Development of the T-ALLiPSC-based Therapeutic Cancer Vaccines for T-cell Acute Lymphoblastic Leukemia

Tao Liu (✉ tao2020@sohu.com)

Shenzhen Luohu Hospital Group Luohu People's Hospital <https://orcid.org/0000-0002-1199-0076>

Zhu Li

Shenzhen University

Xuemei Chen

Shenzhen Luohu Hospital Group Luohu People's Hospital

Luning Liu

Shenzhen Luohu Hospital Group Luohu People's Hospital

Meiling Zhou

Shenzhen Luohu Hospital Group Luohu People's Hospital

Guangqian Zhou

Shenzhen University

Research Article

Keywords: Induced pluripotent stem cells, Cancer vaccine, T-ALL, Cytotherapy

Posted Date: January 28th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-917074/v2>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

The T-cell acute lymphoblastic leukemia (T-ALL) is a kind of hematological malignancy in children. Despite the significant improvement in the cure rate of T-ALL upon treatment with chemotherapy regimens, steroids, and allotransplantation there are relapses. This study focuses on the tumor-specific therapeutic vaccines derived from the induced pluripotent stem cells (iPSC) to address the issue of T-ALL recurrence. Patient-derived tumor cells and healthy donor cells were reprogrammed into the iPSCs and the RNA-seq data of the T-ALL-iPSCs and H-iPSCs were analyzed. *In vitro*, the whole cell lysate antigens of iPSCs were prepared to induce the dendritic cells (DC) maturation, which in turn stimulated the tumor-specific T cells to kill the T-ALL tumor cells (Jurkat, CCRF-CEM, MOLT-4). The cytotoxic T lymphocyte (CTL) stimulated by the DC-loaded T-ALL-iPSC-derived antigens showed specific cytotoxicity against the T-ALL cells *in vitro*. In conclusion, the T-ALL-iPSC-based therapeutic cancer vaccine can elicit a specific anti-tumor effect on T-ALL.

Introduction

Childhood acute lymphoblastic leukemia (ALL) is a genetically heterogeneous cancer accounting for 10–15% of the T-cell ALL (T-ALL) cases. The T-ALL event-free survival rate (EFS) is as high as 80–90%[1]. It has a higher risk of relapse than the B-lineage ALL with has a poor outcome after relapse [2]. Here, the potential of using the tumor-associated proteins in the induced pluripotent stem cells (iPSC) was explored as the basis of a T-ALL therapeutic vaccine.

A therapeutic cancer vaccine is based on the principle of inducing a specific anti-tumor immune response by introducing a tumor-related antigen. Generally, the antigens of the cancer vaccines constitute the tumor cells, tumor antigen-related proteins and peptides synthesized *in vitro*, and antigen-related genes[3, 4]. Studies performed a century ago showed that immunizing mice with embryonic tissues triggered the rejection of the transplanted tumors[5]. Furthermore, the embryonic stem cells (ESC)-vaccinated mice received obvious anti-tumor immunity, protecting them from lung cancer formation and development [6]. Emerging data have demonstrated ESCs to share common transcriptome features and antigens with the tumor cells, however, ethical concerns limit their usage in the cancer vaccines.

Since the discovery of iPSCs [7, 8], the autologous iPSCs have been found to share almost the same gene expression and surface markers as ESCs[9, 10]. In addition, the tumorigenicity and immunogenicity of the autologous iPSCs indicate their potential efficacy in cancer vaccination[11, 12]. This highlights that iPSCs can provide more accurate and representative patients' tumor immunogen than the non-autologous ESCs[12].

Latest studies have shown that [12, 11] iPSCs inactivation can be harnessed as a cancer vaccine inducing extensive anti-tumor immune responses. Through RNA sequencing (RNA-seq), the researchers found that iPSCs from both humans and mice express tumor-associated antigens, indicating that all of these iPSCs could be utilized as an anti-cancer vaccine. Injecting the iPSC-based vaccine can effectively

prevent the growth of breast cancer, mesothelioma, and melanoma in tumor-bearing mice. The iPSC-based vaccine injection can also effectively kill the tumor tissue *in vivo*. These results indicated that the iPSC-based vaccine can induce an effective immune response in the mice, and exert widespread killing effects on the different types of tumors. Compared to the current immunotherapy strategy, the iPSC vaccine can reactivate the immune system and target cancer without any side effects, proving a novel method for developing a clinical cancer vaccine.

This study first reprogrammed the tumor cells from the T-ALL patients into iPSCs, preparing the whole cell lysate antigens of iPSCs for obtaining a specific T-ALL-iPSC-based vaccine. Subsequently, it was presented to the T cells through the dendritic cells (DC), stimulating specific immune reactions against T-ALL.

