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Induced Pluripotent Stem Cell-derived T cells for Cancer Immunotherapy

Sunny Patel, BS[1,2],
Medical College of Georgia, 1120 Fifteen Street, Augusta, GA 30912-3600

Takayoshi Yamauchi, PhD[1], and
[1]Department of Molecular Enzymology, Faculty of Life Sciences, Kumamoto University, Kumamoto, 860-8556, Japan.

[2]Center for Metabolic Regulation of Healthy Aging, Faculty of Life Sciences, Kumamoto University, Kumamoto, 860-8556, Japan.

Fumito Ito, MD, PhD[1,3,4]
Center for Immunotherapy, Department of Surgical Oncology, Roswell Park Comprehensive Cancer Center, Elm and Carlton Streets, CCC-539, Buffalo, NY 14263

[1]Center for Immunotherapy, Roswell Park Comprehensive Cancer Center, Buffalo, NY, United States

[2]Medical College of Georgia at Augusta University, Augusta, GA United States

[3]Department of Surgical Oncology, Roswell Park Comprehensive Cancer Center, Buffalo, NY, United States

[4]Department of Surgery, Jacobs School of Medicine and Biomedical Sciences, State University of New York at Buffalo, Buffalo, NY, United States

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Background

T cells for cancer immunotherapy

$CD8^+$ T cells play a critical role in adaptive immunity by virtue of their ability to initiate killing following receptor-mediated engagement by antigens expressed on the surface of

Corresponding author: Fumito Ito fumito.ito@roswellpark.org.

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tumor cells.[1] $CD8^+$ T cell-mediated cytotoxicity requires direct contact with target cells, thereby limiting damage to bystander cells. The attractiveness of target-specific approaches lies in avoidance of the serious side effects of other conventional treatments such as chemotherapy and radiation that have relatively non-specific mechanisms of action. A unique feature of the immune response, unlike conventional cancer therapies, is that it can elicit long-term protection from recurring disease (immunological memory).[2] Another significant advantage of T cell-based immunotherapies is that T cells can search out and traffic to widely disseminated heterogeneous tumor cell targets by using chemokine-chemokine receptor interaction and generalized Lévy walks.[3,4]

Current status of adoptive T cell therapy

Significant advances have been made in the development of adoptive cell therapy (ACT) aiming to boost the immune response directed against chronic viral infections and various cancers.[5–14] ACT using autologous tumor-infiltrating lymphocytes (TILs) has been used clinically for several decades, and was found to mediate objective tumor regression in 50–70% of patients with IL-2 refractory metastatic melanoma in combination with lymphodepleting chemotherapy and systemic high-dose IL-2 administration.[14,15] Additionally, ACT using genetic modification of peripheral blood lymphocytes with a T cell receptor (TCR) or a chimeric antigen receptor (CAR) specific for tumor-specific antigen can mediate regression in multiple cancer histologies.[5–12] Both technologies can augment T cell function by altering receptor specificity and signaling functions that control proliferative capacity and other cellular functions.[5–12] In the former approach, T cells with enhanced affinity or novel specificity are created by expression of TCR α/β heterodimers in peripheral blood T cells.[10–13] The endogenous repertoire for TCRs is generally of low affinity when targeting shared tumor-associated antigen because of the impact of central tolerance; however, TCRs targeting neoantigens, where mutations in the cancer genome create neo-epitopes, have high affinity.[16,17] Furthermore, the affinity and functional avidity of tumor-antigen specific TCRs can be enhanced by high-throughput genetic approaches.[18–20] NY-ESO-1 is expressed in a variety of cancers, but not in normal adult tissues, except for germ cells of the testis, making it an ideal target for immunotherapy.[21,22] NY-ESO-1-specific TCR-engineered T cells have generated clinical responses in patients with advanced multiple myeloma, melanoma, and synovial cell sarcoma.[11–13] TCR-based targeting approaches are; however, often susceptible to the common tumor escape mechanisms of major histocompatibility complex (MHC) down-modulation and altered peptide processing.[23]

The concept of CAR technology dates to the 1980's when Eshhar and colleagues engineered and expressed chimeric T-cell receptor genes comprising antigen-binding domains fused to T-cell signaling domains.[24] Because the target-binding moiety is derived from antibodies with higher affinity than TCRα/β, CARs enable highly specific targeting of antigen in an MHC-independent fashion.[5–9] Adoptive transfer of CD19-directed CAR-T cells has generated complete and durable remissions in patients with refractory and relapsed B cell malignancies such as acute lymphoblastic leukemia, chronic lymphocytic leukemia, and non-Hodgkin' lymphoma.[6–9] The growing optimism in the field as we develop a better understanding of these technologies continues to unveil the potential present in adoptive T cell therapy.

