial infections.

Given the pivotal role of T_{SCM} in maintaining life-long immunological memory, it would be desirable to develop vaccines capable of inducing significant numbers of T_{SCM}. The majority of clinical vaccine formulations designed to stimulate $CD8^+$ T-cell-mediated immunity induce predominantly T_{EM}, with only little emergence of memory cells[52,53]. These vaccines are generally poorly efficient and rarely protective compared to those inducing protective antibodies[2,54]. Indeed, current T-cell vaccines appear unable to trigger mechanisms that are key for the development of memory T cells, including optimal signaling *via* the TCR and induction of appropriate metabolic programs, transcription factors, and chromatin reorganization[55]. Considering that the activation of $CD8^+$ T cells under conditions of low-level inflammation enhances memory cell formation, one may consider that novel vaccines should preferentially activate T cells without triggering the excessive release of pro-inflammatory cytokines[56]. 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}, despite initial predominance of effector cells[43]. Much work remains to be done in this area, however, the induction of T_{SCM} 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[57–59]. Ideally, new vaccines will be able to recreate the large heterogeneity of memory cells, including long-lived T_{SCM}, that human pathogens and their pathophysiological properties induce *in vivo*[60,61].

T_{SCM} cells in human diseases

The complex biology of T_{SCM} can make it difficult to discriminate between their protective and pathogenic effects because the very characteristics that enable T_{SCM} to represent the backbone of life-long cellular immunity under physiologic conditions may empower these cells to drive disease pathogenesis[62]. 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} in individuals with aplastic anemia, a disease mediated by autoreactive cytotoxic T cells targeting hematopoietic progenitors[63]. Moreover, an elevated number of $CD8^+$ T_{SCM} after immunosuppressive treatment was associated with treatment failure and subsequent disease relapse[63]. Elevated quantities of T_{SCM} were also noted in patients with uveitis, but not with systemic lupus erythematosus, an immune-mediated disease primarily characterized by autoreactive humoral responses[63]. Further pointing towards a role of T_{SCM} 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}[22]. How T_{SCM} can influence autoimmune diseases will have to be studied in dedicated investigations, but based on current knowledge it is reasonable to hypothesize that long-lasting autoreactive or abnormally activated T_{SCM} may induce self-renewing inflammatory cellular responses that are responsible for the durable, and in most cases life-long persistence of such diseases[64]. The possible role of T_{SCM} in other diseases with profound disturbance of cellular immune

responses, such as autoimmune hepatitis, thyroiditis, type I 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} may 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} can effectively support both productive viral replication and a transcriptionally-silent form of infection[65]. Moreover, by infecting long-lived $CD4^+$ T_{SCM}, 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, perpetuating a disease they are meant to restrict[66]. Indeed, the half-life of HIV-1-infected T_{SCM} in ART-treated individuals has been estimated to last for 277 months, a time period significantly longer than that observed for viral reservoirs established in more short-lived T-cell populations[67]. 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} after almost a decade of suppressive ART[66]. 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} can act as progenitors for dominant circulating ATL clones, and efficiently repopulate ATL clones upon transplantation in animal models[68] suggesting that they can serve as a cancer stem cell population responsible for propagating and maintaining 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 T_{SCM} an ideal cell population to employ in adoptive immunotherapy (Fig. 2). Driven by the growing success of clinical trials 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[69,70]. Although these regimens can induce complete and durable tumor regressions in patients with advanced cancer, current response rates remain mostly inadequate underscoring the need for further improvements[69,70]. There is now extensive evidence indicating that objective responses strongly correlate with the level of T-cell engraftment and peak of expansion earlier after transfer[71–77]. T-cell persistence, though not strictly indispensable in certain conditions[72–75,78], has also been associated with the likelihood of objective responses in numerous trials [76,77,79–83] and might be required to sustain durable remissions[84]. These parameters are considerably influenced by the composition of the infused T-cell product as T-cell subsets differ widely in terms of proliferative capacity, immune reconstitution and long-term survival[10,85]. Indeed, the administration of cells with longer telomeres[81,86] or cell products comprising higher fractions of $CD62L^+$, $CD28^+$ or $CD27^+$ T cells has been shown to correlate with objective tumor responses in patients[81,86–88], suggesting that less-differentiated T cells are

