### Induced pluripotent stem cells in hematology: current and future applications

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<td>Published Version</td>
<td>doi:10.1038/bcj.2014.30</td>
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<td>Accessed</td>
<td>June 16, 2017 1:38:48 PM EDT</td>
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REVIEW

Induced pluripotent stem cells in hematology: current and future applications

D Focosi1,6, G Amabile2,3,6, A Di Ruscio2,3, P Quaranta4, DG Tenen2,5 and M Pistello4

Reprogramming somatic cells into induced pluripotent stem (iPS) cells is nowadays approaching effectiveness and clinical grade. Potential uses of this technology include predictive toxicology, drug screening, pathogenetic studies and transplantation. Here, we review the basis of current iPS cell technology and potential applications in hematology, ranging from disease modeling of congenital and acquired hemopathies to hematopoietic stem and other blood cell transplantation.

Blood Cancer Journal (2014) 4, e211; doi:10.1038/bcj.2014.30; published online 9 May 2014

INDUCED PLURIPOTENT STEM CELLS

Stem cells, generally defined as cells able to undergo self-renewal by asymmetric cell division, can be classified according to potency. Pluripotent cells, able to differentiate into any of the three germ layers, can be isolated from blastocysts (embryonic stem (ES) cells) or generated by reprogramming of adult somatic cells (induced pluripotent stem (iPS) cells)1 (Figure 1). Despite that ES cells represent the most promising type of cells for scientific and clinical applications, their use poses a set of concerns (Table 1).

The core technology of iPS cell generation consists of ectopic expression of master reprogramming factors (RFs). The iPS cells have been generated for the first time from murine fibroblasts in 2006 by Takahashi and Yamanaka2 using the transcription factors Oct4, Klf4, Sox2 and c-Myc (OKSM). In 2007, the teams of Yamanaka3 and Thomson4 successfully reprogrammed primary human fibroblasts using the OKSM cocktail5 and Klf4, Oct4, Sox2 and LIN28,6 respectively. Many groups have been able to avoid use of the proto-oncogene c-Myc because of transformation concerns5,6 by replacing it with less dangerous genes.7,8 In order to overcome the low transfection efficiency of primary cells, several retroviral or lentiviral vectors (LVs) have been used to introduce RFs into cells. However, the insertional mutagenesis associated with these vectors still represents a major downside of this type of approach.9 Vector integration raises additional concerns related to the stable permanence of the RFs. Prolonged and uncontrollable duration of RF expression, as well as RF silencing and spontaneous reactivation, have been shown to affect iPS cell biological properties both in vivo and in vitro.10,11 For these reasons, many efforts have been made to increase the safety of delivery approaches and to provide tightly RF controllable expression systems. To date, a wide array of delivery systems have been tested ranging from adeno-associated vectors whose parental viruses integrate in ‘safe harbor’ genomic sites12 to nonintegrating viral vectors (for example, Sendai virus) and to inducible vectors in which RF expression is tightly regulated by external switches.13

IMMUNOGENICITY OF IPS CELL-DERIVED SOMATIC CELLS

Although iPS cells are ideal for patient-tailored treatments for genetic disease, their derivatives could be used to treat diseases in non-histocompatible recipients. Apart from containing necrotic culture debris or induced vasculature, iPS cell products do not contain donor antigen-presenting cells, and hence in principle they would not be able to induce direct allorecognition. This property makes iPS cells immunogenic only through recipient antigen-presenting cells: a minor ‘indirect pathway’ leading to chronic but not acute rejections. As soon as more complex tissues will be generated, the possibility of having cells with antigen-presenting capacity would significantly increase. Until then, human leukocyte antigen (HLA) compatibility remains a suitable objective, and the creation of regional ‘haplobanks’ of iPS cells has been encouraged.14 Few cell types differentiated from iPS cells appear less immunogenic than their native counterpart. For example, human iPS cell-derived hematopoietic progenitor cells induce T-cell anergy in in vitro-generated alloreactive CD8+ T cells, and are not susceptible to natural killer (NK) cell cytototoxicity.15 This assumption cannot be considered universal, as demonstrated by the increasing immunogenicity of allogeneic cardiomyocytes derived in vivo from iPS cells.16 Furthermore, abnormal gene expression in specific differentiated cell types derived from iPS cells is able to induce T-cell dependent immune response in syngeneic recipients.17 Despite these findings, Araki et al.18 found only a limited immunogenicity of transplanted cells differentiated from iPS and ES cells. Therefore, the immunogenicity of therapeutically valuable cells derived from patient-specific iPS cells should be evaluated before any clinical application. Indeed, OCT4-specific T cells can be readily detected in freshly isolated T cells from > 80% healthy donors, 35% of patients with newly diagnosed germ cell tumors and nearly 100% of these patients after chemotherapy.19