Materials And Methods

Cell culture

The human T-ALL cell lines (Jurkat, CCRF-CEM, MOLT-4) and NSCLC cell line NCI-H1299 were cultured in the RPMI 1640 medium supplemented with 10% FBS. The human cervix adenocarcinoma, HeLa cells were cultured in Eagle's minimum essential medium supplemented with 10% FBS and 1% NEAA (10 mM). The breast cancer cell line, MCF-7 cells were cultured in the DMEM supplemented with 10% FBS.

T-ALL reprogramming and preparation of the T-ALL-iPSC lysates

The peripheral blood mononuclear cells (PBMC) from the T-ALL patients and healthy donors were infected with the Cyto Tune-iPS 2.0 Sendai virus reprogramming Kit (Thermo Fisher). The obtained ESC-like clones were identified for pluripotency, using methods like alkaline phosphatase staining[13], flow cytometry (2.4), and immunofluorescence staining[13] for the pluripotency markers, and teratoma formation[14]. The T-ALL-iPSCs were cultured with mTeSR plus medium (STEMCELL Technologies).

To prepare cell lysates, the iPSCs derived from the healthy cells (HiPSCs) (1×10^7 /ml) and T-ALL-iPSCs (1×10^7 /ml) were frozen in liquid nitrogen and disrupted by three freeze-thaw cycles. The cell lysates were then spun at 13000g for 20 min, and the supernatants were collected as iPSC antigens. The total protein concentration of the cell lysates was quantified using the BCA Protein Assay Kit (Beyotime Institute of Biotechnology, China).

Generation of the different types of DC-CTLs

The DCs were prepared from the monocytes of the healthy donors. The PBMC were isolated by Lymphoprep (Stemcell, USA) density gradient centrifugation at 800g for 15 min at 20 °C. After 2 h of incubation, the adherent cells were cultured in an Alys-505 complete culture medium (Zhuhai Baso Cell Science & Technology Co. Ltd, China) supplemented with 100 ng/ml GM-CSF and 30 ng/ml IL-4 induction of DCs. The non-adherent cells were cultured in the Alys-505 complete culture medium containing 1000 U/ml IL-2 and 200 ng/ml CD3 monoclonal antibody for generating the CTLs.

On day 3, the immature DCs were treated with 30µg/ml of different antigens and 1000 U/ml TNF-α. On day 7, the CTLs were co-cultured with autologous DCs at a 5:1 ratio. There were four groups, T group (only CTLs), DC-T group (CTLs were co-cultured with DCs), DC^{H-iPSC}-T group (CTLs were co-cultured with H-iPSC lysates-stimulated DCs), and DC^{T-ALL-iPSC}-T group (CTLs were co-cultured with T-ALL-iPSC lysates-stimulated DCs). All the groups were cultured at 37°C in a humidified 5%CO₂ incubator and passaged every 3 days in an Alys-505 complete culture medium with 1000 U/ml IL-2.

Flow cytometry

The T-ALL-iPSC were stained with PE anti-human TRA-1-60, APC anti-human SSEA-4 (Biolegend, USA) for 20 min at 4°C and analyzed by the flow cytometry software. T cells were stained with FITC anti-human CD3, PerCp/Cyanine5.5 anti-human CD4, APC anti-human CD8a, PE anti-human CD25, PE anti-human PD-1, and PE anti-human CD57 (Biolegend, USA) for 20 min at 4°C and analyzed by the flow cytometry software.

In vitro cytotoxicity assay

The CTLs from each group were examined for the cytotoxic activity toward the target tumor cells by performing a lactate dehydrogenase (LDH) release assay using the LDH cytotoxicity assay kit (Beyotime Institute of Biotechnology). The CTLs and tumor cells were co-cultured at the effector-to-target (E/T) ratios of 5:1, 10:1, 20:1 for 24 h. The specific lysis was calculated as follows: Percentage of cytotoxicity = $\frac{[(\text{experimental release} - \text{effector spontaneous release}) - (\text{target spontaneous release})]}{[(\text{target maximum release} - \text{target spontaneous release})]} \times 100\%$.

Cytokine Release Assay

The T cells were co-cultured with or without DCs for 24 h. The supernatants from each group were collected for assessing IL-6, IL-13, IFN-γ, and TNF-α (Biolegend Human Th Cytokine Panel (12-plex) with V-bottom Plate V02, USA) using flow cytometry.

CTLs from each group were co-incubated with the target cells at an E:T ratio of 10:1. Following 24 h of incubation at 37°C, the supernatant was harvested and the concentration of the released IFN-γ was measured using the human IFN-γ Precoated ELISA Kit (Dakewe Bio-engineering Co. Ltd, Shenzhen, China).

Degranulation assay

The CTLs from each group were co-cultured with the target cells at an E:T ratio of 10:1. The PE anti-CD107a antibody (Biolegend, USA) was included during the culture. After 1 h, 2µM monensin (Biolegend, USA) was added to the co-cultured system and incubated for an additional 4 h, and then the cells were stained with the APC anti-human CD8a and analyzed using flow cytometry. All the CD8⁺ CD107a⁺ cells were regarded as the degranulated T cells.

