Generation and clinical potential of functional T lymphocytes from gene-edited pluripotent stem cells

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Abstract
Engineered T cells have been shown to be highly effective in cancer immunotherapy, although T cell exhaustion presents a challenge for their long-term function. Additional T-cell sources must be exploited to broaden the application of engineered T cells for immune defense and reconstitution. Unlimited sources of pluripotent stem cells (PSCs) have provided a potential opportunity to generate precise-engineered therapeutic induced T (iT) cells. Single-cell transcriptome analysis of PSC-derived induced hematopoietic stem and progenitor cells (iHSPC)/iT identified the developmental pathways and possibilities of generating functional T cell from PSCs. To date, the PSC-to-iT platforms encounter several problems, including low efficiency of conventional T subset specification, limited functional potential, and restrictions on large-scale application, because of the absence of a thymus-like organized microenvironment. The updated PSC-to-iT platforms, such as the three-dimensional (3D) artificial thymic organoid (ATO) co-culture system and Runx1/Hoxa9-enforced iT lymphopoiesis, provide fresh perspectives for coordinating culture conditions and transcription factors, which may greatly improve the efficiency of T-cell generation greatly. In addition, the improved PSC-to-iT platform coordinating gene editing technologies will provide various functional engineered unconventional or conventional T cells. Furthermore, the clinical applications of PSC-derived immune cells are accelerating from bench to bedside.

Keywords: T-cell generation, Pluripotent stem cells, Conventional T cells, Engineered T cells, Gene editing

Introduction
Embryonic stem cells (ESCs) isolated from blastocytes can be cultured in vitro and used to generate engineered cells and animal models. Furthermore, the technology of reprogramming somatic cells to induced pluripotent stem cells (iPSCs) [1, 2] provides a possible way to explore the applications of stem cells in regenerative medicine, without ethical and immune rejection concerns [3]. A recent study showed that the application of clinical-grade iPSC-derived functional retinal pigment epithelium is feasible and safe [4]. However, the biggest challenge is the inefficient reconstitution of iPSC-derived phenotypic cells in vivo.

Edited T cells are being studied for engineering chimeric antigen receptor (CAR) T cells which are a form of major cellular therapy for hematological malignancies [5–7]. However, the application of cell immunotherapy is limited by the availability of autologous T cells and associated complications and resistance [8–10]. Meanwhile, the tumor-killing ability of patient-derived engineered T cells is suppressed by the senescent and exhausted T compartments, or by increasing the Treg subset [11, 12]. Compared with rare HSPCs, PSCs have unique advantages, such as their efficient gene editing and long-term self-renewal properties
in vitro. These advantages make them the best candidates for T-cell generation. However, the progress of PSC-to-iT technology is still facing hurdles because the thymic niche cannot be accurately simulated in vitro. With these challenges, another straightforward approach is to use the in vivo microenvironment to educate PSC-derived thymus-seeding progenitors (TSPs). In this review, we describe recent progress in understanding T cell development in the thymus, single-cell transcriptomes of PSC-iHSPC/iT, PSC-based T lymphocyte generation, and the potential applications of gene editing in the PSC-to-iT platform.

**T lymphopoiesis in the embryo and adult**

T lymphocytes play an essential role in adaptive immunity, including pathogen elimination [13], host homeostasis [14], and anti-tumor activity [15]. During hematopoiesis, fetal liver [16] and bone marrow-derived hematopoietic stem cells (HSCs) [17] differentiate into TSP, such as lymphoid-primed multipotent progenitors (LMPP) [18]. Current evidence demonstrates that non-HSC-derived TSP supports T lymphopoiesis before the emergence of HSCs [19–23] (Fig. 1a). Particularly, Flt3 signals induce CCR9 expression in TSP [24], which is then recruited into the thymus through CCL25 (TECK),
secreted by thymic epithelial cells (TEC), and recruits the TSP into the thymus [25].

Once the TSP cells seed into the cortico-medullary junction (CMJ) in the thymus [26], a new identity is acquired as early thymic progenitors (ETP) [27]. Published studies have shown that ETP have lineage pattern similar to that of LMPP with T cells, B cells, NK cells, and myeloid potential [28–30]. And ETP lack the megakaryocytic and erythroid potential. As a specialized organ for T lymphopoiesis, the thymus provides a complex, highly ordered, and unique niche. Although the microenvironment is complex, the thymus provides cascaded and conserved signaling pathways such as Notch signaling, morphogenic pathway, and protein tyrosine kinase signaling. The thymus mainly contains hematopoietic cells (T cells, B cells, NK cells, monocytes, dendritic cells [DCs], and macrophages) and non-hematopoietic stromal elements (TEC, fibroblasts, vascular smooth muscle cells, lymphatic endothelial cells, and endothelial cells) [31].