therapeutically superior to T_{TE}. Notably, the engraftment and expansion of T cells engineered to express a CD19-specific CAR[39] or a suicide gene[35] correlated with the frequency of infused $CD8^+CD45RA^+CCR7^+$ T_{SCM}. Adoptive transfer experiments in mice using defined T-cell subsets have formally demonstrated that the infusion of less-differentiated $CD62L^+$ T-cell populations results in enhanced T-cell engraftment, expansion and persistence, ultimately leading to more profound and durable tumor regressions[18,19,89–93]. Consistent with the developmental hierarchy, minimally differentiated T_{SCM} mediate more potent antitumor responses than T_{CM}, which in turn are more effective than highly differentiated T_{EM}[18,19,94]. Some level of plasticity, however, must be granted to the hierarchical model of memory T-cell differentiation. In NHP, genetically-engineered CMV-specific effectors derived from purified T_{CM} proved superior to effectors derived from T_{EM} in terms of *in vivo* expansion and persistence, showing that even after *in vitro* manipulation 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[95].

Despite overwhelming preclinical data indicating a therapeutic advantage to transferring tumor-reactive $CD62L^+$ T-cell subsets[18,19,89–93], 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 making the isolation of early memory T-cell subsets impractical[96,97]. However, the selection of less-differentiated T-cell subsets becomes realistic and desirable in the context of immunotherapies aiming 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 consistent and defined T-cell products. Indeed, PBMC composition can vary significantly between individuals as a consequence of age[98], pathogen exposure[99], and prior systemic treatments[100]. Moreover, unselected populations containing high proportions of T_{EM} and effector cells might fail to generate viable clinical products due to poor *in vitro* cell expansion[101]. Recently, two clinical trials in which CD19-specific CAR T cells were generated from isolated T_{CM} have been reported[84,102,103]. This strategy led to the generation of infusion products comprising significantly more T_{EM} than those originating from unselected PBMC indicating that, in the absence of culture conditions restraining T-cell differentiation[18,104–108], the benefit of depleting highly-differentiated T cell subsets is outweighed by the concomitant removal of naïve and T_{SCM}[102]. Notwithstanding the reduction of less-differentiated T-cell subsets, the rates of objective remissions in acute lymphoblastic leukemia (ALL) patients were comparable to trials using unselected T-cell populations[72,73,76,102,109,110]. Whether differences in manufacturing and T-cell product composition will affect rates and duration of clinical responses in other diseases and settings remains to be shown.

So far, the clinical exploitation of T_{SCM} has been hindered by their relative paucity in the circulation[19,20] and the lack – until recently – of robust, clinical-grade manufacturing protocols capable of generating and maintaining this cell type *in vitro*. These strategies rely on programming and redirecting T_{SCM} from naïve-like T cells isolated from PBMC[23,111] (Fig. 2). Although the isolation of naïve T cells adds complexity to the manufacturing