Analysis of RNA-seq data

The human RNA-seq data for the cancer cell lines were downloaded from the Cell Model Passports Database (<https://cellmodelpassports.sanger.ac.uk/>). The cancer-related genes list was downloaded from the Network of Cancer Genes (http://ncg.kcl.ac.uk/cancer_genes.php). The list of over-expressed genes in T-ALL was screened from the Oncomine's Haferlach Leukemia Dataset[15]. The heatmap was generated using GENE-E.

Statistical analysis

The GraphPad Prism 5 software was used for data analysis. All data are presented as the mean $\pm$ the standard deviations. The one-way ANOVA with Bonferroni's post hoc test was used to analyze the differences between the groups. $P < 0.05$ indicates a statistical difference.

Results

The identification of pluripotency of the T-ALL-iPSC

The pluripotency of the T-ALL-iPSC was identified by the alkaline phosphatase staining (Figure 1A) and the expression of pluripotent markers (Figure 1B, 1C, and 1D). Figure 1E shows the karyotype analysis of the T-ALL-iPSC. When the T-ALL-iPSC were transplanted to the immunodeficient mice, these cells could successfully form the teratomas with three germ layers (Figure 1F). Collectively, these data show that the patient's T-ALL cells could be successfully reprogrammed to T-ALL-iPSCs.

The T-ALL-iPSCs have more similar T-ALL-related gene expression profiles than the H-iPSCs

Human induced pluripotent stem cells (hiPSCs) have epigenetic memory and biased propensities for differentiation[16]. To investigate whether the autologous iPSCs have the potential to yield tumor-specific therapeutic vaccines, the transcriptome expression correlation matrix was used to analyze the similarity between the T-ALL-iPSC, H-iPSC, and T-ALL cell lines (Jurkat, CCRF-CEM, MOLT-4). The T-ALL-iPSC had a higher similarity with T-ALL cell lines than the H-iPSC (H-iPSC-1, H-iPSC-2) (Figure 2A). The upregulation of a subset of genes was identified in the T-ALL-iPSCs which were over-expressed in the T-ALL cell lines (Figure 2B). Collectively, these data show that the T-ALL-iPSCs were more similar to the T-ALL-related gene profiles than the H-iPSC, suggesting that the T-ALL-iPSCs are more suitable for yielding cancer vaccines for T-ALL than the H-iPSCs.

DC-CTL phenotype and cytokine secretion

The DCs are currently known as the strongest professional antigen-presenting cells activating the resting T cells[17]. The immature DCs were loaded with the cell lysates of iPSCs, and the ability of the ex vivo-generated mature DCs to stimulate the T cell function was evaluated (Figure 3). The proportion of the activated T cells (Figure 3A), exhausted T cells (Figure 3B), and senescent T cells (Figure 3C) were analyzed in the different groups. As shown in Figure 3A, the proportion of the CD3+CD25+ T cells and

CD3+CD8+CD25+ T cells increased in the DC-T group, H-iPSC group, and T-ALL-iPSC group compared to the T group.

We measured the concentrations of the cytokines IL-6 (Figure 3D), IL-13 (Figure 3E), IFN- γ (Figure 3F), and TNF- α (Figure 3G) in the supernatants secreted by the CTLs. The CTLs stimulated by the DCs had a stronger function and cytokine secretion than the T-group, whereas there were no significant differences between the DC^{H-iPSC}-T and DC^{T-ALL-iPSC}-T groups.

The DC^{T-ALL-iPSC}-Ts and DC^{H-iPSC}-Ts had similar cytotoxicity against the non-T-ALL tumor cells

The iPSCs were reported to share the gene expression profiles with the cancer cells[11,12]. We first identified cytotoxic activity against non-T-ALL cells. The cytotoxicity of CTLs against the non-T-ALL cells including Hela (Figure 4A), MCF-7 (Figure 4B), and NCI-H1299 (Figure 4C) was examined and the level of IFN- γ secreted by CTLs was determined by ELISA at an E:T ratio of 10:1 (Figure 4D–E). The percentage of CD8+ CD107a+ (degranulation assay) was measured by flow cytometry at an E:T ratio of 10:1 (Figure 4G–I). As shown in Figure 4, the DC stimulated CTL groups had higher cytotoxic activity than the non-DC stimulated CTL group, but no significant differences were found between the DC^{H-iPSC}-T and the DC^{T-ALL-iPSC}-T groups.