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Limitations to adoptive T cell therapy for solid malignancies

While adoptive T cell therapy demonstrated impressive clinical response with long-term remission for hematological malignancies, this success has not yet been concluded in solid malignancies.[25] Despite encouraging results in preclinical models and in patients, poor survival of infused T cells and the existence of immune suppressive pathways appear to restrict the full potential of ACT for solid tumors. Current clinical ACT protocols require extensive *ex vivo* manipulation of autologous T cells in order to obtain large numbers, resulting in the generation of fully differentiated effector T cells. While these differentiated T cells are equipped with full effector function, they are severely impaired in their proliferative capacity (Figure 1).[26–28] Trafficking of infused T cells to the tumor is a critical step for successful immunotherapy that correlates with clinical responses in patients.[29] However, the tumor microenvironment (TME) characterized by abnormal tumor vessels and interstitium limits lymphocyte adhesion, extravasation, and infiltration.[30] As a result, only a fraction of *ex vivo* expanded T cells can infiltrate into the tumor tissue.[29]

Cancer cells reprogram their metabolism to meet the rapid energy requirements for proliferation, survival and metastasis.[31] Glycolytic metabolism of glucose results in lactic acid, which can acidify the TME.[32] Acidosis and hypoxia are considered as biochemical hallmarks of the TME[33,34] that not only modulate cancer cell metabolism but also influence T-cell proliferation and effector function.[35] Hypoxia induces FoxP3, a key transcriptional regulator for regulatory T cells (Tregs),[36] and polarizes $CD4^+$ T cells towards a Th2 phenotype,[37] allowing the resultant IL-4 and IL-13 to induce macrophage M2 polarization.[38]

Tumor associated macrophages (TAMs) are the major immunoregulatory cells in tumors, considered to have an M2 phenotype and secrete an array of cytokines, chemokines and enzymes that can suppress T-cell effector function.[39] TAMs secrete chemokines, CCL5, CCL20, CCL22 that recruit natural Treg cells (nTreg) and Arginase I that inhibit TCR ζ chain re-expression in activated T cells by the depletion of L-arginine.[40] IL-10 and TGFβ produced by TAMs can induce regulatory functions by the upregulation of the Foxp3 and cytotoxic T lymphocyte antigen 4 (CTLA-4) in $CD4^+$ T cells, and the expression of the programmed death-ligand 1 (PDL1) in monocytes – a co-inhibitory molecule that can inhibit $CD8^+$ T cell functions.[40–44] Through HIF-1 α signaling, myeloid-derived suppressor cells (MDSCs) and TAMs in the hypoxic TME upregulate PD-L1 on macrophages.[45] Continuous exposure to chronically expressed tumor antigens drives T cells into senescence and exhaustion, characterized by expression of co-inhibitory molecules such as T cell immunoglobulin, mucin domain-containing protein 3 (TIM-3), lymphocyte activation gene 3 protein (LAG-3), programmed cell death protein 1 (PD-1) and CTLA-4 with impaired effector functions and proliferative capacity.[27,46–48]

Ideal T-cell subsets for adoptive T cell therapy

These limitations signify the necessity of identifying T-cell subsets that maintain the ability to proliferate, effectively traffic to the TME, exhibit robust effector function, and mediate regression of tumors for ACT. Accumulating evidence from preclinical and clinical studies has shown that less-differentiated “younger” T cells with longer telomere persist longer and exhibit more potent anti-tumor efficacy than differentiated T cells after adoptive transfer.

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[26,27,49–54] Using murine B16 melanoma model with Pmel-1 T-cell receptor (TCR) transgenic mice specific for the gp100 antigen expressed on B16 tumors, adoptive transfer of central memory T cells (T_{CM}: $CD62L^{hi}$ $CD44^{hi}$) exhibited superior expansion, persistence, and anti-tumor efficacy *in vivo* compared with effector memory T cells (T_{EM}: $CD62L^{lo}$ $CD44^{hi}$ KLRG-1^{lo}) or terminally differentiated effector T cells (T_{EFF}: $CD62L^{lo}$ $CD44^{hi}$ KLRG-1^{hi}).[26,27,49] Even more resounding was the result that stem cell memory T cells (T_{SCM}: $CD62L^{hi}$ $CD44^{lo}$ Stem cell antigen-1^{hi} $CD122^{hi}$) were even more potent than T_{CM} on a per-cell basis.[52–55] Preclinical and clinical studies found a significant correlation between T cell differentiation status and anti-tumor efficacy, indicating the superiority of T_{SCM} cells over other memory $CD8^+$ T-cell subsets.[52–54] Finally, in addition to evaluating memory and effector subsets individually, the ability of natural Ag-specific T_{EFF} derived from different $CD8^+$ T- cell subsets, specifically naïve T cells (T_N) and T_{CM}, has also been assessed in both mice and human.[56,57] Compared to T_{EFF} derived from T_{CM}, naive-derived T_{EFF} retained the ability to release IL-2 while withholding the acquisition of the senesce marker, KLRG-1.[56,57] When adoptively transferred into tumor-bearing mice, T_N -derived T_{EFF} demonstrated superior *in vivo* expansion, persistence, and anti-tumor efficacy relative to T_{CM} -derived T_{EFF}[57]. In humans, these cells also maintained significantly higher CD27 and longer telomere lengths after *ex vivo* expansion, suggesting greater proliferative potential.[56] These results suggest that the ability of T cells to mediate tumor regression decreases with differentiation. Overall, the increased potential to self-proliferate and differentiate into memory and effector T cell subsets allows less differentiated forms, such as T_{SCM} and T_N, to regulate and sustain effective tumor regression and foster superior anti-tumor efficacy relative to differentiated effector cells.

