T cell therapies—are T memory stem cells the answer?

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Abstract: T memory stem cells (TSCM) are the earliest developmental stage of memory T cells, displaying stem cell-like properties and exhibiting a gene profile between naive and central memory (CM) T cells. Their long-lifespan, robust proliferative potential and self-renewal capacity has generated much research and clinical interest particularly for therapeutic use. Here, we discuss recent findings published in *Science Translational Medicine* by Biasco and colleagues [2015 Feb 4;7(273):273ra13], which provided evidence for the persistence of TSCM in humans for up to 12 years after infusion of genetically modified lymphocytes, and we examine the implications for the development of novel immunotherapies using TSCM.

Keywords: T memory stem cells (TSCM); T cells; gene therapy; cancer; clinical trials

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T memory stem cells (TSCM)—the least differentiated memory T cell

TSCM were first described in 2011 by Gattinoni and colleagues (1) as a rare memory T cell subset, constituting only 2-4% of the total $CD4^+$ and $CD8^+$ T cell population in the periphery (1,2). Although TSCM share some phenotypic characteristics with naïve T cells ($CD45RA^+$, $CD45RO^-$, $CCR7^+$, $CD27^+$), they can be distinguished by expression of the memory marker CD95 and IL-2 receptor beta (CD122) (1,3-6). Additional differences in cellular marker expression (including B-cell lymphoma 2 and chemokine receptors CXCR3 and CXCR4) are reported in Gattinoni *et al*. 2011 (1) and reviewed in Flynn and Gorry 2014 (4).

Functionally, TSCM are more similar to memory T cells than naïve cells, as upon T cell receptor (TCR) stimulation TSCM are antigen experienced and secrete cytokines (TNF-α, IFN-γ and IL-2) (1). They also exhibit a lower level of TCR rearrangement circles, similar levels to central memory (CM) and effector memory (EM) T cells, indicating that TSCM have undergone multiple rounds of division (1). Importantly, TSCM are able to differentiate into CM and EM subsets, have a greater self-renewal capacity and are longer lived compared to other memory T cell subsets (1,7); it is these characteristics which make TSCM an attractive candidate for use in T cell therapies.

The clinical significance of TSCM

A critical step forward for the development of TSCM in future therapeutic strategies is demonstrated in the latest study by Biasco *et al*. which was able to uniquely use data from two clinical trials to reveal the longevity of TSCM when transferred into humans (8). This significant study investigated the survival and differentiation of TSCM in two gene therapy clinical trials which used either retrovirally engineered hematopoietic stem cells or mature lymphocytes. These clinical trials were initially developed to validate the safety of gene therapy in the mid-1990s, using gene therapy to treat severe combined immunodeficiency disease (SCID) due to an adenosine deaminase (ADA) deficiency.

These trials [described in more detail in Biasco *et al*. 2015 (8)] importantly showed the use of genetically corrected lymphocytes with no adverse effects but positive

outcomes including immune reconstitution, protection from infection and metabolic correction (9-11). Of further significance, these studies provided a platform to follow the long-term dynamics of genetically modified TSCM in different biological conditions in humans. Thus, this study is the first to describe the long-term fate (*in vivo*) of genetically modified T cells.

Using techniques such as high throughput sequencing of retroviral vector integration sites (IS), Biasco and colleagues could trace the fate or over 1,700 individual T cell clones (8). Notably, this study was able to show that TSCM could persist whilst maintaining precursor potential for up to 12 years post infusion of gene-corrected lymphocytes.

Despite slight variations in the number of $CD8^+$ TSCM between healthy donors and gene therapy treatment conditions, the $CD4^+$ TSCM showed a similar trend of cell frequencies between groups, and in all patients the TSCM counts were within the range for pediatric and adult healthy donors. Thus in these studies, during and after gene therapy, the TSCM counts were similar to normal donors showing no enrichment for TSCM during the clinical trials, nor any adverse effect of the different treatment conditions upon TSCM numbers (8).

Functionally, TSCM displayed IFN-γ production which was lower than effector T cells, and significantly higher than naïve T cells consistent with previous reports (1), and after activation (anti-CD3/CD28) were able to differentiate into CM and EM subsets whilst maintaining a subset of TSCM. Additionally, individual TSCM clones were able to be tracked *in vivo* (for greater than 5 years) and using IS, identical TSCM clones were not only detectable over time but were detected in different T cell subtypes, thus providing evidence that these clones had long-term precursor activity.