The DC^{T-ALL-iPSC}-Ts exhibited specific cytotoxicity against the T-ALL cells in vitro

To assess whether the T-ALL-iPSCs were effective vaccines against the T-ALL cells and whether the stimulated T cells contained antigen-specific CTLs, the cytotoxic activity of the DC^{T-ALL-iPSC}-CTLs against T-ALL cells were analyzed in vitro. The cytotoxicity of the DC^{T-ALL-iPSC}-T group against the T-ALL cells including Jurkat (Figure 5A), CCRF-CEM (Figure 5B), and MOLT-4 (Figure 5C) was significantly higher compared to the DC^{H-iPSC}-T group. To further verify the cytotoxicity, the level of IFN- γ secreted by the CTLs co-cultured with the T-ALL cells at an E:T ratio of 10:1 was measured (Figure 5D–E). Finally, the percentage of the CD8+CD107a+ cells was detected when the CTL killed the T-ALL tumor cells (Figure 5G–I). Collectively, these data show that the DC^{T-ALL-iPSC}-Ts exhibited specific cytotoxicity against the T-ALL cells.

Discussion

Both tumorigenesis, as well as tumor development, comprise highly proliferative and low-immune cells that escape the surveillance of the immune system. Therefore, targeting tumors by reactivating the immune system constitutes a cancer treatment. The novel therapy called the chimeric antigen receptor T (CAR-T) cell therapy for T-ALL is under investigation. The CAR-T cells targeting CD7, CD5, and CD1a have been developed to show therapeutic potential [18–20]. However, CAR-T cell therapy remains a challenge for T-lineage neoplasms owing to its finite ability in distinguishing between the normal and malignant T cells; hence, it requires further development and trials [21].

So far, researchers have utilized ESCs as a vaccine for activating the immune system against cancer, but it has not shown much effectiveness and safety [22, 23]. The clinical applications of ESCs have been obstructed mainly by ethical issues, tumorigenicity, and alloimmunization [22]. Therefore, searching for a new cancer vaccine to overcome these problems is necessary. The iPSC vaccines are undoubtedly a good tumor vaccine candidate to replace ESCs. Joseph et al. used the irradiated autologous iPSCs to produce iPSC vaccines inhibiting the recurrence of a variety of tumors and reducing the burden of metastatic tumors[12]. Tailoring iPSCs by introducing other driver mutations as a vaccine can prevent the initiation and progression of pancreatic cancer[24] and lung cancer[25]. Although the irradiated iPSC-based vaccines are effective at inducing immunity against multiple cancer types in mice, it is still fraught with risks such as tumorigenicity[26] and immunogenicity[27] which need to be overcome[28].

The iPSCs have been successfully derived from a variety of human cancers[29–32]. The differentiation of the leukemia-specific iPSCs is an effective strategy for studying the early stages of the normal and abnormal hematopoietic cells[33]. Kumano's laboratory has successfully constructed the iPSCs from the CML patients and then differentiated the iPSCs into blood cells sensitive to imatinib[34]. Besides, when the AML-iPSCs are differentiated into the blood cells, these cells regain the gene expression pattern of leukemia[35]. Muñoz-López and others also tried to generate iPSCs from the B-cell ALL patients[36]. Compared to the normal cell-derived iPSCs, the tumor-derived iPSCs may possess gene expression and epigenetic characteristics similar to that of the original tumor cells. Consistently, our results support the hypothesis that for T-ALL, the T-ALL-iPSC-based vaccines are more suitable than the H-iPSC-based vaccines.

Joseph C Wu and colleagues demonstrated iPSCs alone to be incapable of stimulating an effective immune response, and also stated that only the combination of iPSCs and adjuvant CpG can cause anti-tumor effects[12]. Our findings specified that the iPSC lysates could serve as complete antigens for stimulating the immature DCs and stimulating the killing effect of the T-ALL-iPSCs vaccine on the tumor cells. They are reportedly pulsed with the tumor cells or tumor cell lysates to obtain the vaccines and providing a variety of specific tumor antigens without prior knowledge of the targets[37]. Recent studies have reported that the fusion of the tumor cells with DCs can induce various anti-tumor immune responses[38]. The only non-antiviral cancer vaccine approved by the FDA is Sipuleucel-T (Provenge), a TAA pulsed autologous dendritic cell -based prostate cancer vaccine[39]. The flow cytometry experiments showed that DC can enhance the function of the T cells, but produces no significant difference between the DC^{H-iPSC}-T and the DC^{T-ALL-iPSC}-T groups. This indicated that DCs had the same antigen presentation effect on the H-iPSC and T-ALL-iPSC.

This study clarified the inhibitory effect of T-ALL-iPSCs on the tumors and found that the DC^{H-iPSC}-T and DC^{T-ALL-iPSC}-T groups had similar cell -killing effects on the non-T-ALL tumor cells. Akin to the study by Nigel et al., the iPSC-based vaccine generated broad tumor immunity against various types of cancer. The iPSC vaccine generates a large number of tumor antigens for the immune system[12]. But co-culturing with the T-ALL cell lines including Jurkat, CCRF-CEM, and MOLT-4 showed that the DC^{T-ALL-iPSC}-T cells

significantly increased the cytotoxicity compared to the DC^{H-iPSC}-T cells. These results indicated that the T-ALL-iPSC have a larger pool of T-ALL-related epitopes, and DC^{T-ALL-iPSC} can induce the T cells to produce a specific killing effect against the T-ALL cell lines.