Nude mouse research has shown that TEC is the most pivotal element [32–34]. TEC and other stromal cells foster natural T lymphopoiesis by providing a microenvironment and expressing chemokines, Notch ligands (JAG1, JAG2, Delta-like ligand 1, and 4), Wnt ligands, Hh proteins, BMPs, SCF, and IL7 [35–52]. Coordinating the thymic niche-provided Notch signal initiates the pre-commitment phase [53]. Also, interfering with the function of Notch results in a complete block of T lymphopoiesis [54, 55].

During T lymphopoiesis in the thymus, ETP differentiate into immature CD4/CD8 double-negative thymocytes, (DN1, DN2, DN3, and DN4 cells), and immature CD4/CD8 double-positive thymocytes (DP cells) (Fig. 1a). After undergoing positive and negative selection, DP cells mature into naive mature CD4 single-positive (CD4SP) thymocytes and CD8 single-positive (CD8SP) thymocytes. In addition to conventional T cell subpopulations (TCRαβ+ CD4SP T subset and TCRαβ+ CD8SP T subset), TSP also differentiate into unconventional T cell subpopulations (γδ T subset, Treg subset, CD8αα+ T cell subset, natural killer T-like [NKT-like] subset, and fetal TH17-like subset) [31]. The rapid deployment of single-cell sequencing technology has helped us to discriminate rare T precursors and unconventional T subsets from the thymus atlas [31], analyze the dynamics of thymocyte development, and simulate T-cell generation in vitro. New unconventional subsets were identified through single-cell RNA sequencing (scRNAseq), such as the CD8αα(I) subset (expressing PD-1, TNFRSF9, CD72, CREB3L3, GNG4, and XCL1 at mRNA level), CD8αα(II) subset (PD-1, ZNF683, and MME), T (agonist) subset (MIR155H), Treg (diff) subset (IKZF4, GNG8, and PTGIR), and Th17-like cell subset (CD40LG, RORC, KLRB1, and ZBTB16) (Fig. 1b). The marker genes CD34, ST18, and IGLL1 were used to identify cells at the double-negative (DN) early stage.

**Single-cell transcriptome analysis of PSC-iHSPC/iT**

Single-cell transcriptional profiling has been used for the analysis of adult/embryonic hematopoietic development and immune states monitoring. In most cases, it is difficult to accurately confirm whether the PSC-derived cells are the desired cell types. Indeed, the desired PSC-derived cells were mixed with undifferentiated PSC, mesodermal progenitors, endothelial cells, lineage-specific hematopoietic progenitors, and other unexpected cell types. Fortunately, the single-cell transcriptional sequencing technology has made it possible to reliably delineate the directed differentiation process of PSC to hematopoietic lineages (Table 1). Using such technology, Guo lab reported that PSCs are heterogeneous cell populations themselves and thereby have variable efficiency of hematopoietic differentiation [56]. The PSC cell lines from different labs also showed obvious differences identified through sc-RNAseq analysis (Fig. 2a). Without mesodermal lineage differentiation-related cytokines, it is difficult for the PSCs to generate hemogenic endothelium cells (HECs) (Fig. 2a). The combination of glycan synthase kinase (GSK) 3 inhibitor (CHIR99021) with BMP4 and Activin A helps PSCs efficiently differentiate into mesoderm progenitors (cytokine-driving differentiation pattern A). VEGF and bFGF further enforce these progenitors to differentiate into EC and HECs/hematopoietic cells (cytokine-driving differentiation pattern B) (Fig. 2b). There is strong evidence indicating that the heterogeneities of embryonic and PSC-derived HECs result in diverse lineage potentials as demonstrated at the sc-RNAseq levels (Fig. 2c)[57–60]. Based on the decision of the hematopoietic fate, HECs can be divided into two groups: primitive hematopoietic development-related HECs (pHECs) and definitive HECs (dHECs); dHECs are major populations producing T-lineage cells. Despite the comprehensive work of embryonic hematopoietic development at the sc-RNAseq level, mimicking hematopoietic development using PSC-derived hematopoietic cells remains a challenge. With the help of embryonic hematopoietic development and adult hematopoiesis at sc-RNAseq levels, the hematopoietic differentiation of PSC is moving closer and closer to physiological hematopoietic development by adding missing critical transcription factors and culture niche [60, 61]. Unlimited functional PSC-derived HSC or T/NK cells are one of the ultimate goals of PSC-based regenerative medicine, and several
problems remain to be solved, such as Q1: how to efficiently get dHECs but not pHECs; Q2: how to enforce the differentiation of dHEC into *bona fide* HSCs and lymphoid-primed HPCs; and Q3: how to provide a suitable niche for T-lineage to mature and harvest both functional CD4\(^+\) T and CD8\(^+\) T cells robustly. Taken together, the single-cell transcriptional profiling of PSC-derived cells clearly shows the possibility of generating functional T cells in vitro, although some problems still remain.