An array of possible approaches has been proposed to enhance the efficacy of ACT. Initial antigen signal strength,[58] quality of costimulation,[59] and the presence of cytokines, such as IL-2, IL-7 and IL-15,[49,60] may influence the relative ratio of T_{CM} to T_{EM} and T_{EFF} generated in response to antigen. Therefore, modulating immunomodulatory cytokines used in ACT along with adapting the duration and nature of T cell *ex vivo* culture conditions can enhance the *in vivo* function of tumor-specific $CD8^+$ T cells by selecting and generating optimal memory T cells in cancer patients. Another strategy involves altering metabolism within T cells, primarily inhibiting glycolytic pathways noted to be drivers of terminal effector differentiation. This may promote long-lived $CD8^+$ T cell immunity and enhanced tumor destruction.[61] *In vitro* culturing of T cells in the presence of small molecules provide cell products with superior engraftment, expansion and anti-tumor immunity after adoptive transfer. Inhibition of GSK3, a vital component of the oncogenic WNT signaling pathway, maintains stemness in mature memory $CD8^+$ T cells providing self-renewal capability and multipotency superior to central memory T cells.[54] Collectively, less-differentiated tumor antigen-specific T cells are ideal T-cell subsets for ACT; however, generating large numbers of these “younger” T cells is problematic.

Classification of stem cells based on differentiation potential

Stem cells are defined by dual hallmark features of self-renewal and differentiation potential.[62–64] These cells are classified into several types according to their capacity to differentiate into specialized cells. A totipotent cell such as zygote (a fertilized egg) and blastomeres

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during early cleavage of the embryo can give rise to a new organism given appropriate maternal support. They can also differentiate into embryonic and extra-embryonic cell types such as the fetal membranes and placenta.[65]

Pluripotent stem cells **(PSCs)** can self-renew and have the ability to form all three embryonic germ layers (i.e., ectoderm, endoderm and mesoderm). Embryonic stem cells (**ESCs**) epitomize quintessential PSCs that can be isolated from the inner cell mass of blastocysts and cultured as immortal cell lines.[66,67] Multipotent stem cells can self-renew, but differentiate into all cell types within one particular lineage.[64] These include neural stem cells that are derived from neural tissues and can give rise to all cell types (neurons, astrocytes, and oligodendrocytes) of the nervous system.[68] Mesenchymal stem cells are also multipotent stromal cells that can be isolated from the bone marrow.[69] They are nonhematopoietic, multipotent stem cells with the capacity to differentiate into mesodermal lineage such as bone cells, cartilage cells, muscle cells, and fat cells.

The rise of induced pluripotency

The understanding of induced pluripotency has developed over the last six decades with the aid of advancing discoveries and technologies. The first PSCs cultured *in vitro* were derived from a type of germ line tumor called teratocarcinoma.[70] The breakthrough in the field came when researchers showed that PSCs can be isolated from mouse blastocysts and propagated *in vitro* as immortalized, non-transformed cell lines.[66,67] Later, Thompson et al. showed PSCs can be derived from human embryos.[71] However, ethical concerns using human zygotes and immune rejection of grafted stem cells limit the use of human ESCs.

In 2006 Takahashi and Yamanaka demonstrated that the transient expression of only four transcription factors (Oct4, Sox2, Klf4, and c-Myc) was sufficient to convert murine fibroblasts into induced pluripotent stem cells (iPSCs), which are ESC-like cells that demonstrate the same pluripotency and self-renewal properties.[72] Only a year later, the successful derivation of human iPSCs from fibroblast was reported.[73,74] Human iPSCs circumvent the ethical controversies and rejection problem associated with using autologous stem cells; they provide a valuable source of patient-specific cells for the study and potential treatment of human diseases. Remarkable progress made in reprogramming technology over the past decade has also facilitated the generation of human iPSCs with a minimally invasive approach from a number of human cell types such as keratinocytes, dental stem cells, oral gingival, oral mucosa fibroblasts, and cord blood cells.[75–80]