1Division of Transfusion Medicine and Transplant Biology, Azienda Ospedaliero-Universitaria Pisana, Pisa, Italy; 2Harvard Medical School, Boston, MA, USA; 3Harvard Stem Cell Institute, Boston, MA, USA; 4Retrovirus Centre and Virology Section, Department of Translational Research, University of Pisa, Pisa, Italy and 5Cancer Science Institute, National University of Singapore, Singapore, Singapore. Correspondence: Dr D Focosi, Division of Transfusion Medicine and Transplant Biology, Azienda Ospedaliero-Universitaria Pisana, via Paradiso 2, 56124 Pisa, Italy.
E-mail: dfocosi@ao-pisa.toscana.it

6These authors contributed equally to this work.

Revised 3 March 2014; revised 26 March 2014; accepted 2 April 2014
DISEASE MODELING
Transgenic mouse models have been generated in the attempt to recapitulate a disease phenotype; however many of these models are, in the best scenario, only similar to the human disease and often reflect incompletely the specific and relevant pathogenetic mechanisms. In particular, as iPS cells represent an early stage of disease, the establishment of in vitro differentiation models recapitulating specific cell-type differentiation would be relevant for dissecting pathogenetic events responsible for disease initiation and progression.

Understanding diseases affecting principally the bone marrow (BM) is quite limited if researchers have to only rely mostly on peripheral blood leukocytes. Specific hemopathies in which tissue samples are scarce, for example, idiopathic myelofibrosis or aplastic anemia, represent an important challenge. Patient-derived iPS cells hold promise for understanding the molecular pathways involved in disease through the establishment of ‘the disease in a dish’. In particular, as iPS cells have the potential to differentiate into every cell of the hematopoietic system, cell types relevant for a specific disease can be generated recapitulating in vitro a specific-disease environment. This approach could lead to the identification of new genetic and epigenetic aberrations including environmental stress inducers that might represent a precipitating event during disease onset and otherwise not detectable.

INHERITED BM FAILURE SYNDROME
Inherited BM failure syndromes are a heterogeneous group of genetic disorders characterized by BM failure, congenital abnormalities and an increased risk of generating malignant diseases. The representative diseases with involvement of all hematopoietic lineages are the Fanconi anemia and the dyskeratosis congenita. Diamond–Blackfan anemia (DBA) is, instead, a disease affecting exclusively the erythroid lineage. To date, the only available...
therapy for these types of diseases is represented by the allogeneic hematopoietic stem cell (HSC) transplantation, even though most patients do not have fully HLA-matched donor, and those who do still have the risk of morbidity and mortality.

Low reprogramming efficiency of patient fibroblasts has been described in inherited disorders associated with activated p53, such as Diamond–Blackfan anemia, Fanconi anemia and ataxia telangiectasia. Transgenic expression of the implicated genes has been shown to correct the phenotype of hematopoietic cells, but in many cases gene therapy attempts have failed mainly because of the low efficiency of gene targeting and inadequate selection of real HSC population. Alternatively, gene editing of somatic cells followed by reprogramming to iPS cells and subsequent expansion and redifferentiation into HSCs can be exploited to overcome the low gene targeting efficiency.