**Generation of T lymphocytes from PSCs in vitro**

Reprogramming of somatic cells to iPSCs [1] provides the possibility of solving the source problem arising from limited T-cell or HSC sources [69]. Early studies have illustrated the ability of ESCs differentiating to T lineage in *vitro* and in vivo [70–75]. Based on the understanding of T-lineage commitment in the thymus, researchers have established OP9-DLL1 as stromal cells to harvest T-lineage commitment cells from PSCs [76]. The OP9-DLL1/4-PSC co-culture system is widely applied to T cell development research in vitro as a stable and efficient culture method [77–81]. Interestingly, OP9-DLL1/4- PSC coculture exhibited unconventional T-subset bias in vitro, such as γδ T cells and NKT cells, compared with T lymphopoiesis in the thymus in *vivo* [77, 78].

The function of PSC-derived T lymphocytes was only clearly shows the possibility of generating functional T lymphocytes [69]. As a classic example of cellular immunotherapy [84], anti-CD19 CAR (CD19-CAR)-modified T-cell therapy provides new ideas for antigen-specific T-cell generation. One study showed the potential of anti-tumor therapeutic CAR-engineered PSCs [82]. Intriguingly, CD19-CAR engineered T cells from iPSCs were innate “γδ-like” CAR-T cells instead of conventional T subsets. Single-cell sequencing technology provides an opportunity to understand rare and unconventional cell subsets. Multiple-development-stage, large-scale, and high-throughput sc-RNAseq analysis of the human thymus revealed a rational framework for the generation of functional T lymphocytes [31]. The iPSC-derived “γδ-like” CD19-CAR-T cells conform the phenotype of TCRα\(^+\)TCRγδ\(^-\)CD8α\(^+\)CD8β\(^-\)flowIL2RB\(^-\)CCR7\(^-\)CD62L\(^-\) (SELL\(^-\)) (Fig. 3a). CD8αβ heterodimers, not CD8αα, provide co-receptor function for CD8-dependent TCR, as an effective co-receptor for TCR signaling [85] and binding to MHC-I molecules efficiently [86].

Following a previous OP9-DLL1/PSC monolayer co-culture protocol [87], Takuya Maeda and his colleagues harvested PSC-derived LMP2-specific CD8αα\(^+\) T cells, with low cytotoxic activity compared with primary CTLs [88] (Fig. 3b). Interestingly, purified iPSC-derived DP cells, but not DN cells, could differentiate into CD8αβ T cells after stimulation with CD3 Ab or agonist peptide (Fig. 3b). To avoid the loss of antigen-specificity caused by TCRα rearrangement, Shin Kaneko’s lab depleted
RAG2 by CRISPR-Cas9 in antigen-specific T-derived iPSCs (T-iPSCs) [89]. Alternatively, myeloid cell-derived iPSCs carrying TCR expression cassettes have overcome the hurdle of mispaired TCRαβ. Song et al. also established a solid protocol for PSC-to-iT based on the OP9-DLL1/PSC monolayer co-culture system, which helped to
harvest functional hepatitis B virus (HBV) Ag-specific T lymphocytes and target HBV Ag+ cells in a mouse model [90]. In summary, without a well-organized thymus-like microenvironment, the designed program of T lineage from PSCs is disrupted by unpredictable factors, such as PSC-derived unfavorable cells, abnormal TCR signal, or endogenous RAG gene expression.