In 2010, three groups reported the generation of human iPSCs from peripheral blood T cells.[81–83] The use of peripheral blood cells as a source for iPSCs is a less invasive procedure compared to having patients undergo skin biopsy for obtaining fibroblasts. Although all three groups used the same four transcription factors (Oct4, Sox2, Klf4, and c-Myc) to generate T-cell derived human iPSCs (T-iPSCs), reprogramming efficiency was different. Seki et al. introduced the four factors with Sendai virus vectors and found that only 1 ml of whole blood was sufficient to generate human iPSCs.[81] In addition to higher induction efficiency, Sendai virus vectors have some advantages for the generation of human iPSCs. Unlike integrating viral (e.g. retroviral or lentiviral) vectors, Sendai virus vectors only replicate in the cytoplasm of infected cells and do not integrate into the host genome.[84]

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Moreover, temperature-sensitive mutations in the viral genome allow for rapid removal of residual viral genomic RNA from reprogrammed cells.[85] Generation of transgene-free iPSCs by non-integrating Sendai virus vectors minimizes the risk of tumor formation associated with random oncogene activation or tumor supressor inactivation.

Of note, during normal αβ T cell development, *TCRA* and *TCRB* genes are rearranged in the thymus. Detection of TCR gene arrangement in iPSCs is indicative of derivation from cells of the T lineage.[81–83] Using Sendai virus vectors, we have also found efficient generation of human iPSCs from peripheral blood T cells (Figure 2). Furthermore, we have shown successful derivation of human iPSCs from melanoma TILs expressing high levels of PD-1.[86] A wide variety of TCR gene rearrangement patterns in TIL-derived iPSCs confirmed the heterogeneity of T cells infiltrating melanomas.[86] These findings also suggest the feasibility of rejuvenating fully differentiated and exhausted antigen-specific T cells by reprogramming and redifferentiation techniques for adoptive T cell therapy.

Potential of iPSCs to generate T cells for adoptive cell therapy

Subsequently, a series of studies have provided insights into the function of rejuvenated antigen-specific T-iPSC-derived T cells. The tumor specificity of T-iPSC-derived T cells can be conferred via two approaches. One is to reprogram tumor-antigen specific T cells and redifferentiate T-iPSCs for the generation of T cells carrying the TCR recognizing the same tumor antigen (Figure 3A). Vizcardo et al. established T-iPSCs from $CD8^+$ T-cell clone specific for the melanoma antigen MART-1 using the Sendai virus reprogramming system.[87] All regenerated $CD8^+$ T cells from T-iPSCs were found to express TCR specific for MART-1 antigen and produce IFN-γ *in vitro*.[87] Nishimura et al. rejuvenated HIV-1 specific $CD8^+$ T cell clone and demonstrated that regenerated iPSC-derived T cells have high proliferative capacity, antigen-specific killing activity and elongated telomere.[88] Wakao et al. generated T-iPSCs from human cord blood mucosal-associated invariant T (MAIT) cells, innate-like T-cells that recognize derivatives of precursors of bacterial riboflavin presented by the MHC class I-related molecule MR1.[89] Regenerated MAIT cells possessed the ability to produce a wide variety of cytokines and chemokines in the presence of bacteria-fed monocytes.[89]

While these studies utilize a strategy of reprogramming T cells with known antigen specificity and redifferentiating T-iPSCs for the generation of rejuvenated antigen-specific T cells, another approach is to genetically transfer a receptor with known specificity for an antigen into established iPSCs (Figure 3B). Themeli et al. have shown that T-iPSCs transduced with CAR specific for CD19 antigen can generate T cells that display anti-tumor immunity in a xenograft model of lymphoma.[90] These studies suggest that iPSCs with CAR genetic modification have the potential to generate functional and expandable T cells specialized for tumor eradication.

In vivo anti-tumor efficacy of iPSC-derived T cells against solid malignancies

Although these studies suggest *in vivo* anti-tumor efficacy of T-iPSC-derived T cells, it remains uncertain whether iPSC-derived T cells escape immune rejection (immunogenicity) and mediate effective regression of established tumor following adoptive transfer in

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immunocompetent host. Some studies have shown that certain iPSC-derived cells such as smooth muscle cells and cardiomyocytes are immunogenic while other cell types such as retinal pigment epithelial, hepatocytes, and neuronal cells exhibit little to no immunogenicity.[91–94]

To this end, we have recently established a preclinical murine model in which Pmel-1 TCR transgenic $CD8^+$ T cells able to recognize gp100 antigen were rejuvenated to iPSC-derived T cells utilizing the Sendai virus reprogramming system (Figure 4).[95] This novel preclinical model allows us to discover a variety of new findings that have unveiled insights of not only the reprogramming process of iPSC technology, but also its therapeutic potential through *in vitro* and *in vivo* analysis in an immunocompetent mouse model. We demonstrated for the first time that murine T cells, like human T cells, can be reprogrammed into iPSCs with the Sendai virus reprogramming system without the use of gene knockout mice or drug-inducible gene expression systems.[96,97] Of equal importance was our finding that dual inhibition (2i) of both prodifferentiation MEK and GSK-3 pathways that was shown to support the establishment of mouse iPSCs from partially reprogrammed cells[98] was required for reprogramming of Pmel-1 T cells. Rejuvenated iPSC-derived T cells were less-differentiated phenotypes that expressed memory T cell markers and acquired effector functions producing IFN-γ and TNF-α after stimulation with the cognate antigen, gp100.[95] Furthermore, adoptive transfer of iPSC-derived regenerated T cells significantly delayed B16 tumor growth and improved overall survival in a lethal murine model of melanoma (Figure 5A and B).[95] Importantly, an establishment of antigen-specific immunological memory provides insight into immunogenicity of iPSC-derived T cells, and reveals the feasibility of generating long-lived tumor-specific T cells via reprogramming to pluripotency and redifferentiation (Figure 5C).[95]