HEMOGLOBINOPATHIES

Hemoglobinopathies are genetic inherited conditions that originate from the lack or malfunction of the hemoglobin protein. The severe anemia combined with the complications associated with the most aggressive subtypes raises the necessity for a cure to restore the hemoglobin function. Routine therapies for these conditions, namely transfusion and iron chelation, have significantly improved the quality of life of the patients over years, despite that the pathogenetic mechanism of this group of disease remains largely unknown. A curative option is the allogeneic HSC transplantation. However, this approach is limited by both the availability of suitable donors and by the graft-versus-host disease. Gene therapy offers an alternative approach to cure hemoglobinopathies by the direct recovery of the hemoglobin function via globin gene replacement. In the past two decades, gene transfer tools based on LV development have been significantly improved and proven to be curative in several animal models for sickle cell disease (SCD) and thalassemia.

THALASSEMIAS

The β-thalassemias are one of the most prevalent inherited disorders worldwide. They are caused by over 200 different types of either point mutations or deletions of nucleotides in β-globin gene, resulting in reduced, abnormal or no synthesis of β-globin chains. Patients affected by β-thalassemia have severe anemia and a shortened lifespan. Compound β²/β⁰-thalassemia is the most common form of severe thalassemia in Southeast Asian countries and their diasporas. The β²-globin allele bears a point mutation that causes alternative splicing. The abnormally spliced form is noncording, whereas the correctly spliced mRNA expresses a mutated β²-globin with partial instability. When this is compounded with a nonfunctional β⁰ allele, a profound decrease in β-globin synthesis is observed, and approximately half of β²/β⁰-thalassemia patients are transfusion dependent. Gene therapy for β-thalassemia is particularly challenging given both the requirement for massive hemoglobin production in a lineage-specific manner and the lack of selective advantage for corrected HSCs. To date, only one adult with transfusion-dependent β²/β⁰-thalassemia major was transplanted with globin LV-transduced autologous CD34⁺ cells. This patient (who is still transfusion independent at 4.5 years of follow-up) showed a semidominant myeloid-biased cell clone bearing a globin lentivirus within the HMGGA2 gene. The clonal dominance that accompanies therapeutic efficacy may be coincidental and stochastic or result from a hitherto benign cell expansion caused by dysregulation of the HMGGA2 gene in stem/progenitor cells.

A relevant issue to hemoglobinopathies is whether persistence of fetal hemoglobin or embryonic–fetal–adult globin class switching can occur in vivo after transplantation with human iPS cell-derived HSCs. LV-transfected iPS cell-derived HSCs were compared with their LV-transfected natural isogenic somatic counterparts. In particular, NSG immunodeficient mice transplanted with the corrected cells showed an embryonic to fetal and a partial fetal to adult globin class switching. This finding suggests that the β-globin gene transfer is likely necessary for iPS cell-based therapy of the β-hemoglobinopathies. If HSCs will be safely derived from iPS cells, it is envisaged that patient-specific autologous iPS cells will also be routinely generated for patients affected by hemoglobinopathies and HSCs obtained by in vitro differentiation. In fact, HSCs derived from iPS cells may be corrected by gene editing (for example, transcription activator-like effector nucleases (TALENs) or modified by lentiviral transfer carrying a therapeutic gene. A screening to assess viral integration in chromosomal areas of lower genotoxic potential is likely to become an important step for this kind of approach. An alternative to β-globin gene transfer might be the erythroid-specific knockdown of BCL11A by means of small hairpin RNA, thereby forcing γ-globin expression and β-globin downregulation.

SICKLE CELL DISEASE

In the majority of SCD patients, the mutation of A to T (also known as β⁰ to β⁴ mutation) in both alleles of the β-globin (HBB) gene changes codon 6 from Glu (GAG) to Val (GTG), resulting in a defective form of adult hemoglobin. An ideal iPS cell-based gene therapy for SCD would require both precise correction of disease-causing mutation and a complete switching from fetal-type globin to adult-type globin.