One concern with the use of retroviral vectors in gene therapy is that they could activate nearby genes, which may include oncogenes. Furthermore, using this technology with long-term survival precursor T cells (TSCM) rather than mature T cells may enable the possibility of a greater number of mutations to develop. Of importance clinically, in this study there was no selection for clones with integrations in specific gene loci in any of the gene therapy patients, and there was no over representation of proto-oncogenic insertions (8). These observations suggest that retrovirally engineered TSCM function was preserved in the absence of clonal selections. Thus, this study demonstrated that TSCM were enriched and phenotypically and functionally detectable 12 years post gene therapy. Clinically, this research validated the safe, functional, survival and differentiation potential of using engineered TSCM for future therapies.

It is important to note, that prior to the study by Biasco *et al*. (8) the potential of using TSCM in a clinical setting had been highlighted, where the use of TSCM had showed promising results particularly for cancer research (1,12). These studies characterized the role and function of TSCM and their clinical potential in murine models. The murine studies demonstrated the ability of TSCM to survive adoptive transfer, providing a long-lived T cell with enhanced anti-tumor properties, promoting tumor regression and cure (1). TSCM were also shown to have a greater replicative and survival ability compared to naïve and other memory T cell subsets (CM and EM) (1).

Cieri and colleagues tested the self-renewal capacity of $CD8^+$ TSCM in a graft-versus-host-disease murine model. In this study, TSCM were able to exhibit xenoreactivity over serial transplantations and expand in numbers (12). Importantly, Cieri *et al*. (12) were also able to demonstrate the ability of human TSCM to differentiate, expand, self-renew and undergo genetic modification; thus providing a vital proof of principle for the expansion and use of TSCM for the development of new human immunotherapies.

Both these TSCM studies [Gattinoni *et al*. (1), Cieri *et al*. (12)] however, required expansion of the TSCM as the cell numbers are naturally very low. This could be a potential limitation for using TSCM as a therapy. However Cieri *et al*. was able to demonstrate the use of IL-7 and IL-15 for human TSCM expansion in a clinical setting (12). Additionally, Gattinoni and colleagues have been developing and validating methods for the expansion of TSCM through targeting the Wnt/β-catenin pathway in naïve T cells through the use of glycogen synthase (GSK)-3β, as described in their studies (1,7,13). Phase I clinical trials to test the safety of these approaches will be an important step for the use of expanded numbers of TSCM in a human clinical setting.

The future of TSCM as a T cell therapy

The study by Biasco *et al*. (8) is the first to show the safe use and longevity of genetically engineered T cells, in particular TSCM, for gene therapy, thus highlighting the potential clinical use of TSCM. Additional to gene therapy, TSCM may also prove an important part of other T cell therapies for treating cancer.

Particular interest in using TSCM has arisen for current T cell therapies, where the use of a less differentiated

T cell has been associated with improved therapeutic responses. These have included targeting solid cancers such as melanomas with tumor-infiltrating lymphocytes (TILs) (14,15), and engineering T cells to express chimeric antigen receptors (CARs, for example expression of tumor associated antigen-specific TCR) (16).

Commonly, CM T cells have been used for developing CD19-specific CAR for the treatment of B-cell leukemia and lymphoma, with successful tumor eradication reported (4,17-19) and CM T cell have been used to develop virus-specific T cells to be used in combination with CAR technology for adoptive immunotherapies (20). Additionally, methods to expand virus-specific TSCM for clinical applications have recently been reported (21).

However, whether the use of genetically modified TSCM for T cell therapies and adoptive immunotherapies would improve treatment outcomes is unknown. The study by Biasco *et al*. (8) showed the safety and longevity of using genetically modified TSCM in clinical trials. In these trials enzyme replacement therapy (ERT) was used and cells may have had improved survival through ADA-correction (8). It remains to be investigated whether the functional and phenotypic properties of TSCM, in particular their potential for differentiation and survival, are maintained when used in cancer immunotherapies, particularly as CAR T cells. It is likely that the future use of TSCM as a T cell therapy will require cell expansion prior to transfer, and that such strategies may require a combination of therapies, with a different approach likely for the treatment of different cancers.