Challenges / Future Directions

ESCs and iPSCs are tumorigenic cells that can give rise to teratoma upon transplantation.[99] For clinical translation of iPSC-derived T-cell therapies, the tumorigenic potential of contaminated iPSCs and the malignant transformation of differentiated iPSCs (tumorigenicity) are major safety concerns.[100,101] While the tumorigenic risks of iPSC-derived products can be reduced by several methods,[102–105] they may not be satisfactory because tumorigenic risk arises not only from contamination with undifferentiated iPSCs, but also from intermediate products having altered proliferation potential and/or with tumorigenic transformed cells.[100]

Current method of *in vitro* differentiation of T lymphocytes from human iPSCs uses co-culture with murine OP9 bone marrow stromal cells expressing the Notch ligand Delta-like 1 (OP9-DL1).[106] To translate this strategy into routine clinical practice, it will be essential to find a way to differentiate iPSCs under xeno-free conditions. Furthermore, regenerated human iPSC-derived T cells express CD3, TCRαβ, and CD8α, but not CD8β. Therefore, these regenerated iPSC-derived T cells possess CD8αα homodimers, not CD8αβ heterodimers.[107] CD8αα homodimer has been found only on a small portion of developing thymocytes, gut intraepithelial lymphocytes (IEL) and a subset of NK cells and dendritic cells.[108] Although both forms of the CD8 molecule bind to MHC class I with similar

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affinity, studies have shown that the CD8αα homodimer is a functionally weaker co-receptor than CD8αβ for TCR-based activation.[109,110] Moreover, Themeli et al. have shown that CAR-iPSC-derived T cells possess an innate γδ T cell-like profile.[90] In contrast, we have found that regenerated murine iPSC-derived T cells express both CD8α and CD8β (CD8αβ heterodimer), which might be because of the use of the sorting procedure performed before activation with the cognate antigen.[95] In line with our study, Maeda et al. have recently shown that isolating $CD4^+CD8^+$ double positive (DP) T cells before activation with anti-CD3 antibody (Ab) can generate human CD8αβ iPSC-derived T cells.[107]

Nevertheless, development of feeder-free and xeno-free culture procedures for the generation of CD8αβ T cells will be ideal for clinical use of iPSC-derived T cells. Of note, Vizcardo et al. recently developed a 3D thymic culture system in preclinical model and showed successful generation of murine CD8αβ iPSC-derived T cells without the use of OP9-DL1 feeder cells.[111]

Lastly, TCRα gene rearrangement takes place when T cells are at the $CD4^+CD8^+$ DP stage in thymus.[112,113] Additional rearrangement of TCR α chain may occur when iPSC-derived T cells become $CD4^+CD8^+$ DP T cells. This may produce T cells with unpredictable antigen specificity, and adoptive transfer of these T cells may cause unpredictable autoimmune reactions because they do not go through thymic positive and negative selection. A possible solution would be to downregulate the expression of the recombination activating genes 1 and 2 (RAG-1 and RAG-2) to stop further endogenous TCRα gene rearrangement by CRISPR/Cas9 genome editing technique.[114–116]

Conclusions

Adoptive cell therapy with antigen-specific T cells is a promising approach for treating patients with a variety of malignancies. Despite remarkable success seen in the treatment of hematological malignancies, difficulty with generating sufficient numbers of tumor-specific T cells harboring characteristics necessary for *in vivo* effectiveness remains a major roadblock to ACT for solid malignancies. Use of iPSCs to provide an unlimited number of autologous less-differentiated antigen-specific T cells can theoretically overcome these limitations, and hold great promise for adoptive T cell therapy. While autologous iPSC-derived T cells provide a bright future for personalized cancer treatment, many challenges still remain before these cells can be utilized clinically in patients. Safety and therapeutic efficacy of iPSC-derived T cells need to be further evaluated in preclinical models before they are translated into clinic.