Although SCD was one of the first described molecular diseases, the goal for treating this monogenic disorder using gene therapy approaches has not been fully accomplished in humans. Gene correction of β⁰ in mouse ES cells by homologous recombination (HR) has previously been reported. Similarly, correction of the β⁰ mutation in murine iPS cells derived from a humanized SCD mouse model, followed by successful transplantation of differentiated hematopoietic cells into isogenic mice, has been applied to cure SCD phenotypes. Demonstrated a site-specific gene correction of the silent HBB gene correction in human patient-specific iPS cells. Such corrected iPS cells could be redifferentiated in red blood cells (RBCs) expressing 25–40% of the wild-type β⁰ allele.

TRANSPLANTATION OF GENETICALLY UNEDITED, IPS CELL-DERIVED HSCS

HSCs derived from BM, umbilical cord and mobilized or nonmobilized peripheral blood have been successfully used as a source for iPS cell generation. On the other hand, iPS cells obtained from different somatic cell types of humans and nonhuman primates can be successfully redifferentiated to HSCs (Figure 2).

To date, there are at least three efficient ways to differentiate HSCs from iPS cells. (1) Coculture with OP9 cells, a murine mesenchymal stem cell line established from newborn B6C3F1 osteopetrotic mouse calvaria not producing functional macrophage colony-stimulating factor. Coculture of OP9 cells with mouse ES cells has been used to develop a preferential differentiation induction system of pluripotent cells into hematopoietic cells other than monocytes. Although the use of mouse stroma provides a barrier to clinical translation, this barrier might be overcome if a master cell bank is produced. (2) Transduction of iPS cells with Lhx2, a LIM-homeobox
transplantation of iPS cell-derived HSCs. 42

leukemia nor tumors were observed in recipients after immunodeficiency mice can be treated by HSCs derived from this of the hematolymphopoietic system in serial transfers. Using expression. 48 (4) Culture with MEDII (medium conditioned by HOXB4 cell-derived HSCs had limited proliferative and migratory capacity through centrifugation 45 in the absence of murine stroma. 46

methods such as ‘spin-EBs’ aggregate undifferentiated human ES differentiation into hematopoietic progenitor cells. Alternative may be utilized, although it tends to be more variable in system of EB-mediated differentiation of human ES cells/iPS cells 42 et al.

Summary of blood cell types successfully used as a source for iPS cell generation, and of blood cells successfully redifferentiated to blood cells to date (see text for references).

transcription factor. 40 (3) Dissociation of teratoma-generated immunodeficient mice. Our group recently showed that human iPS cells differentiate within teratomas generating functional myeloid and lymphoid cells. Similarly, HSCs can be isolated from teratoma parenchyma and reconstitute a human immune system when transplanted into NOD.Cg-Pkd1(scid) Il2rg(tm1Wjl)/SzJ immunocompromised (NSG) mice. 41 Suzuki et al. 42 reported that human iPSC cell-derived HSCs migrate from teratomas into the murine BM and their intravenous injection into irradiated recipients resulted in multilineage and long-term reconstitution of the hematolymphopoietic system in serial transfers. Using this in vivo generation system, X-linked severe combined immunodeficiency mice can be treated by HSCs derived from gene-corrected clonal iPS cells. It should also be noted that neither leukemia nor tumors were observed in recipients after transplantation of iPS cell-derived HSCs. 42

Other strategies have been successfully implemented with ES cells, and could theoretically be adapted to iPS cells. (1) Dissociation of human embryoid body (EB) at days 7–10; 43 however, human ES cell-derived HSCs had limited proliferative and migratory capacity compared with somatic HSCs. 44 The traditional ‘feeder-free’ system of EB-mediated differentiation of human ES cells/iPS cells may be utilized, although it tends to be more variable in differentiation into hematopoietic progenitor cells. Alternative methods such as ‘spin-EBs’ aggregate undifferentiated human ES cells through centrifugation 45 in the absence of murine stroma. 46 (2) Coculture with monolayers of cells derived from mouse aorta-gonads-mesonephros and fetal liver, or with stromal cell lines derived from these tissues. 47 (3) Ectopic expression of CD44 and HOXB4 expression. 48 (4) Culture with MEDII (medium conditioned by HepG2 cells, a human hepatocarcinoma cell line). 49