A recent study compared the OP9-DLL1/PSC monolayer co-culture with 3D thymic co-culture and identified aberrant physiological developmental signals of T development in the OP9-DLL1/PSC monolayer co-culture system. The ectopic-expressing Notch ligands on stromal cells enhanced the T-lineage commitment, the following immature T cells mature into CD8αα+ T cells or CD8αβ+ T cells under different culture conditions. T-cell generation models in 3D co-culture system. 2-deoxyguanosine-treated thymus lobes, and MS5-DLL1/4-constructed ATO, can be used for 3D coculture system, which may provide a thymus-like microenvironment. MC, monolayer culture. d A scalable iPSC-to-iT platform under Ff condition.
CD8αα/CD8αβ/DN cells in the OP-9-DLL1/PSC co-culture system, and anti-TCR antibody stimulation leading to NKT-like cells separately. To generate an in vitro physiological thymic microenvironment, a fetal thymic organ culture (FTOC) system was used to facilitate the maturation of iPSC-derived immature T cells to CD8αβ T cells. As designed, 2-deoxyguanosine-treated fetal thymic lobes enforced the generation of functional CD62L+CD69- MHC-I+ CD8αβ T cells.

Although the 3D thymic co-culture system has unique advantages, the source of primary organs, production expansion, and quality control are irreconcilable challenges. The strategy of the 3D ATO co-culture system ensures positive selection and harvests conventional T cells from HSPCs in vitro, which provides a new method for conventional T-subset generation [92]. Crooks extended the ATO strategy to the PSC-to-iT field [93] (Fig. 3c). Purified PSC-derived CD326-CD56+ embryonic mesodermal progenitors (EMPs) were aggregated into 3D embryonic mesodermal organoids (EMO) with MS5-DLL1/4 in low-serum conditions. After two weeks, the T-lineage commitment medium was used for ATO culture where derivation of PSCs produced a dominant CD8αβ T subset with transit CD8αα T subset and a few CD4SP T cells. The pattern of CDR3 lengths and DNTT expression indicated that PSC-ATO could provide a fetal thymus-like microenvironment. By applying the same strategy, Shin Kaneko’s lab could also harvest CD4+ T helper (Th) cells with Th1 or Th2 function mediated by knocking out IL4 or TBX21, respectively [94]. Altogether, the FTOC system and ATO systems provide CD8αβ+ TCRαβ+ T-cell and CD4+ Th cell generation platforms, which are closer to the thymic microenvironment. However, these approaches must be optimized to save time, reduce complex steps, and become operation friendly.

Shin Kaneko’s lab developed an efficient and scalable feeder-free (Ff) differentiation system that can regenerate cytotoxic T-cells from iPSCs [95]. This Ff system drives a well-defined T lineage commitment in vitro: iPSCs → CD235a+CD14-CD34+CD43+ iHPCs → CD7+CD5+ T-cell progenitors → CD4+CD8αβ+ DP cells → CD8αβ iT cells. The combination of several factors (CXCL12, SB203580, retinoblastoma, IL-7, IL-15, IL-12, IL-18, IL-21, TL1A, and so on) in synergy helps to establish a novel strategy of large-scale production of CD8αβ+ T cells from iPSCs (Fig. 3d). Notably, CXCL12 and SB203580 can expand iT by approximately 3000-fold during T-cell differentiation. This culture system could avoid safety issues, such as replacing OP9-DLL4 stromal cells with DLL4 protein, FBS with BIT (BSA supplemented with insulin and transferrin) or serum-free medium. This is a credible and comprehensive culture system of PSC-to-iT; however, reducing the tedious technical process will be a serious challenge.

Reconstitution of T lymphopoiesis from PSCs in vivo

Reconstitution of T lymphopoiesis from PSC-derived TSP

Obtaining engraftable functional PSC-derived mature lineage cells is the most important challenge in the field of regenerative medicine, owing to the challenges of the recipient’s immunological rejection, dysfunctional cell survival/proliferation/differentiation signal, or inability of the cells to migrate to a suitable microenvironment [76, 80]. PSC-derived cells cannot effectively exert their physiological functions in vivo. However, under specific circumstances, PSC-derived T progenitors can produce CD4SP T cells and CD8SP T cells in subcutaneously implanted FTOCs, which indicates that these T progenitors lack thymus-seeding ability [76]. The latest platform of physiological conventional T-subset generation in vivo provides a novel idea for the practical application of PSC-to-T technology [60, 96].