The flip side of TSCM

TSCM in other diseases have not displayed the same therapeutic promise as that demonstrated in the study by Biasco *et al*. (8). In human immunodeficiency virus type 1 (HIV-1) in particular, TSCM have been shown to be infected (3,5,6,22) and can become a long-lived reservoir of HIV-1 (3,23). Despite low cell numbers of $CD4^+$ TSCM, over-time their contribution to the HIV-1 reservoir increases (3). This is likely to be influenced by a decline in contribution to the HIV-1 reservoir from shorter-lived memory T cells such as EM, thus the survival of HIV-1 infected TSCM assists to preserve the HIV-1 reservoir and viral persistence. HIV-1 infection of TSCM raises treatment and eradication issues due to the longevity of TSCM survival, and their proliferation and differentiation potential into other memory T cell subsets (4).

Gattinoni and colleagues are researching whether the induction of the Wnt signaling pathway using GSK-3β can lead to the accumulation of β-catenin and block T cell differentiation (7), and whether this approach would be beneficial for treatment of HIV-1. The Wnt/β-catenin signaling pathway is one pathway which is likely to be involved in influencing whether TSCM undergoes self-renewal or differentiation (24,25). Such inhibitors are currently being used to target cancer stem cells, which can persist following treatment to kill proliferating tumor cells (4,26,27). Treatments such as β-catenin inhibitors, if effective at promoting cell differentiation may be useful for assisting in the treatment of HIV-1, acting as a method for the reactivation of a long-lived latent reservoir promoting TSCM to differentiate into shorter lived memory subsets (28).

TSCM have also been shown to play a detrimental role in other diseases such as adult T cell leukemia (ATL), which is a $CD4^+$ T cell malignancy associated with human T cell leukemia virus type 1 (HTLV-1). Recently, Nagai and colleagues (29) have provided evidence for TSCM to be infected by HTLV-1, and with their longevity, TSCM have the ability to preserve ATL clones and give rise to ATL, acting as ATL initiating stem cells (29). One concern is that ability of TSCM to repopulate ATL, combined with their longevity, may cause them to potentially act as an ATL reservoir. Thus, strategies including targeting the self-renewal and survival pathways of TSCM such as the suggested Wnt-β catenin pathway may provide a method to disrupt the formation of viral HTLV-1 reservoirs in ALT.

The propensity of HTLV-1-infected TSCM to transform and potentially cause ATL is perhaps a concern for the use of retrovirally transformed TSCM in gene therapy. However, the recent study by Biasco *et al*. showed that the modified TSCM lasted over a decade without the emergence of clonal dominance, which suggested safe use of genetically engineered TSCM in a clinical setting. The long-term persistence of TSCM may become a challenge in the eradication of some diseases, however, the survival of TSCM has also been demonstrated to play a positive role in gene therapy (8) and adoptive T cell immunotherapies (13,15,30), and additionally in vaccination studies. A recent report has shown the persistence of yellow fever specific $CD8^+$ TSCM for 25 years post vaccination (31). These cells were capable of self-renewal and displayed markers and mRNA profiling consistent with that reported for TSCM. Thus, vaccine specific TSCM are able to persist for decades in humans, suggesting TSCM are capable of inducing durable cellular immunity, a desirable trait for many

immunotherapies.

Conclusions

Biasco and colleagues are the first to demonstrate the potential of TSCM to be used safely in human gene therapy, notably with TSCM persisting for up to 12 years post infusion. Significantly, this study has validated the safe, functional, survival and differentiation potential of using engineered TSCM for future therapies. The longevity of TSCM has also shown promise in vaccination studies where viral specific TSCM have been demonstrated to last for decades, and new methods for the expansion for TSCM in a clinical setting show promise for the trial of TSCM in adoptive immunotherapies. The long-term survival of TSCM, however, can be detrimental in some diseases where TSCM are able to form a viral reservoir. Thus, it is likely the clinical significance of TSCM as a T cell therapy will be disease specific depending upon the nature of the disease and the role TSCM are required to play.

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Footnote

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