

Rise of iPSCs as a cell source for adoptive immunotherapy

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Abstract

Adoptive T-cell transfer is a potentially effective strategy for treating cancer and viral infections. However, previous studies of cancer immunotherapy have shown that T cells expanded in vitro fall into an exhausted state and, consequently, have limited therapeutic effect. One way to overcome this obstacle is to use induced pluripotent stem cells (iPSCs) as a cell source for making effector T cells. In recent years, there have been several reports on generating effector T cells suitable for adoptive immunotherapy. The reported findings suggest that by using iPSC technology, it may be possible to stably derive large numbers of juvenile memory T cells targeted to cancers or viruses. In this review, we describe a strategy for applying iPSC technology to immunotherapy and the characteristics of T cells derived from iPSCs. We also discuss how these

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2 technologies can be applied clinically in the future.
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8 **Introduction** 9

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11 T cells play a central role in acquired immunity against pathogens. Since the
12 identification of tumor-specific antigens and their epitopes in the 1990's (1), substantial
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14 progress has been made in our understanding of T cell-mediated antitumor responses.
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18 From that understanding emerged adoptive cell transfer (ACT) therapy for cancer,
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21 which makes use of tumor-infiltrating lymphocytes (TILs) and has achieved some
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24 success. For example, Rosenberg's group in National Cancer Institute reported that after
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27 lymphodepleting systemic chemotherapy, adoptive transfer of TILs induced clear and
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reproducible responses in nearly 50% of melanoma patients (2). Despite the therapeutic potential of TIL infusion therapy against cancer and chronic viral infections, there remain two important biological problems must be overcome (3-5). One is that cancer and viruses often hamper or escape T cell immunity by increasing regulatory T cell (Treg) counts, expressing one or more inhibitory molecules (e.g., PD-1L), or suppressing antigen presentation. This can be partially remedied by removing/remodeling the host immune system using chemoradiotherapy or newly developed monoclonal antibodies that have immunoregulatory effects (2, 6). The other

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2 problem relates to the quality of T cells expanded ex vivo. As currently performed, in
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5 vitro expansion for ACT induces T cells to differentiate into a late effector state and
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8 increase killer activity; however these cells then fall into an exhausted state and do not
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11 survive or proliferate in vivo after infusion. Consequently, they have little therapeutic
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14 effect (2,7). Optimization of the in vitro culture protocol may improve outcomes, but for
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17 now the problem remains to establish antigen-specific T cell clones that need repeated
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21 stimulation in long-term culture periods.
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24 To overcome that problem, the therapeutic potential of induced pluripotent cells
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27 (iPSCs) is drawing attention (8). iPSCs have the capacity for self-renewal while
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30 maintaining pluripotency and could potentially be a major cell source for induction of
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33 juvenile T cells suitable for ACT therapy. T cells recognize antigens via their T cell
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36 receptors (TCRs). Because TCR genes are irreversibly rearranged in the thymus during
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39 T cell maturation, iPSCs derived from T cells (T-iPSCs) retain the rearranged TCR
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42 genes of the original cell. It would therefore be expected that redifferentiating T-iPSCs
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45 derived from antigen-specific T cells would produce large numbers of juvenile
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48 antigen-specific T cells. In mouse, the therapeutic potential of this approach was
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51 successfully demonstrated through nuclear transplantation and subsequent in vivo
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54 maturation using toxoplasma gondii antigen-specific T cells and alpha-GalCel-specific
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2 Valpha14 NKT cell nuclei (9,10).
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5 Generation of iPSCs from T cells was initially difficult. But over the years several
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8 groups, including ours, have succeeded in using integrated viral vector systems to
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11 produce T-iPSCs from mouse and human polyclonal and antigen-specific T cells (11-16).
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14 This has enabled the potential of T-iPSCs as a cell source for ACT to be explored all
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17 around the world.
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24 **Generation of rejuvenated antigen-specific T cells using T-iPSCs**