Transcription factors (TFs) are the core organizers of cell fate [97, 98]. Among them, Runx1 is the master regulator of embryonic hematopoietic development [99, 100]. This factor helps the generation of T cells from PSCs. Transient expression of Runx1 during hematopoietic commitment, enforced the emergence of pre-HSC-like (CD31+CD41lowCD45− c-Kit+CD201high) inducible hemogenic endothelial cells (iHECs) and HPC-like cells, but not T cells in vitro or in vivo in the further differentiation, indicating that Runx1 alone is not sufficient to initiate the PSC-to-iT program. Further scRNA-seq analysis showed that inducible Runx1-mESC-derived iHEC has divergent gene expression patterns when compared with those from mouse E11 Type I pre-HSC (T1-pre-HSC), especially missing the expression of some important hematopoietic TFs, such as Hoxa family members, Hif1, Ikaros, Setbp1, and Nkx2-3. Using the strategy of “Runx1 +1”, the combination of Runx1 and Hoxa9 can enforce strong T lineage commitment markedly, but not other combinations (Fig. 4). The Hoxa family is essential for the proliferation of HSPC and lymphoid commitment, especially Hoxa9 [101]. The inducible Runx1-p2a-Hoxa9 mESC (iR9-ESC)-derived iHEC showed molecular features between E11 EC and T1-pre-HSC, and then differentiated to TSP-like (Lin− c-kit−CD127+/CD135+) progenitors. Also iR9-ESC-derived iHECs gave rise to T cells at the single-cell level efficiently, regardless of in vitro or in vivo conditions. After iR9-ESC-derived pre-thymic progenitors were transplanted into irradiated B-NDG mice, these progenitors generated inducible T (iT) cells, which showed features of abundant TCR diversity, multi-organ distribution, and conventional T
development pattern. More importantly, different stages (DN1, DN2, DN3, DN4, DP, and conventional SP) of T lymphopoiesis were detected in the thymus. These PSC-derived iT cells have a physiological adaptive immune response, which has been identified by allogeneic skin transplantation. TCR-edited iPSC-derived iT cells efficiently eradicated E.G7-OVA tumor cells. Furthermore, these iPSC-derived functional iT cells can be further engineered with CD19-CAR T cells, which can robustly eliminate lymphoma cells both in vitro and in vivo [102]. Combining this strategy with those of Notarangelo’s lab or Mikkers’ lab reconstituted T lymphopoiesis in vivo and rescue severe combined immune deficiency (SCID) patients early in life [103, 104]. Altogether, regenerated bona fide TSP-like cells through transient expression of Runx1 and Hoxa9 are effective, allow normal conventional T development in the thymus, and avoid the generation of abnormal cells because of in vitro unfavorable factors.

**Reconstitution of T lymphopoiesis from PSC-derived induced HSC**

HSCs become the major source of thymopoiesis once these rare cells emerge. Reconstituting T lymphopoiesis by HSCs is an additional feasible way, but there is no robust culture method for expanding HSCs ex vivo. Generation of HSCs from pluripotent stem cells (PSCs) is a useful idea for cell therapy. Exogenous expression of hemogenic transcription factors to guide conversion of PSC-derived mesodermal cells to HSCs is a mainstream method reported by different research groups [105] (Fig. 5). Daley laboratory provides several options,
such as Hoxb4 [106], Cdx4 [107], the combination of HOXA9/ERG/RORA/SOX4/MYB with shEZH1 targeting [108], and the combination of ERG/HOXA5/HOXA9/HOXA10/LCOR/RUNX1/SPI1 [61]. LIM-homeobox gene Lhx2 can drive the in vitro generation of HSC-like cells from mPSCs, but the inappropriate persistence of Lhx2 expression suppresses the developmental program at the DN stage in the thymus [109]. Terminating the Lhx2 expression can pave the way to mature T cells from the DN stage in vivo [110], which helps to reconstitute T lymphopoiesis from PSC-derived TSP-like cells. Tan et al. found that inducing MLL-AF4 expression promotes the generation of PSC-derived engraftable induced HSPCs (iHSPCs) with T lineage potential [111]. PSC-derived teratoma as a disorganized and spontaneous differentiation system may occasionally produce rare engraftable HSPCs [112–114] (Fig. 5), but this must be optimized to control the risk of tumorigenesis. For example, large animal models, such as gene-edited immunodeficient pigs [115], can be used as containers to avoid the risk of teratoma formation or leukaemogenesis in patients, and produce sufficient engraftable iHSPCs from PSCs. Naturally, current strategies for PSC-derived iHSPC generation need to be modified by reducing the number of tumorigenesis-related TFs (such as MYB and MLL-AF4), or avoiding the formation of PSC-derived abnormal cells.