process, it is a critical step because the presence of more differentiated T-cell subsets during naïve T-cell stimulation accelerates naïve T-cell differentiation into T_{EM} and T_{TE} cells[112]. It should also be considered that purifying large numbers of specific cell subsets over multiple parameters under GMP conditions is becoming increasingly accessible thanks to recent developments in clinical cell sorting technologies[85,113]. IL-7 and IL-15 have successfully been used to generate tumor-redirected or suicide gene-modified T_{SCM} from naïve cell precursors[23] (Fig. 2). IL-7 is essential for the development of these cells[23,114], while IL-15 primarily sustains their expansion[23]. IL-7 and IL-15-programmed T_{SCM} possess a core gene signature of naturally occurring T_{SCM}, display an enhanced proliferative capacity 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} by combining Zinc Finger Nuclease sets specific for the endogenous TCR gene loci with lentiviral vectors encoding tumor-specific TCRs[115] (Fig. 2). Moreover, the ability of IL-7 and IL-15 to support the formation and expansion of T_{SCM} makes it an ideal strategy to generate T_{SCM} without the need to redirect their specificity. This may be particularly suitable for the generation of virus-specific T_{SCM} for the treatment and prevention of life-threatening infections after transplantation (Fig. 2) as infection control can be obtained by transferring relatively small numbers of virus-specific memory cells[116]. A demonstration that IL-7 and IL-15 could be successfully employed to generate and expand virus-specific T_{SCM} starting from isolated naïve-like cells was recently provided by Volk and colleagues[117]. This protocol could also be adapted to generate CAR-modified virus-specific T_{SCM}, which may lower the risk of GVHD given the restricted TCR repertoire and exhibit additional proliferative and survival advantages as result of the *in vivo* triggering of the native virus-specific TCRs by antigens from persistent viruses[80,118]. Another clinical-grade strategy promoting the generation of tumor-reactive T_{SCM} is based on the activation of naïve-like lymphocytes in the presence of IL-7, IL-21 and the WNT agonist TWS119[111]. Although both IL-15[119,120] and IL-21[121-123] have been implicated in the generation and maintenance of memory T cells, IL-21 is more effective in restraining T-cell differentiation[105] due to its specific ability to activate STAT3 signaling[124] and to sustain the expression of WNT-β-catenin transcription factors *TCF7* and *LEF1*[105]. TWS119 provides a synergistic effect with IL-21 to induce maximal expression of *TCF7* and *LEF1* by stabilizing β-catenin[111]. CAR-modified T_{SCM} generated under these culture conditions are phenotypically, functionally, and transcriptionally equivalent to their naturally occurring counterparts[111]. Moreover, they exhibit metabolic features characteristic of long-lived memory T cells such as a high spare respiratory capacity[125] and low glycolytic metabolism[126]. Although these culture conditions profoundly inhibit T-cell proliferation, T_{SCM} can be efficiently redirected against a tumor antigen and expanded to clinically relevant numbers[111]. More importantly, CAR-modified $CD8^+$ T_{SCM} mediated superior and more durable anti-tumor responses than cells generated with protocols currently employed in clinical trials[111]. CAR-modified T_{SCM} may also provide an attractive approach for immunotherapy in the setting of non-malignant diseases, such as HIV-1 infection or other chronic viral illnesses[127,128] (Fig. 2). Altogether, these studies provide both a strong scientific rationale and practical methodologies for the rapid advancement of T_{SCM} in human clinical trials of adoptive immunotherapy[129].

Disrupting T_{SCM} cell reservoirs in retroviral infections and autoimmune diseases

The emerging role of $CD4^+$ T_{SCM} in the pathogenesis of chronic viral infections such as HIV-1 and HTLV-1 infection may 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} may 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 governing the stem cell-like behavior of T_{SCM} continue to be understood, new molecules regulating proliferation and self-renewal of T_{SCM} may represent attractive targets for reducing viral persistence in $CD4^+$ T_{SCM}. For instance, WNT-β-catenin signaling has been identified as a key driver for the homeostasis of T_{SCM}[18], and pharmaceutical inhibition of this pathway may therefore translate into a more limited ability of HIV-1 to use the T_{SCM} compartment for maintaining survival of virally-infected cells (Fig. 2). This approach may be facilitated by the availability of existing pharmacological inhibitors of WNT-β-catenin designed to target cancer stem cells[130]. Although such as strategy might be not entirely specific for eliminating HIV-1-specific $CD4^+$ T_{SCM}, advances in nanotechnology may allow for 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[131,132] (Fig. 2). Similar strategies are also conceivable to target HTLV-1-infected T_{SCM} in the setting of ATL or to disrupt long-lasting reservoirs of autoreactive T_{SCM} in autoimmune diseases. Additionally, recent advances in *ex-vivo* gene editing may allow the design of $CD4^+$ T_{SCM} that are intrinsically resistant to HIV-1, through e. g. targeted deletion of the chemokine receptor CCR5, which is necessary for viral entry[133], thus mimicking the CCR5Δ32 mutation known to confer resistance to HIV-1 infection[134] (Fig. 2). 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 may allow for drug-free remission of HIV-1 infection.