Modern HSC mobilization drugs (granulocyte colony-stimulating factor and CXCR4 antagonists) make access to this type of cells particularly easy. From this point of view, transiting through the pluripotent status in order to achieve HSCs seems to be a complication. However, differentiation of HSCs from iPS cells can be particularly useful in the presence of congenital or acquired settings where HSC harvest is poor (for example, idiopathic myelofibrosis or aplastic anemia) or whenever expansion to large numbers is required. As previously stated, apart from reconstituting hematopoiesis, human iPS cell-derived HSCs also retain the unique and interesting ability to induce T-cell anergy in in vitro-generated alloreactive CD8+ T cells. 55

TRANSPANTATION OF IPS CELL- DERIVED, GENETICALLY ENGINEERED AUTOLOGOUS HSCS IN GENETIC HEMOPATHIES

GENETIC engineering in inherited hemopathies can be performed directly on autologous HSCs provided that they are available and prone to transfection and transduction. As both conditions are difficult to achieve, iPS cell technology may result in a valuable alternative. 51

Gene manipulation has been performed on iPS cells with several technologies: (1) suppression of expression, either transient or sustained, 52 of disease-related genes in patient-specific iPS cells by RNA interference; (2) gene knockout; 53 (3) HR that can be used for phenotypic correction of loss-of-function mutations, 54 and correction or replacement of underlying disease-causing mutations at the endogenous loci. Differently to conventional gene therapy approaches, HR ensures that the corrected gene will be expressed in an appropriate temporal and tissue-specific manner under the regulation of endogenous cis-elements. HR can be pursued with several and constantly improving methods such as: (1) zinc-finger nucleases; 55 (2) TALENs; TALENs efficiently recognize and cleave any given DNA sequences and, compared with zinc-finger nucleases, exhibit lower unspecific cleavage, with a reduced nuclease-associated cytotoxicity. 56 (3) Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) systems in bacteria and archaea use short RNA to direct degradation of foreign nucleic: this activity has been exploited for gene targeting in iPS cells, 57, 58 even at multiple loci simultaneously; 59 and (4) helper-dependent adenoviral vectors. 60

Following HSC expansion at a suitable dose and, if applicable, ex vivo or in vivo selection for corrected/modified cells, appropriate pretransplantation conditioning (myeloablation) of the patient can eventually be applied before intravenous or intrabone infusion of HSCs.

TRANSPANTATION OF DIFFERENTIATED BLOOD CELL TYPES

Anucleated cells

Because of short half-life and absence of nucleus, such cells spare investigators and patients from risks of oncogenicity, and are hence the most immediate candidates for clinical trials. This category includes most likely mature RBCs and platelets. An efficient screening to separate accurately all the anucleated cells from the nucleated counterpart appears to be a limiting step for this kind of application.

RBCs are unique, highly specialized and the most abundant cells in humans. The primary function of RBCs is transportation of the respiratory gases O2 and CO2. Meanwhile, RBCs are also the main antioxidant reservoir for the whole body. Blood transfusion is a common procedure in modern medicine, and it is practiced throughout the world; however, many countries report a less than sufficient blood supply. Even in developed countries where the supply is currently adequate, projected demographics predict insufficiency in this supply as early as 2050. Once alloimmunization occurs, such patients require RBCs from donors with a different blood group antigen combination, making it a challenge to find donors after every successive episode of alloimmunization. Alternative blood substitutes such as synthetic oxygen carriers have so far proven unsuccessful. 51 Transfusing iPS cell-derived RBCs (safer than transplanting genetically engineered iPS cell-derived HSC) suffers from two major limitations: short half-life and need for repeated, life-long transfusions. Nevertheless, iPS cell-derived RBCs have the potential to alleviate shortages and produce pathogen-free O Rh-negative ‘universal donor’ RBCs. The major limitations for translating iPSC-derived RBCs into clinic are: (1) inefficient enucleation, (2) difficult switching to adult-type (b) globin form and (3) the possibly insurmountable number of RBCs (10^12) needed to generate one unit.
The IPS cell-derived autologous platelets have potential to alleviate supply shortages because of high demand and limited shelf-life. Production of pathogen-free O universal donor platelet concentrates with negligible isoagglutinin titers would be the ideal aim. The IPS cell-derived platelets have been generated and will be soon tested in clinical trials (Advanced Cell Technologies). The main methods are handpicking ES sacs with two-step stroma coculture or HB method with one-step stroma coculture. The major limitation is reliance on stroma and inefficiency/poor yield in megakaryocyte to platelet differentiation step.