**T-cell generation meets gene editing**

Several forms of adoptive T-cell transfer (ACT), such as tumor-infiltrating lymphocytes (TILs), TCR-engineered T cells (TCR-T), CAR-T, and T cell antigen coupler-engineered T cells (TAC-T) [116], have been developed for antitumor therapy [6, 117], antivirus therapy [118], and targeting cardiac fibrosis [119]. CAR-T cells have unique advantages, such as MHC-independent recognition, which can kill tumor cells without MHC-associated antigens. Compared to CAR-T, MHC-dependent TCR-T cells have the advantages of intracellular targeting, lifelong persistence, robust ability to enter the solid tumor microenvironment, and reduced cytokine release syndrome. CAR-T and TCR-T cells can both effectively eliminate tumor cells and prolong patient survival. However, the efficiency of gene editing in patient-derived primary T cells remains an obstacle, which limits the purity of antigen-specific T cells and restrict the scope of gene editing at the genomic level.

The advent of the PSC-to-T technique provides a scalable system that can produce large doses of gene-edited...
T cells in one batch, reduces substantial economic burden, increases product consistency, and easily achieves predetermined genetic engineering. Theoretically, the existing gene-editing approaches in primary T cells can be applied to PSCs more efficiently (Fig. 6). To avoid graft-versus-host-disease (GVHD), Hiroki Torikai and his colleagues made universal CD19-CAR T cells by curbing the expression of endogenous αβ TCR [120]. Eliminating B2M in transplanted cells prevents the stimulation of allogeneic T cells, and expressing HLA-E can help avoid allogeneic rejection by preventing host NK-mediated lysis [121]. Therefore, we can introduce multiplex gene editing in the TCR/B2M locus and HLA-E expression in PSCs for universal engineered iT generation. Moreover, inactivation of HLA-A and HLA-B, but not HLA-C, is another ideal strategy which could cover a large population [122]. T-iPSCs with RAG2 knockout and non-T-iPSCs with transduced TCR can also help avoid unpredictable TCR generation [89]. It is notable that CAR, as an artificial fusion molecule, may disturb the normal pattern of T lymphopoiesis [123] and this can be overcome by constructing conditional expression cassettes at the stem cell level. Additionally, eliminating the expression of GM-CSF in CAR-T cells mitigates neurotoxicity and cytokine release syndrome (CRS) [124–126]. Notably, multi-target CAR-T cells are entering clinical trials [127, 128], which might help us to cope with more complex disease processes. Defects in CTLA-4, PD-1, or HPK1 in T cells enhance T cell function [129–131]. This notion prompts the disruption of these genes to generate function-enhanced T cells from PSCs. The safety concern for engineered T cells or regenerated cells relates to the off-tumor side effects and potential tumorigenicity. These risks can be solved by employing inducible suicide gene systems, such as HSVTK/GCV and iCasp9/AP1903 [132, 133]. The synNotch AND-gate circuit is another unique strategy for reducing the adverse effects on bystander tissues [134]. According to treatment purposes, we can perform precise gene editing mentioned above in PSCs to obtain multiplex engineered PSC-iT cells. However, the current PSC-to-T platforms are inefficient, limiting the development of immune cell-based regenerative therapies. When an efficient and stable PSC-to-T platform is established, diversified immunotherapy strategies through precise gene editing technologies will quickly translated to the clinic.
| Organization                        | Product       | Cell type | Tumor type                             | Characteristic                                                                 | Phase/ClinicalTrials.gov Identifier |
|------------------------------------|---------------|-----------|----------------------------------------|-------------------------------------------------------------------------------|--------------------------------------|
| National Cancer Institute (NCI)    | iPSC T        | T         | Gastrointestinal Cancers              | 1) Generation of an iPSC-derived thymic organoid 2) Cancer antigen-specific T-cells | Preclinical studies/ NCT03407040      |
|                                    |               |           | Breast Cancer                         |                                 |                                      |
|                                    |               |           | Pancreatic Cancer                      |                                 |                                      |
|                                    |               |           | Melanoma                               |                                 |                                      |
|                                    |               |           | Lung Cancer                            |                                 |                                      |
| Fate Therapeutics                  | FT819         | T         | B-cell Malignancies                    | 1) CAR19 1XX placed under the control of endogenous TCR activity; 2) TCR KO   | Phase 1 /NCT04629729                 |
|                                    | FTS00         | NK        | Advanced solid tumors                 | –                               | Phase 1 /NCT03841110                 |
|                                    | FTS96         | NK        | r/r B Lymphoma                         | 1) CD19 CAR 2) High-affinity 158 V, non-cleavable CD16 (hnCD16) Fc receptor 3) IL-15 receptor fusion (IL-15RF) | Phase 1 /NCT04245722                 |
|                                    | FTS16         | NK        | r/rAML r/rB-cell lymphoma Advanced solid tumors | hnCD16 Fc receptor             | Phase 1 /NCT04551885/ NCT04023071    |
|                                    | FTS36         | NK        | –                                      | 1) MICA/B CAR 2) IL15RF 3) CD38 KO                                        | Preclinical studies/-               |