The patient-derived iPSCs were used as an antigen source for the anticancer vaccines because they can prime the immune system to respond to multiple patient-specific tumor antigens[40]. Hence, the patient's T-ALL cells were successfully reprogrammed into the T-ALL-iPSCs. When the H-iPSCs from the healthy people were compared to the T-ALL-iPSCs there were more similar gene expression profiles with the T-ALL cell lines, and the T-ALL-iPSC vaccine was found to stimulate more powerful toxic effects on the T-ALL cells. Therefore, T-ALL-iPSCs as a therapeutic tumor vaccine induces a better anti-tumor effect on the T-ALL. These beneficial properties make the iPSCs vaccine a potential choice for personalized adjuvant immunotherapy soon after the primary cancer treatment. Future studies should verify these effects through various *in vivo* experiments as they will establish the foundation for solid tumor cell derived-specific iPSC vaccine. Hence, Kooreman et al. describe a novel immunotherapeutic vaccination strategy using iPSCs that is highly effective in preventing multiple tumor growth in preclinical mouse models. Our results suggest that patient-derived iPSCs may have an advantage if targeted at a particular disease.

Declarations

Authors' contributions

Z.L. performed the main experiments and wrote the manuscript. X.M.C., L.N.L and M.L.Z. assisted with all experiments. G.Q.Z and T.L. designed the project. All authors have read and approved the final manuscript.

Funding

This work was funded by the Shenzhen Science and Technology Innovation Committee (Project No. JCYJ20170412155231633).

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. Kato M, Manabe A (2018) Treatment and biology of pediatric acute lymphoblastic leukemia. *Pediatr Int* 60 (1):4-12. doi:10.1111/ped.13457
2. Patrick K, Vora A (2015) Update on biology and treatment of T-cell acute lymphoblastic leukaemia. *Curr Opin Pediatr* 27 (1):44-49. doi:10.1097/MOP.0000000000000171

3. Rosenberg SA, Yang JC, Restifo NP (2004) Cancer immunotherapy: moving beyond current vaccines. *Nature medicine* 10 (9):909-915. doi:10.1038/nm1100
4. Xiang X, Wang J, Lu D, Xu X (2021) Targeting tumor-associated macrophages to synergize tumor immunotherapy. *Signal transduction and targeted therapy* 6 (1):75. doi:10.1038/s41392-021-00484-9
5. Brewer BG, Mitchell RA, Harandi A, Eaton JW (2009) Embryonic vaccines against cancer: an early history. *Experimental and molecular pathology* 86 (3):192-197. doi:10.1016/j.yexmp.2008.12.002
6. Dong W, Du J, Shen H, Gao D, Li Z, Wang G, Mu X, Liu Q (2010) Administration of embryonic stem cells generates effective antitumor immunity in mice with minor and heavy tumor load. *Cancer immunology, immunotherapy : CII* 59 (11):1697-1705. doi:10.1007/s00262-010-0899-9
7. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S (2007) Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 131 (5):861-872. doi:10.1016/j.cell.2007.11.019
8. Takahashi K, Yamanaka S (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 126 (4):663-676. doi:10.1016/j.cell.2006.07.024
9. Mallon BS, Hamilton RS, Kozhich OA, Johnson KR, Fann YC, Rao MS, Robey PG (2014) Comparison of the molecular profiles of human embryonic and induced pluripotent stem cells of isogenic origin. *Stem cell research* 12 (2):376-386. doi:10.1016/j.scr.2013.11.010
10. Bock C, Kiskinis E, Verstappen G, Gu H, Boulting G, Smith ZD, Ziller M, Croft GF, Amoroso MW, Oakley DH, Gnirke A, Eggan K, Meissner A (2011) Reference Maps of human ES and iPS cell variation enable high-throughput characterization of pluripotent cell lines. *Cell* 144 (3):439-452. doi:10.1016/j.cell.2010.12.032
11. Ouyang X, Liu Y, Zhou Y, Guo J, Wei TT, Liu C, Lee B, Chen B, Zhang A, Casey KM, Wang L, Kooreman NG, Habtezion A, Engleman EG, Wu JC (2021) Antitumor effects of iPSC-based cancer vaccine in pancreatic cancer. *Stem cell reports* 16 (6):1468-1477. doi:10.1016/j.stemcr.2021.04.004
12. Kooreman NG, Kim Y, de Almeida PE, Termglinchan V, Diecke S, Shao NY, Wei TT, Yi H, Dey D, Nelakanti R, Brouwer TP, Paik DT, Sagiv-Barfi I, Han A, Quax PHA, Hamming JF, Levy R, Davis MM, Wu JC (2018) Autologous iPSC-Based Vaccines Elicit Anti-tumor Responses In Vivo. *Cell stem cell* 22 (4):501-+. doi:10.1016/j.stem.2018.01.016
13. Yue G, Liao J, Wang Y, He L, Wang T, Zhou G, Lei B (2021) Quality evaluation of induced pluripotent stem cell colonies by fusing multi-source features. *Comput Methods Programs Biomed* 208:106235. doi:10.1016/j.cmpb.2021.106235
14. Nelakanti RV, Kooreman NG, Wu JC (2015) Teratoma formation: a tool for monitoring pluripotency in stem cell research. *Curr Protoc Stem Cell Biol* 32:4A 8 1-4A 8 17. doi:10.1002/9780470151808.sc04a08s32
15. Haferlach T, Kohlmann A, Wieczorek L, Basso G, Kronnie GT, Bene MC, De Vos J, Hernandez JM, Hofmann WK, Mills KI, Gilkes A, Chiaretti S, Shurtleff SA, Kipps TJ, Rassenti LZ, Yeoh AE, Papenhausen PR, Liu WM, Williams PM, Foa R (2010) Clinical utility of microarray-based gene