Monocytes, monocyte-derived dendritic cells (DCs) and macrophages

Current methods for generating human primary macrophages vary in cell yield, purity and activation status, often resulting in conflicting and difficult to interpret results. The circulating monocytes are heterogeneous and vary in size, granularity, morphology and protein expression profile. Several different monocyte subsets have been characterized. Moreover, the method of isolation influences the properties of differentiated macrophages and monocyte-derived DCs. The primary human monocytes have a limited potential for proliferation in vitro and are difficult to transfet. Thus, development of new approaches to produce a homogenous population of macrophages is of large interest. Furthermore, the phagocytic activity of these cells limits the ability for additional genetic manipulation. Genetically modified macrophages differentiated from human IPS cells can serve as a useful model for understanding the etiology of, for example, macrophage-tropic HIV-1 disease, and facilitating the development of novel therapeutic interventions.

Because of HLAA restriction of the adaptive immune response, it was soon realized that the unavailability of human ES cells genetically identical to the patients was a problem in the clinical application. Genetic modification of ES cell-derived DCs, and laborious modification of the β2m or TAP gene, has been attempted to solve the problem of HLAA class I allele mismatch between human ES cells and the recipients to be treated. Theoretically, the histocompatibility issue may be resolved by autologous IPS cell-derived DCs. In fact, DCs derived from xeno-free human IPS cells have been shown to be fully functional. However, animal models are needed to test the in vivo efficacy and safety of this procedure.

The monocye-derived DCs have been widely used in cancer immunotherapy showing significant donor-to-donor variability and low capacity for the cross-presentation of tumor-associated antigens to CD8+ T cells. These fundamental properties reside only in CD141+ DC81. DCs that are present only in trace in peripheral blood and for this reason are not suitable for clinical application. The ability to generate a potentially unlimited source of DCs from IPS cells offers the possibility of harnessing their capacity for cross-priming cytotoxic T lymphocytes inducing a tumor-specific immune response.

Assuming allogeneic IPS cells will be used, once a clone of TAP- or β2m-deficient human IPS cells is established, a premade library of clones expressing various types of HLAA class I can be generated by the introduction of various HLAA class I genes. Currently, TAP2-deficient IPS cell clones have been generated using zinc-finger nucleases, and other could be achieved from patients with type I bare lymphocyte syndrome caused by mutation of the TAP1 or TAP2 gene. This will not solve the issue of HLAA class II antigen matching.

NK lymphocytes

The natural lifespan for NK cells in the human body is ~ 2 weeks. Only few human permanent NK-like cell lines are available for research, mostly derived from NK cell leukemia or lymphoma patients, and hence lacking important features of normal NK cells.

Furthermore, several of these NK-like cell lines are latently infected with Epstein-Barr virus (EBV), and the contribution of viral effectors to cell proliferation and survival has not been studied; only recently in vitro growth transformation of functional human NK cells by Herpesvirus saimiri has been reported. NK lymphocytes are capable of killing cells with missing self (that is, HLAA class I expression). NK cells have been generated from IPS cells and have the potential application in both cancer therapy and infectious diseases. Interleukin-2 is generally used to expand effector cytotoxic T or NK cells in vitro and in vivo, and it also promotes the expansion of T regulatory cells expressing the interleukin-2 receptor β-chain CD25. As T regulatory cells are able to directly suppress both T- and NK-cell responses, the therapeutic effect of effector cells is limited. In addition, it is not completely clarified why NK cell-based immunotherapy is effective for acute myeloid leukemia but not for other hematological or solid malignancies. Genetic engineering is difficult to achieve in primary peripheral blood NK cells, whereas high-efficiency genetic modification is routinely feasible in human IPS cell-derived NK cells that result in high effectiveness in animal models. Strictly considering the cell number, it seems feasible and reasonable to generate a significant amount of human IPS cell-derived NK cells for adoptive immunotherapy (~ 107–108 NK cells per patient). Major limitation is reliance on two steps of stroma coculture, and the need for sorting of rare CD34+CD45− cells may hinder clinical scale-up.