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Synopsis:

Current approaches to adoptive T cell therapy for solid malignancies are limited by the difficulty of obtaining sufficient numbers of less-differentiated tumor-specific T cells with superior *in vivo* expansion, persistence, and anti-tumor efficacy relative to differentiated effector T cells. The use of induced pluripotent stem cells (**iPSCs**) that self-renew and provide an unlimited number of autologous less-differentiated antigen-specific T cells can theoretically overcome these limitations. Accumulating evidence suggests T cell-derived iPSCs can generate less-differentiated antigen-specific T cells that harbor long telomeres and increased proliferative capacity, and exhibit potent anti-tumor efficacy *in vitro* and *in vivo*. While this strategy holds great promise for adoptive T cell therapy, development of clinically applicable protocol for the generation of human iPSC-derived T cells is required prior to the translation of iPSC technology into the clinical setting.

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Key Points

1. Despite full effector function, differentiated T cells currently available for adoptive cell therapy (ACT) exhibit less expansion, persistence, and anti-tumor efficacy *in vivo* against solid malignancies compared with less-differentiated T cells.
2. Induced pluripotent stem cells (iPSCs) can self-renew and provide unlimited number of autologous less-differentiated antigen-specific T cells that can mediate effective regression of established tumor and establish antigen-specific immunological memory *in vivo*.
3. Development of highly reproducible and robust differentiation protocols for clinically applicable large scale production of tumor-specific iPSC-derived T cells is needed.

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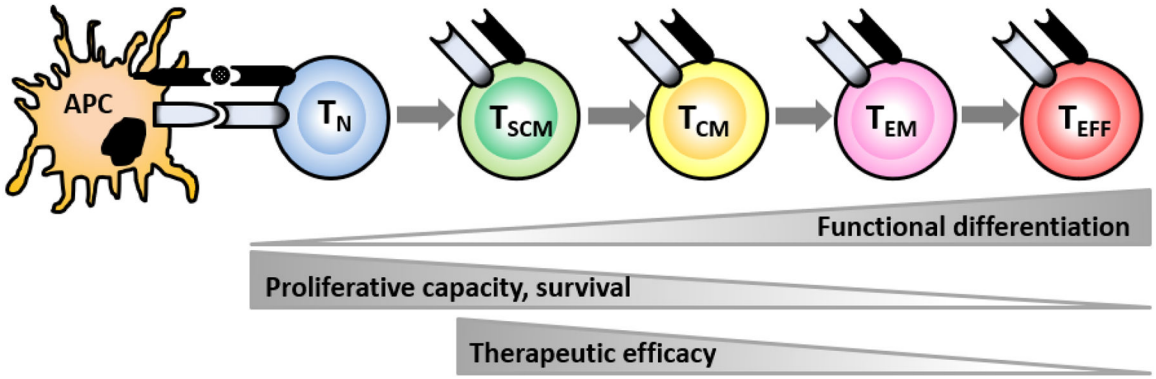

Figure 1. Differentiation status of adoptively transferred T cells inversely correlates with therapeutic efficacy.

APC: antigen-presenting cell, T_N: naïve T cells, T_{SCM}: stem cell memory T cells, T_{CM}: central memory T cells, T_{EM}: effector memory T cells, T_{EFF}: effector T cells.

Data from Sallusto F, Lanzavecchia A. Memory in disguise. Nat Med 2011; 17(10): 1182–1183.

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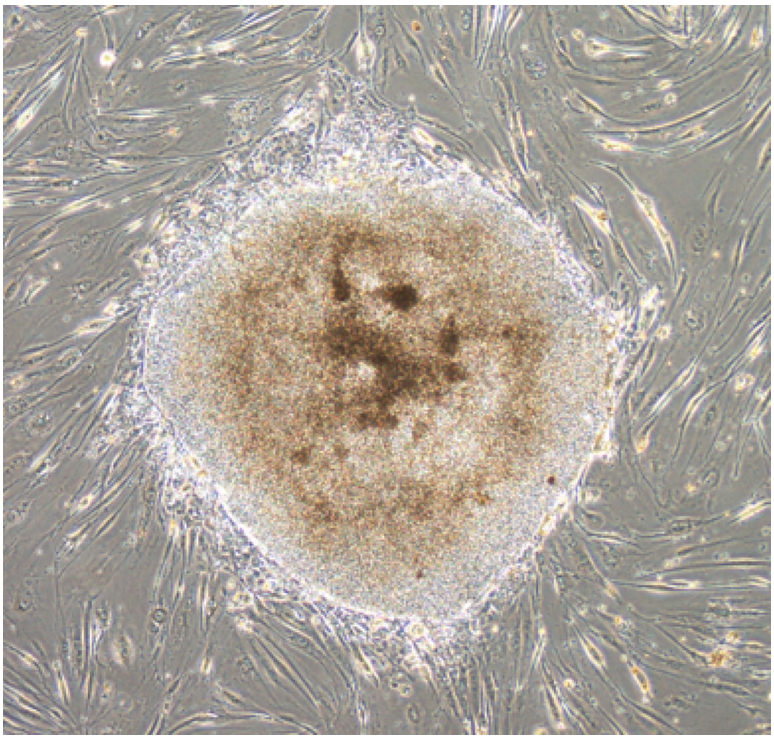

Figure 2. Human iPSC derived from peripheral blood T cells under on-feeder condition.