Concluding Remarks

T_{SCM} are rare antigen-experienced T cells, likely generated directly from naïve lymphocytes and endowed with long-term self-renewal capacity and multipotency. Compelling evidence in mice, NHP, and humans points towards a scenario in which T_{SCM} 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} in human diseases. The increasingly recognized protective role of T_{SCM} 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 T_{SCM} an attractive target to tame for these pathological conditions. Several issues regarding T_{SCM} biology remain to be addressed: characterization of their metabolic requirements, epigenetic and transcriptional programs, and anatomical niches (Box 1) will possibly guide innovative T_{SCM}-based therapeutic interventions for human diseases.

T_{SCM} epigenetic and transcriptional programs.

Genome-wide transcriptomic analyses of whole[19,135] and yellow fever-specific T_{SCM}[36,37] have shown a high relatedness between these cells and central memory T cells (T_{CM}). These findings suggest that the majority of signaling pathways and transcriptional factors that shape the development and maintenance of T_{CM} might be to a certain degree involved in the regulation of T_{SCM}. This reasoning is exemplified by the demonstration that WNT-β-catenin signaling, which is essential for T_{CM} formation and long-term survival[141–144], is also critical for the generation of T_{SCM}[18,19,111]. Likewise, tempering mTOR signaling has been shown to enhance the development of both T_{CM}[145,146] and T_{SCM}[135]. Whether specific transcriptional networks are uniquely activated to influence T_{SCM} fate is unknown. It is also currently unclear what is the role of CD95-FASL signaling in T_{SCM} homeostasis. The epigenetic programs in T_{SCM} remain largely undefined. Emerging genome-wide analysis of histone methylation on two histone H3 lysine residues (H3K4me3 and H3K27me3) in naïve and *in vitro*-generated murine $CD8^+$ T cell subsets have revealed that chromatin accessibility is mostly regulated in a progressive fashion further supporting a hierarchical model of T-cell differentiation, in which T_{SCM} represent the least differentiated antigen-experienced T cell subset[136].

T_{SCM} metabolism.

A growing body of work has recently highlighted the importance of cell metabolism in regulating the activity and fate commitment of T lymphocytes[147]. Fatty acid oxidation, increased mitochondrial biomass and spare respiratory capacity (SRC) have been shown to support the development of memory T cells and confer on them a bioenergetic advantage necessary to sustain rapid recall responses[125,148]. Conversely, aerobic glycolysis has been associated with the formation of short-lived terminally differentiated effector cells and defects in the establishment of T cell memory[126]. Emerging findings indicate that naturally-occurring and *in vitro*-generated human T_{SCM} also exhibit the prototypical ‘metabolic signature’ of memory cells with reduced glycolytic flux, preferential lipid oxidative metabolism, and high SRC[111,135]. Recently, HSC and T cell stemness have been linked to presence of decreased mitochondrial membrane potential ($\Delta\Psi m$)[149]. Consistent with these findings, T_{SCM} display lower $\Delta\Psi m$ than other antigen-experienced T cell subsets, including T_{CM}[135,149]. Whether T_{SCM} maintain fused mitochondrial networks with tight cristae organization, which have been shown to facilitate electron transport chain activity in conventional memory T cells[150] remains to be determined. Future area of research also include a more global characterization of the T_{SCM} metabolome and a deeper understanding of the role of amino acids in T_{SCM} metabolism and function.

T_{SCM} anatomical niches.

Stem cell niches are instrumental in regulating stem cell behavior and tissue homeostasis[151]. Specialized niches in the bone marrow provide not only physical support but also soluble factors and cellular interactions that guide HSCs’ decision to either self-renew or differentiate[151]. Increasing evidence has recently underscored the critical role of the bone marrow also in sustaining life-long persistence of conventional memory T cells[152–155]. Whether the bone marrow can similarly serve as a niche for T_{SCM} is a fundamental question

that needs to be addressed. Alternatively, it might be evaluated whether, akin to naïve T cells[156], T_{SCM} rely on homeostatic cues provided by fibroblastic reticular cell niches within T-cell zones of secondary lymphoid organs. Finally, it would be critical to characterize cell contact-dependent cross-talk, cytokine networks and metabolite constituents regulating T_{SCM} differentiation and function in their niches.