- expression profiling in the diagnosis and subclassification of leukemia: report from the International Microarray Innovations in Leukemia Study Group. *J Clin Oncol* 28 (15):2529-2537. doi:10.1200/JCO.2009.23.4732
16. Lee JH, Lee JB, Shapovalova Z, Fiebig-Comyn A, Mitchell RR, Laronde S, Szabo E, Benoit YD, Bhatia M (2014) Somatic transcriptome priming gates lineage-specific differentiation potential of human-induced pluripotent stem cell states. *Nat Commun* 5:5605. doi:10.1038/ncomms6605
 17. Katz T, Avivi I, Benyamini N, Rosenblatt J, Avigan D (2014) Dendritic cell cancer vaccines: from the bench to the bedside. *Rambam Maimonides Med J* 5 (4):e0024. doi:10.5041/RMMJ.10158
 18. Gomes-Silva D, Srinivasan M, Sharma S, Lee CM, Wagner DL, Davis TH, Rouce RH, Bao G, Brenner MK, Mamonkin M (2017) CD7-edited T cells expressing a CD7-specific CAR for the therapy of T-cell malignancies. *Blood* 130 (3):285-296. doi:10.1182/blood-2017-01-761320
 19. Mamonkin M, Rouce RH, Tashiro H, Brenner MK (2015) A T-cell-directed chimeric antigen receptor for the selective treatment of T-cell malignancies. *Blood* 126 (8):983-992. doi:10.1182/blood-2015-02-629527
 20. Sanchez-Martinez D, Baroni ML, Gutierrez-Aguera F, Roca-Ho H, Blanch-Lombarte O, Gonzalez-Garcia S, Torreadell M, Junca J, Ramirez-Orellana M, Velasco-Hernandez T, Bueno C, Fuster JL, Prado JG, Calvo J, Uzan B, Cools J, Camos M, Pflumio F, Toribio ML, Menendez P (2019) Fratricide-resistant CD1a-specific CAR T cells for the treatment of cortical T-cell acute lymphoblastic leukemia. *Blood* 133 (21):2291-2304. doi:10.1182/blood-2018-10-882944
 21. Lato MW, Przysucha A, Grosman S, Zawitkowska J, Lejman M (2021) The New Therapeutic Strategies in Pediatric T-Cell Acute Lymphoblastic Leukemia. *Int J Mol Sci* 22 (9). doi:10.3390/ijms22094502
 22. Yaddanapudi K, Mitchell RA, Putty K, Willer S, Sharma RK, Yan J, Bodduluri H, Eaton JW (2012) Vaccination with embryonic stem cells protects against lung cancer: is a broad-spectrum prophylactic vaccine against cancer possible? *PLoS One* 7 (7):e42289. doi:10.1371/journal.pone.0042289
 23. Li Y, Zeng H, Xu RH, Liu B, Li Z (2009) Vaccination with human pluripotent stem cells generates a broad spectrum of immunological and clinical responses against colon cancer. *Stem Cells* 27 (12):3103-3111. doi:10.1002/stem.234
 24. Lu S, Zhang Z, Du P, Chard LS, Yan W, El Khouri M, Wang Z, Zhang Z, Chu Y, Gao D, Zhang Q, Zhang L, Nagano A, Wang J, Chelala C, Liu J, Chen J, Liu P, Dong Y, Wang S, Li X, Dong J, Lemoine NR, Pei D, Wang Y (2020) A Virus-Infected, Reprogrammed Somatic Cell-Derived Tumor Cell (ViReST) Vaccination Regime Can Prevent Initiation and Progression of Pancreatic Cancer. *Clin Cancer Res* 26 (2):465-476. doi:10.1158/1078-0432.CCR-19-1395
 25. Zhang Z, Lu S, Dunmall LSC, Wang Z, Cheng Z, Zhang Z, Yan W, Chu Y, Gao D, Wang N, Li Y, Wang J, Li Y, Ji Y, Shan D, Li K, Wang P, Dong Y, Dong J, Lemoine NR, Pei D, Zhang L, Wang Y (2020) Treatment and Prevention of Lung Cancer Using a Virus-Infected Reprogrammed Somatic Cell-