|                                    | FTS38         | NK        | Advanced hematologic malignancies      | 1) hnCD16 Fc receptor 2) IL15RF 3) CD38 KO                                  | Phase 1 /NCT04614636                |
|                                    | FTS73         | NK        | Solid/hematologic malignancies         | 1) B7H3 CAR 2) hnCD16 Fc receptor 3) IL15RF 4) CD38 KO                      | Preclinical studies/-               |
|                                    | FTS76         | NK        | MM                                     | 1) BCMA CAR 2) hnCD16 Fc receptor 3) IL15RF 4) CD38 KO                      | Preclinical studies/-               |
| Allogene Therapeutics + Notch Therapeutics | iPSC-AlloCAR | T/NK      | NHL Leukemia MM                        | Generating from synthetic Engineered Thymic Niche (ETN) platform              | Preclinical studies/-               |
| Century Therapeutics               | –NTY-101      | NK        | r/r B-cell lymphoma                    | 1) CD19 CAR 2) expressing soluble IL-1S 3) EGFR safety switch                | Preclinical studies/-               |
|                                    | CNTY-103      | NK        | Recurrent glioblastoma                 | CD133 + EGFR CAR CD19 + CD79b CAR                                           | Preclinical studies/-               |
|                                    | CNTY-102      | T/NK      | r/r B-cell lymphoma Other B-cell malignancies |                               |                                      |
|                                    | CNTY-104      | T/NK      | AML                                    | Multi-specific                                                               | Preclinical studies/-               |
| CiRA + Takeda                      | iCART         | T         | –                                      | –                                                                             | Preclinical studies/-               |
| Cartherics                         | –             | NK        | ovarian cancer                         | 1) TAG72 CAR 2) Delete immune suppression gene 3) Multiple anti-cancer functionality | Preclinical studies/-               |
| Shoreline Biosciences              | –             | NK        | –                                      | –                                                                             | Preclinical studies/-               |
|                                    | –             | Mac       | –                                      | –                                                                             | Preclinical studies/-               |
| CellOrigin                         | –             | NK        | Hematological malignancies             | –                                                                             | Preclinical studies/-               |
|                                    | –             |          | –                                      | –                                                                             | Preclinical studies/-               |
|                                    | –             | NK        | Solid Tumor                            | –                                                                             | Preclinical studies/-               |
|                                    | –             |          | –                                      | –                                                                             | Preclinical studies/-               |
|                                    | –             |          | –                                      | –                                                                             | Preclinical studies/-               |
|                                    | –             |          | –                                      | –                                                                             | Preclinical studies/-               |
|                                    | –             |          | –                                      | –                                                                             | Preclinical studies/-               |
Clinical applications of PSC-derived immune cells

The PSC-derived immune cells are being quickly translated from bench to bedside. Many factors, such as safety issues and cell purity, that hinder the clinical applications of PSC-derived immune cells, are being addressed. Furthermore, various technologies have been developed to shorten the time to generate the patient-derived iPSC lines from somatic cells with adequate efficiency and safety (Fig. 7A)[135]. Unlimited sources and efficient gene-editing of iPSC show the high prospects for their clinical application and commercialization. Recently, a group reported a stromal cell/serum-free DL4-μbeads-based approach that supports the development of PSC-derived CD34⁺ cells to T lineage progenitors, which can eliminate the concerns over the safety of animal-derived substances (Fig. 7b). However, this study did not show the function or developmental progress of PSC-derived T lineage in vivo [68]. Many researchers and organizations are promoting the commercialization of iPSC-derived immune cells (Table 2). Interestingly, almost all iPSC-derived immune cell therapy products are NK cells (NK: 17/27, NK and/or T: 4/27; T: 4/27, Mac: 2/27). The reason for this may be that NK cell-mediated cytotoxicity does not require HLA-matching [136]. Several trials have demonstrated the safety of adoptive transfer of allogeneic NK cells [137]. These universal and “off-the-shelf” iPSC-derived NK cells can be produced easily. Furthermore, knocking out the HLA gene in iPSCs can help harvest universal iPSCs, which can subsequently be used for generation of universal iPSC-derived CAR-T cells.