Peripheral blood T cells were reprogramed by viral transduction of a Sendai-virus vector carrying a cassette of the OCT3/4, SOX2, KLF4, and c-MYC. One day after reprogramming, cells were replated on to feeder cells. A human iPSC on feeder cells on day 19 is shown. Scale bar: 500 μm.

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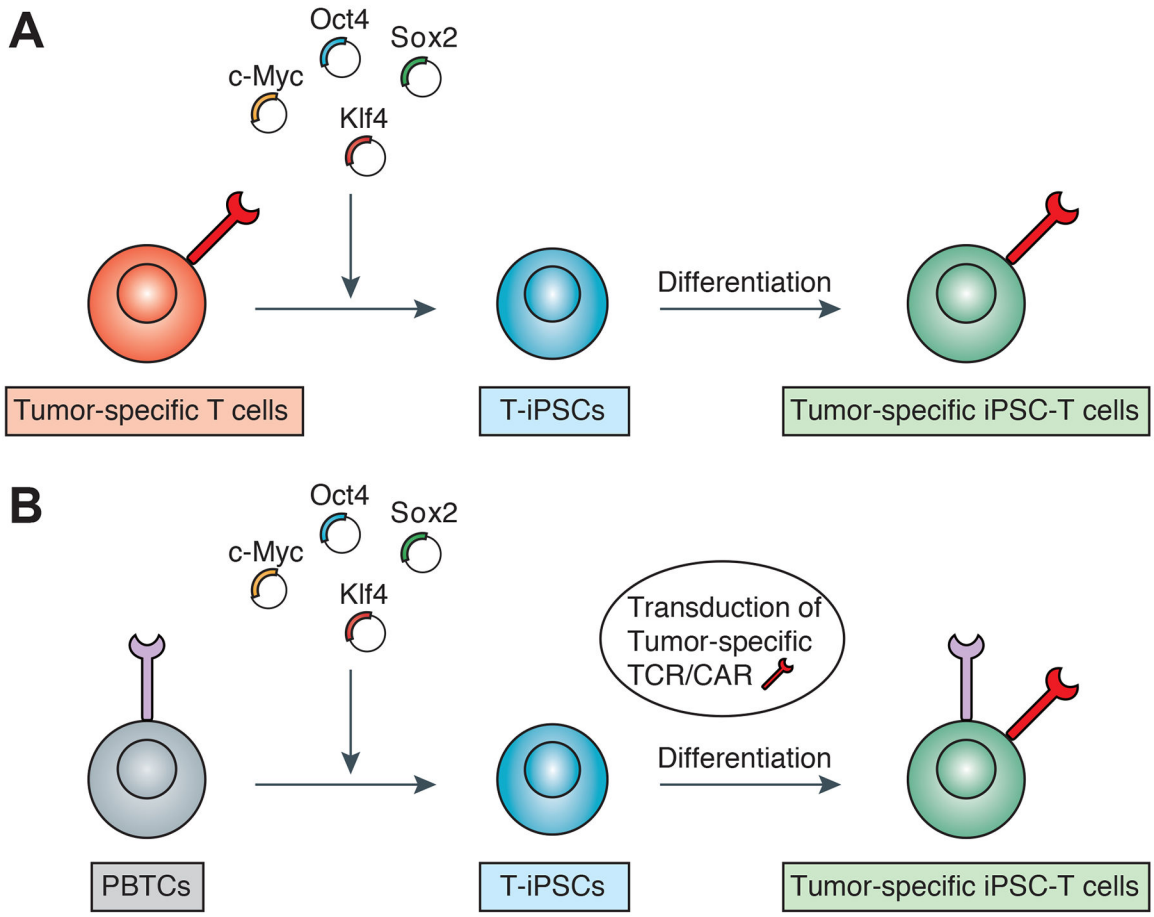

Figure 3. Two approaches of generating tumor-specific T cells using autologous induced pluripotent stem cells (iPSCs) and reprogramming technology.

(A) Reprogramming of tumor-specific T cells to generate T-cell derived human iPSCs (T-iPSCs) followed by redifferentiation to naïve tumor-specific iPSC-derived T cells. (B) Reprogramming of peripheral blood T cells followed by transduction of T-cell receptor (TCR) or chimeric antigen receptor (CAR) recognizing tumor antigen to T-iPSCs. Genetically engineered T-iPSCs differentiated to naïve tumor-specific TCR/CAR-transduced iPSC-derived T cells.