Derived Tumor Cell Vaccination (VIREST) Regime. *Front Immunol* 11:1996.

doi:10.3389/fimmu.2020.01996

26. Lee AS, Tang C, Rao MS, Weissman IL, Wu JC (2013) Tumorigenicity as a clinical hurdle for pluripotent stem cell therapies. *Nature medicine* 19 (8):998-1004. doi:10.1038/nm.3267
27. de Almeida PE, Meyer EH, Kooreman NG, Diecke S, Dey D, Sanchez-Freire V, Hu S, Ebert A, Odegaard J, Mordwinkin NM, Brouwer TP, Lo D, Montoro DT, Longaker MT, Negrin RS, Wu JC (2014) Transplanted terminally differentiated induced pluripotent stem cells are accepted by immune mechanisms similar to self-tolerance. *Nat Commun* 5:3903. doi:10.1038/ncomms4903
28. Yaddanapudi K, Li C, Eaton JW (2018) Vaccination with induced pluripotent stem cells confers protection against cancer. *Stem Cell Investig* 5:23. doi:10.21037/sci.2018.07.03
29. Amabile G, Di Ruscio A, Muller F, Welner RS, Yang H, Ebrallidze AK, Zhang H, Levantini E, Qi L, Martinelli G, Brummelkamp T, Le Beau MM, Figueroa ME, Bock C, Tenen DG (2015) Dissecting the role of aberrant DNA methylation in human leukaemia. *Nat Commun* 6:7091. doi:10.1038/ncomms8091
30. Kotini AG, Chang CJ, Boussaad I, Delrow JJ, Dolezal EK, Nagulapally AB, Perna F, Fishbein GA, Klimek VM, Hawkins RD, Huangfu D, Murry CE, Graubert T, Nimer SD, Papapetrou EP (2015) Functional analysis of a chromosomal deletion associated with myelodysplastic syndromes using isogenic human induced pluripotent stem cells. *Nat Biotechnol* 33 (6):646-655. doi:10.1038/nbt.3178
31. Lee DF, Su J, Kim HS, Chang B, Papatsenko D, Zhao R, Yuan Y, Gingold J, Xia W, Darr H, Mirzayans R, Hung MC, Schaniel C, Lemischka IR (2015) Modeling familial cancer with induced pluripotent stem cells. *Cell* 161 (2):240-254. doi:10.1016/j.cell.2015.02.045
32. Suva ML, Rheinbay E, Gillespie SM, Patel AP, Wakimoto H, Rabkin SD, Riggi N, Chi AS, Cahill DP, Nahed BV, Curry WT, Martuza RL, Rivera MN, Rossetti N, Kasif S, Beik S, Kadri S, Tirosh I, Wortman I, Shalek AK, Rozenblatt-Rosen O, Regev A, Louis DN, Bernstein BE (2014) Reconstructing and reprogramming the tumor-propagating potential of glioblastoma stem-like cells. *Cell* 157 (3):580-594. doi:10.1016/j.cell.2014.02.030
33. Li T, Zhang YW, Li Y, Wang X, Bao WQ, Huang J, Ma YN, Li SS, Wang SQ, Yang Y, Liu YF, Gao YJ, Feng HZ, Li YX (2021) Modeling leukemia with pediatric acute leukemia patient-derived iPSCs. *Stem cell research* 54:102404. doi:ARTN 102404 10.1016/j.scr.2021.102404
34. Kumano K, Arai S, Hosoi M, Taoka K, Takayama N, Otsu M, Nagae G, Ueda K, Nakazaki K, Kamikubo Y, Eto K, Aburatani H, Nakauchi H, Kurokawa M (2012) Generation of induced pluripotent stem cells from primary chronic myelogenous leukemia patient samples. *Blood* 119 (26):6234-6242. doi:10.1182/blood-2011-07-367441
35. Chao MP, Gentles AJ, Chatterjee S, Lan F, Reinisch A, Corces MR, Xavy S, Shen J, Haag D, Chanda S, Sinha R, Morganti RM, Nishimura T, Ameen M, Wu H, Wernig M, Wu JC, Majeti R (2017) Human AML-iPSCs Reacquire Leukemic Properties after Differentiation and Model Clonal Variation of Disease. *Cell stem cell* 20 (3):329-344 e327. doi:10.1016/j.stem.2016.11.018