Conclusions and future perspectives

The study of HSPC transplantation [138–142], as well as disorders of hematopoiesis, lymphatics, and immunity [143] has facilitated the understanding of the HSC differentiation cascade. T lineage commitment not only involves a precise transcription factor regulatory network, but also an organized thymus microenvironment [23]. Indeed, extensive research has demonstrated the feasibility of PSC-to-T [69]. To identify the T lymphopoiesis in the thymus, several single-cell transcriptional atlas of T lymphopoiesis and embryonic/adult thymus organogenesis have been established [23, 31, 144], which help us to identify the features of TSP, the interaction of thymocytes and stromal cells, and rare unconventional T subsets. Furthermore, several published scRNA-seq datasets of PSC-derived cells clearly showed the differentiation pathways and possibilities of generating physiological T-lineage cells (Table 2). More importantly, by deconstructing T lymphopoiesis in the thymus and eliminating unnecessary factors, an organized thymus-like microenvironment was reproduced in vitro for functional PSC-derived T-cell generation. The ATO co-culture system indicated the feasibility of conventional T-subset generation by constructing thymus-like niche in vitro. Defined TFs (Runx1 and Hoxa9) were used to generate transplantable PSC-derived TSP. Furthermore, the improved PSC-to-T platforms through gene editing technology will likely facilitate the clinical application of PSC-T, NK and macrophage cells for cancer immunotherapy [145–152].

Abbreviations

PSCs: Pluripotent stem cells; iT: Induced T; 3D: three-dimensional; ATO: Artificial thymic organoid; ESCs: Embryonic stem cells; iPSCs: Induced pluripotent stem cells; CARs: Chimeric antigen receptors; HSPCs: Hematopoietic stem/progenitor cells; TSP: Thymus-seeding progenitors; HSCs: Hematopoietic stem cells; LMPP: Lymphoid-primed multipotent progenitors; CMJ: Corticomedullary junction; ETP: Early thymic progenitors; thymic TEC: Epithelial cells; TFs: Transcription factors; FTOCs: Fetal thymic organ cultures; ATO: Artificial thymic organoid; EMP: Embryonic mesodermal progenitors; EMO: Embryonic mesodermal organoids; iHECs: Inducible hemogenic endothelial cells; scRNA-seq: Single cell RNA-seq; GvHD: Graft-versus-host-disease; ACT: T-cell transfer; TILs: Tumor-infiltrating lymphocytes; TCR-T: TCR-engineered T cells; TAC-T: T cell antigen coupler-engineered T cells.

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Table 2 (continued)

| Organization         | Product     | Cell type | Tumor type | Characteristic                           | Phase/ClinicalTrials.gov Identifier |
|----------------------|-------------|-----------|------------|-----------------------------------------|-------------------------------------|
| HebeCell             | –           | NK        | –          | 1) Generating from 3D bioreactors       | Preclinical studies/-               |
|                      |             |           |            | 2) CAR construction                      |                                     |
| Neukio Biotherapeutics | –          | NK        | –          | CAR construction                         | Preclinical studies/-               |
| nuwacell             | –           | T/NK      | –          | CAR construction                         | Preclinical studies/-               |
| Biotheus + iCAMUN    | iPS-CAB-NK  | NK        | Solid tumor| –                                       | Preclinical studies/-               |
| PersonGen            | iPS-CAR-NK  | NK        | –          | CAR construction                         | Preclinical studies/-               |

NK: natural killer, MM: multiple myeloma, AML: acute myeloid leukemia
Author contributions
GR, SY, and JZ designed the study. GR drafted the manuscript. All authors were involved in manuscript preparation and revisions. All authors read and approved the final manuscript.

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Availability of data and materials
This is not applicable for this review.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
No individual data were used in this study.

Competing interests
The authors declare that they have no competing interests.

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