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Timeline: T-cell stemness and TSCM: milestones and key discoveries

T_{SCM}	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

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Box 1:

T_{SCM} cell biology: outstanding questions

Several questions regarding T_{SCM} biology have yet to be addressed. A major unresolved issue is how T_{SCM} physiologically form during the course of an infection and the impact of the strenght of antigen stimulation. Is T_{SCM} fate immediately programmed at the time of naïve T cell priming or is it shaped throughout the number of antigen encounters and the diverse inflammatory environments that their progenies experience in the acute phase of the infection? A glimpse into T_{SCM} transcriptional and epigenetic landscapes[19,36,37,135,136], and early work exploring T_{SCM} metabolism[111,135] have begun to shed light on the molecular and metabolic programs regulating T_{SCM} formation and homeostasis, but much ground remains to be covered. For instance, it is still unknown whether and to what extent asymmetric partitioning of key transcription factors[137,138] and metabolic master regulators[139,140] is programming T_{SCM} formation. Additionally, studies examining T_{SCM} anatomical niches are entirely missing. Progress in these area of investigation has been hampered to some extent by the rareness of T_{SCM} in the circulation, which is a limiting factor for epigenetic, proteomic and metabolomic studies. A major hurdle is the lack of mouse infection models capable of generating robust numbers of T_{SCM}, which so far has precluded researchers to precisely evaluate specific gene contributions to T_{SCM} physiology and physiopathology with genetic tools, and to image T_{SCM} dynamics in tissues by real-time *in vivo* microscopy.