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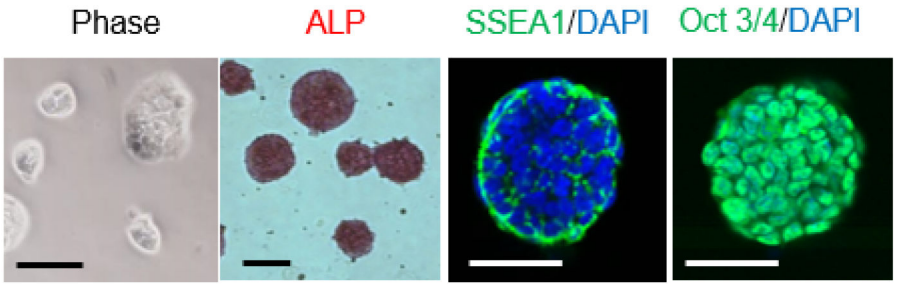

Figure 4. Generation of iPSCs from Pmel-1 TCR transgenic CD8$^+$ T cells.

Morphology, alkaline phosphatase (ALP) activity and expression of pluripotency and surface markers (SSEA1 and Oct3/4) in Pmel-1 iPSCs. Scale bar: 200 μm.

From Saito H, Okita K, Chang AE, et al. Adoptive Transfer of CD8+ T Cells Generated from Induced Pluripotent Stem Cells Triggers Regressions of Large Tumors Along with Immunological Memory. Cancer Res 2016;76(12):3473–3483; with permission.

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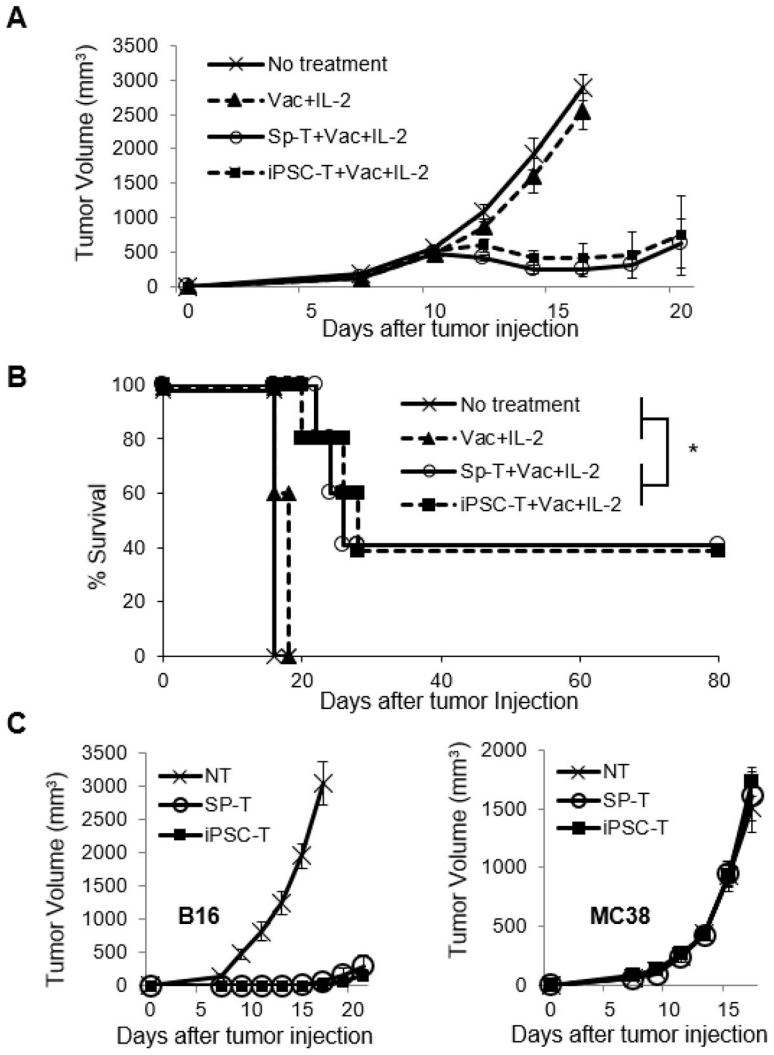

Figure 5. Adoptively transferred iPSC-derived CD8$^+$ T cells mediate effective regression of large tumors and establishes immunological memory.

(A and B) Tumor growth curves (A) and survival curves (B) in C57BL/6 mice bearing B16 melanomas established for 11 days in different treatment groups. Vac: vaccination with the gp100 antigen, anti-CD40 mAb, poly (I:C), and imiquimod cream. Tumor volume results are the mean of measurements from 5 mice per group. (*=$P < 0.0001$ using log-rank (Mantel-Cox) test.) (C) Surviving mice (n=4) after adoptive transfer of Pmel-1 iPSC-derived or splenic T cells, vaccination and IL-2 were rechallenged with B16 cells into the contralateral flank and MC38 cells on back on day 80. Tumor growth curves are depicted in which T=0 corresponds to the time of injection of secondary tumors. As a control, tumor growth was monitored following inoculation of the same tumor cell dose into non-tumor (NT) experienced naive C57BL/6 mice (n=5).

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