It seems that NK cells express all of the components to signal through the T-cell receptor (TCR) makes them particularly suitable to be used as a platform to utilize ectopic TCR expression. This approach seems to be particularly important because of lack of competition for TCR binding between endogenous and exogenous α/β subunits.

T lymphocytes

The IPS cells, by rejuvenating the original adult somatic cell, represent an interesting technology to achieve large doses of functional autologous antigen-specific T lymphocytes for immunotherapy. If the TCR specificity is too rare or absent in the patient, autologous polyclonal lymphocytes can eventually be cleared of their endogenous TCR and then transfected with a chimeric antigen receptor. Figure 3 represents the potential approaches to achieve large doses of functional, antigen-specific autologous effector T lymphocytes, avoiding off-target toxicity.

T cells are usually derived from IPS cells through the OP9 coculture system or by the way of teratoma formation. The OP9 cell line ectopically expressing the Notch ligand Delta-like 1 (DLL1) or DI4 (OP9-DL1 and OP9-DL4, respectively) gains the ability to recapitulate thymus-like function, supporting T-cell differentiation of both murine and human progenitors.

T lymphocytes with invariant α-chain

T lymphocytes with invariant α-chain act as innate T lymphocytes acting as a connection between the innate and acquired immune systems. Both type I natural killer T (NKT) cells or invariant NKT (iNKT) cells75 and mucosa-associated invariant T cells76 have been generated from IPS cells. To date, there is no report about germline-encoded, mycolyl-reactive T cells yet.

This cell population seems to be important for the defense against mycobacteria, fungi and cancer. In fact, once iNKT cells are activated by α-GalCer-pulsed DCs, they are capable to eliminate both MHC− and MHC+ tumor cells. Both these types of tumor cells are simultaneously present in patients, and at present iNKT cells are the only known cell type capable to eliminate them. Apparently, two-thirds of patients were ineligible for this adoptive therapy, because of the limited number of iNKT cells in their bodies.
As all these cell types are very rare, iPS cell technology for the first time will likely pave the way to adoptive cell therapies.

**B lymphocytes**

Despite initial issues, few reports focusing on the generation of iPS cells from B cells have been published. In parallel, it has been shown that iPS cells undergo lymphoid differentiation when cocultured with OP9 stroma cells77 or through intra-teratoma lymphopoiesis.41

Large collections of EBV-immortalized B-cell lines (EBV-B) from patients affected by various diseases have been maintained in a large number of institutions. These EBV-B cells can be an excellent resource for disease-specific iPS cell generation and banking for a variety of human diseases, especially for those patients with rare diseases whose tissues are no longer available, except as preserved EBV-transformed B cells.78

**CONCLUSIONS**

The advent of iPS cell technology produced an important milestone in hematopoiesis. The availability of patient-specific pluripotent stem cells will undoubtedly improve disease modeling, drug development and will pave the way to autologous cell therapy for many monogenic and acquired diseases. Many biotech companies are nowadays focused on iPS cells in order to generate novel effective therapeutic molecules. Currently, biotech companies are already significantly engaged and invested in the development and commercialization of iPS cells. It has been estimated that the iPS cell market is projected to grow to US$1 billion by 2016.83 Provided regulatory authorities will not pose limits to iPS cell research,84 the growing engagement of all pharmaceutical companies appear today a crucial step for translating iPS cells from bench to bedside in the coming years.

**CONFLICT OF INTEREST**

The authors declare no conflict of interest.

**ACKNOWLEDGEMENTS**

We are grateful to the Italian Society of Hematology (SIE) and the Italian League against Leukemia (AIL) for supporting funds. GA was supported by the American Italian Cancer Foundation during this work.

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