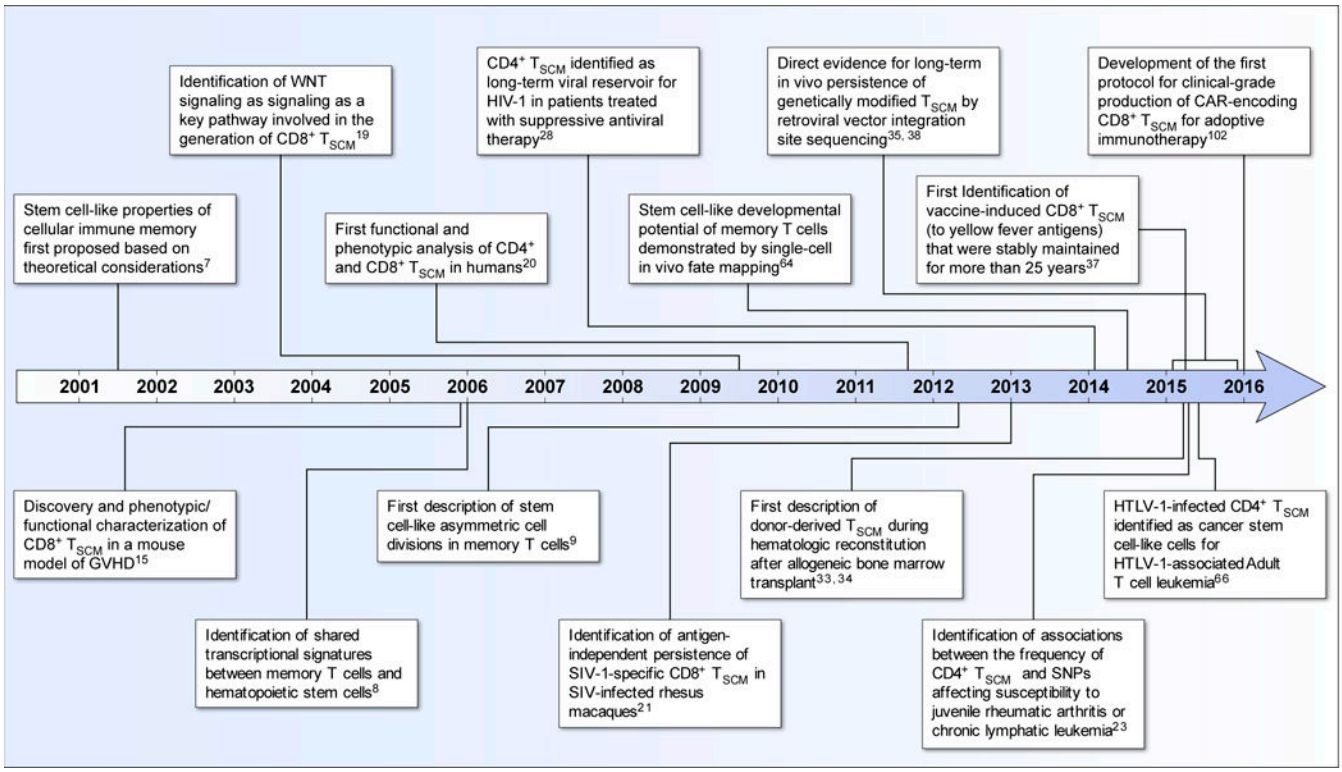

Figure 1: Hierarchical model of human T-cell differentiation.

Following antigen priming, naïve T cells (T_N) progressively differentiate into diverse memory T-cell subpopulations and ultimately into terminally differentiated effector T cells (T_{TE}). T-cell subsets are distinguished by the combinatorial expression of the indicated surface markers. As T_N progressively differentiate into T_{TE}, they lose or acquire specific functional and metabolic attributes. T_{SCM}, T memory stem cell; T_{CM}, central memory T cell; T_{EM}, effector memory T cell; $\Delta\Psi m$, mitochondrial membrane potential.

	T_N	T_{SCM}	T_{CM}	T_{EM}	T_{TE}
CD45RA	+	+	−	−	+
CD45RO	−	−	+	+	−
CCR7	+	+	+	−	−
CD62L	+	+	+	−	−
CD28	+	+	+	+/−	−
CD27	+	+	+	+/−	−
IL-7Rα	+	+	+	+/−	−
CXCR3	−	+	+	−	−
CD95	−	+	+	+	+
CD11a	−	+	+	+	+
IL-2Rβ	−	+	+	+	+
CD58	−	+	+	+	+
CD57	−	−	−	+/−	+

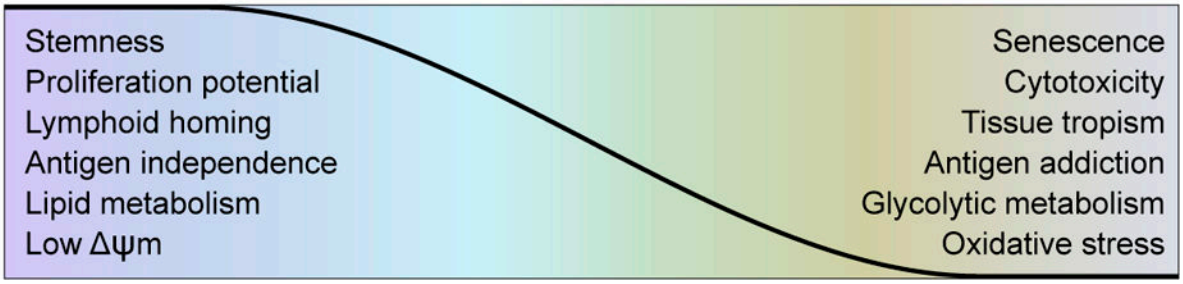

Figure 2: T_{SCM}-based therapeutic interventions for human diseases.

T memory stem cells (T_{SCM}) can be either tamed (left panel) to treat T_{SCM}-driven diseases such as autoimmunity, T-cell leukemia and T-cell tropic infections or exploited (right panel) to potentiate T cell-based immunotherapies against cancer and infectious diseases. Left panel: 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} reservoirs by driving them to differentiate into short-lived subsets such as effector memory T cells (T_{EM}). Nanoparticle or aptamer technology could be employed to specifically target $CD4^+$ T cells or virally-infected T cells. Right panel: patient- or donor-derived naïve-like T cells can be used to generate and *in vitro* expand T_{SCM} 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 in the context of donor lymphocyte infusion following hematopoietic stem cell transplantation, and CCR5 deletion in the setting of

HIV-1 infection. Virus-specific T_{SCM} can also be expanded from the naturally-occurring antigen-specific TCR repertoire through *in vitro* sensitization protocols favoring the generation of T_{SCM}. T_N, naïve T cell; T_{CM}, central memory T cell; APC, antigen presenting cell.