36. Munoz-Lopez A, Romero-Moya D, Prieto C, Ramos-Mejia V, Agraz-Doblas A, Varela I, Buschbeck M, Palau A, Carvajal-Vergara X, Giorgetti A, Ford A, Lako M, Granada I, Ruiz-Xiville N, Rodriguez-Perales S, Torres-Ruiz R, Stam RW, Fuster JL, Fraga MF, Nakanishi M, Cazzaniga G, Bardini M, Cobo I, Bayon GF, Fernandez AF, Bueno C, Menendez P (2016) Development Refractoriness of MLL-Rearranged Human B Cell Acute Leukemias to Reprogramming into Pluripotency. *Stem cell reports* 7 (4):602-618. doi:10.1016/j.stemcr.2016.08.013
37. Robbins PF, Morgan RA, Feldman SA, Yang JC, Sherry RM, Dudley ME, Wunderlich JR, Nahvi AV, Helman LJ, Mackall CL, Kammula US, Hughes MS, Restifo NP, Raffeld M, Lee CC, Levy CL, Li YF, El-Gamil M, Schwarz SL, Laurencot C, Rosenberg SA (2011) Tumor regression in patients with metastatic synovial cell sarcoma and melanoma using genetically engineered lymphocytes reactive with NY-ESO-1. *J Clin Oncol* 29 (7):917-924. doi:10.1200/JCO.2010.32.2537
38. Markov O, Oshchepkova A, Mironova N (2019) Immunotherapy Based on Dendritic Cell-Targeted/-Derived Extracellular Vesicles-A Novel Strategy for Enhancement of the Anti-tumor Immune Response. *Front Pharmacol* 10:1152. doi:10.3389/fphar.2019.01152
39. Cheever MA, Higano CS (2011) PROVENGE (Sipuleucel-T) in prostate cancer: the first FDA-approved therapeutic cancer vaccine. *Clin Cancer Res* 17 (11):3520-3526. doi:10.1158/1078-0432.CCR-10-3126
40. Goyvaerts C, Breckpot K (2018) Towards a personalized iPSC-based vaccine. *Nature biomedical engineering* 2 (5):277-278. doi:10.1038/s41551-018-0237-7

Figures

Figure 1

Pluripotency identification of the T-ALL-iPSCs. The pluripotency of the T-ALL-iPSC was identified by the alkaline phosphatase staining (A) and the expression of the pluripotent markers (B–D). Also, the karyotype analysis of the T-ALL-iPSCs was shown (E). When the T-ALL-iPSCs were transplanted into the immunodeficient mice, these cells could successfully form teratomas with three germ layers (F).

Figure 2

Analysis of the RNA sequencing data from the H-iPSCs, T-ALL-iPSCs and T-ALL tumor cells. (A) The RNA seq data of 9 H-iPSC clones generated from the healthy donors and T-ALL patients. The cell lines and iPSCs were subjected to a transcriptome expression correlation matrix, based on 15,687 filtered genes, which were generated. The healthy donor 1 (L1-iPS, L2-iPS, L3-iPS); healthy donor 2 (X1-iPS, X2-iPS, X3-iPS); T-ALL patient (W4-iPS, W6-iPS, W10-iPS); (B) Based on more than 1,000 over-expressed genes in the

T-ALL patients, we were able to identify the presence of 61 genes that were highly expressed in the T-ALL-iPSC.

Figure 3

The phenotype and cytokine secretion after CTL stimulation by the antigen-loaded DCs. The proportion of the activated T cells (A), exhausted T cells (B), and senescent T cells (C) was measured by flow cytometry. IL-2 (D), IL-6 (E), IL-13 (F), IFN- γ (G), and TNF- α (H) secreted by the CTL cells were detected. Data were represented as mean $\pm$ SEM from three independent experiments. *** $p < 0.001$, **** $p < 0.0001$.

Figure 4

The cytotoxic activity of the CTL stimulated by the antigen-loaded DC on the non-T-ALL cells *in vitro*. The cytotoxicity (A), secretion of IFN- γ (D), and proportion of CD8+CD107a cells (G) analysis of the CTLs against the HeLa cells were detected. The cytotoxicity (B), secretion of IFN- γ (E), and proportion of the CD8+CD107a cells (H) analysis of CTLs against the MCF-7 cells were detected. The cytotoxicity (C), secretion of IFN- γ (F), and proportion of the CD8+CD107a cells (I) analysis of CTLs against the NCI-H1299 cells were detected. Data were represented as mean $\pm$ SEM from three independent experiments.

Figure 5

CTLs from the T-ALL-iPSC group show specific cytotoxicity against the T-ALL cells *in vitro*. The cytotoxicity (A), secretion of IFN- γ (D), and proportion of the CD8+CD107a cells (G) analysis of CTLs against the Jurkat cells were detected. The cytotoxicity (B), secretion of IFN- γ (E), and proportion of the CD8+CD107a cells (H) analysis of CTLs against the CCRF-CEM cells were detected. The cytotoxicity (C), secretion of IFN- γ (F), and proportion of CD8+CD107a cells (I) analysis of CTLs against the MOLT-4 cells were detected. Data were represented as mean $\pm$ SEM from three independent experiments. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.