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27 In 2013, a series of studies were reported on the establishment of T-iPSCs from
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29 antigen-specific cytotoxic T cells (CTLs) and redifferentiation of the T-iPSCs into
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31 functional CTLs (16,17). Two Japanese groups, including ours, each succeeded in using
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34 a Sendai viral vector to establish T-iPSCs, our group from a HIV nef protein-specific
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37 CTL clone and Kawamoto's group from a melanoma MART-1 antigen-specific CTL
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40 clone. To differentiate T-iPSCs into CTLs, we employed an in vitro differentiation
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43 protocol entailing co-culture with C3H10T1/2 and Delta-like 1-expressing
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46 OP9(OP9-DL1) stromal cells. After 35-40 days of differentiation, we obtained CD3+
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49 CD4+ CD8+ double-positive (DP) stage T cells. It is at this stage during physiological
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52 thymocyte development that TCR- α gene rearrangement occurs (18, 19). With
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2 T-iPSC-derived DP cells, activation of Rag-1 and Rag-2, two genes related to
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5 recombination machinery, was observed, as was a reduction in the percentage of
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8 original antigen-specific cells. To create mature CD8 single-positive (SP) CTLs from
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11 T-iPSCs without TCR gene revision, it was necessary to artificially end the TCR- α
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14 rearrangement. TCR signaling via the peptide-major histocompatibility complex (MHC)
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17 during positive selection is known to downregulate RAG expression and prevent further
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20 rearrangement of TCR (19). In addition, stimulation using a anti-CD3 antibody is
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23 known to mimic TCR signals (20). Both our group and Kawamoto's stimulated
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26 differentiated T-iPSCs on days 35-40 using anti-CD3 antibody or PHA and succeeded in
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29 producing CD8 SP cells. In our hands, redifferentiated CD8 SP cells recognized the
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32 same peptide on HIV antigen as the original CTLs, and immunological assays revealed
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35 that these cells exhibit normal cytolytic activity, INF- γ secretion and degranulation
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38 when stimulated by their target peptide. In addition, proliferation assays revealed that
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41 redifferentiated T-iPSCs had greater expansion potential and longer telomeres than the
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44 original cells, indicating CTLs can become "rejuvenated" by passing through the
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47 T-iPSC state.
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53 Clonal expansion of acquired immune cells is very useful for clinical application,
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56 as has been seen with B cell monoclonal antibodies. This strategy to generate T cells by
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2 passing them through T-iPSCs enables us to expand clinically applicable T cell clones
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5 stably and without limit, which could drive innovation in ACT therapy.
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8 Decades of research into cancer immunology have established that strong
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10 lymphocyte infiltration is associated with a good outcome in many types of tumors,
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12 including melanoma and head and neck, breast, renal, bladder, urothelial, ovarian,
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14 colorectal and pancreatic cancers, among others. This provides hope that ACT could
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16 become an effective therapy for numerous tumor types (21). At present, however,
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18 clinical trials of ACT are performed only in a few tumors, like melanoma. This is in part
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20 because of the difficulty of achieving proliferation of tumor-specific CTLs, which must
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22 differ radically depending on the immunogenicity of the tumor (22). A stable and
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24 unlimited supply of tumor-specific CTLs derived from T-iPSCs will facilitate research
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26 into ACT therapy for many types of cancer not treatable with immunotherapy at present.
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43 **iPSC based expansion of Mucosal-associated invariant T**

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46 In T cells there are some subsets of innate T cells as represented by natural killer T
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48 (NKT) cells other than acquired immune T cells. Mucosal associated invariant T
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50 (MAIT) cells belong to the innate T cells and are abundant in humans, representing up
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52 to 50% of the resident T cells in the Liver, 10% of the peripheral blood mononuclear
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2 cells (PBMCs) were known to play a pivotal role in host defense against a wide range of
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5 bacterial and fungal infection including mycobacterium. Recently, Wakao et al reported
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8 generating T-iPSCs from human cord blood MAIT cells by using Sev vector and
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11 redifferentiated them into MAIT cells. Redifferentiated MAIT (re-MAIT) cells could
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14 produce various cytokines such as IFN- γ , TNF- α in the presence of bacteria-fed
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17 monocyte as original MAIT cells do. Infusion of re-MAIT cells into
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20 immunocompromised mice showed their antimycobacterial activity in vivo (23).
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24 This result not only demonstrate the clinical potential of T-iPSCs derived innate T cells,
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27 but also show the possibility of T-iPSC technology as a device to explore the rare or
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30 difficult to culture T cell populations
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34 35 36 37 **Combination of T-iPSC and CAR technology**

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40 Genetic engineering of T cells to express chimeric antigen receptors (CARs) has
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43 recently emerged as a promising approach to rapidly generating tumor-targeted T cells
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46 endowed with enhanced antitumor properties (24). Most CARs utilize an
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49 antigen-derived antigen-binding motif to recognize their target (25). This enables them
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52 to recognize cell surface antigens with a higher affinity than TCRs and to function in an
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55 HLA-independent fashion, thereby eliminating the need to consider HLA restriction and
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2 overcoming some tumor escape mechanisms. Recently clinical trials of CD19
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5 CAR-modified T cells demonstrated their efficient targeting of acute and chronic
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8 lymphoblastic leukemias (26-30). Moreover, promising results on the use T-iPSCs as a
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11 cell source for CAR-modified T cells were recently reported (31). By itself, use of CAR
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14 technology is a new and potentially problematic strategy, and severe adverse effects
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17 have been reported in several clinical trials. But although many aspects of this approach
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20 require improvement, the combination of iPSC and CAR technologies offers a
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23 promising avenue to the treatment of cancers.
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31 **Discussion**

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34 T-iPSCs possess great potential as a cell source for ACT therapy. Establishing T-iPSCs
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37 from a patient's own antigen specific T lymphocytes and redifferentiating them for ACT
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40 therapy is an ideal way to tailor immunotherapy. But this strategy still has significant
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43 problems, including the time required and cost. For example, it takes at least 3 months
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46 to establish T-iPSCs and redifferentiate them into T cells, and establishing iPSCs for
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49 each patient may be too costly for routine use. One possible solution is to generate a
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52 T-iPSC bank for common combination of cancer antigen and HLA haplotypes (i.e
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55 HLA-A2 restricted cancer antigen WT1 derived specific peptide) and repress
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2 allo-antigenic HLA expression through genomic modification (32,33). If such an
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5 antigen-HLA-identified T-iPSC bank could be established, then the dream of
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8 coordinating antigen-specific CTLs for timely ACT therapy based on T-iPSCs with
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11 matched HLA-type might come true. This banking concept would match well with
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14 CAR or exogenous TCR transduction technology to T-iPSC.
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18 Increasing the efficiency of T-iPSC generation and redifferentiation,
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21 establishment of feeder-free culture systems for T cells, cost reduction, and many
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24 biological and technical challenges lie ahead. But if it were possible to achieve T-iPSCs
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27 as a cell source for ACT therapy, then the treatment of malignancies and chronic viral
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30 infections would be completely changed, and the impact on medicine would be
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34 enormous.
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