CAR-NK cells from engineered pluripotent stem cells: Off-the-shelf therapeutics for all patients

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Abstract
Clinical success of adoptive cell therapy with chimeric antigen receptor (CAR) T cells for treating hematological malignancies has revolutionized the field of cellular immunotherapy. However, due to the nature of utilizing autologous T cells, affordability and availability are major hurdles, in addition to scientific challenges relating to CAR-T therapy optimization. Natural killer (NK) cell is a specialized immune effector cell type that recognizes and kills targets without human leukocyte antigen (HLA) restriction and prior sensitization. CAR-NK cells do not cause graft vs host disease and can be obtained from unrelated donors as well as pluripotent stem cells (PSC), representing an ideal off-the-shelf therapeutics readily available for patients. Furthermore, unlike cytotoxic T cells, NK cells specifically target and eliminate cancer stem cells, which are the cells causing relapse and metastasis. PSCs can be genetically manipulated and engineered with CARs at the pluripotent stage, which allows the establishment of permanent, stable, and clonal PSC-CAR lines for the manufacture of unlimited homogenous CAR-NK cells. Multiple master PSC-CAR cell banks targeting a variety of antigens for cancer, viral infection, and autoimmune diseases provide inexhaustible cell sources for all patients. Development of a next-generation 3D bioreactor platform for PSC expansion and NK cell production overcomes major barriers related to cost and scalability for CAR-NK product.

KEYWORDS
CAR-T, CAR-NK, cellular immunotherapy, off-the-shelf therapeutics, pluripotent stem cells

Significance statement
Critical to the success of chimeric antigen receptor (CAR) engineered immune effector cell therapies will be the industrialization—converting the technologies into universal and cost-effective therapies for a large number of patients. Autologous CAR-T cell therapy has been successful for the treatment of certain blood malignancies; limited cell availability and high manufacturing cost make it difficult to be used for most patients. The development of a 3D PSC-CAR-NK platform will allow scalable, reproducible, and efficient production of homogenous functional CAR-NK cells, which can be rapidly deployed worldwide for all patients.
1 | CAR-T THERAPY: PROMISE AND CHALLENGES

1.1 | The promise

Adoptive cell transfer (ACT) for the treatment of human diseases has advanced rapidly in the past few years, especially for cancer. Chimeric antigen receptor (CAR), artificially engineered to express on the surface of immune effector cells, functions as a GPS and redirects effector cells toward their targets such as tumors and has revolutionized the ACT therapeutic field. The success of autologous CD19 CAR-T cell therapy against hematological malignancies, such as chronic lymphocytic leukemia (CLL), acute lymphocytic leukemia (ALL), and non-Hodgkin lymphoma (NHL), represents one of the most exceptional breakthroughs in cancer immunotherapy in the past decade. This success and the approval of CD19 CAR-T cell therapy by the U.S. Food and Drug Administration (FDA) have prompted the exploration, all over the world, of CAR-T clinical trials targeting different antigens for different tumors.

1.2 | The challenges

The success of CD19 CAR-T therapy, however, is not accomplished without side effects. The eradication of tumors by CD19 CAR-T cells leads to the release of several inflammatory cytokines, including IL-6 and TNF-α, which is termed cytokine release syndrome (CRS). In most patients, CRS is associated with fever and hemodynamic compromise, which can be fatal without proper intervention. Neurotoxicity has also been observed concurrent with or following CRS in some patients treated with CD19 CAR T cells, the cause of which is not clear although there are reports that CD19 CAR-T cells may penetrate the blood-brain barrier and attack cells expressing CD19.

The translation of CAR-T therapy beyond B-cell malignancies to other tumors (including other blood malignancies) still faces several obstacles that need to be addressed.

First, specificity: most CARs are targeting tumor-associated antigens (TAAs) that are also expressed on normal tissues, which leads to severe toxicity. Although there are different approaches to minimize the scale of toxicity, on-target/off-tumor toxicity can be solved completely by identifying neoantigens, which are derived from tumor-specific gene mutations (drivers), as their formation and expression are restricted to malignant tumor cells.

Second, resistance/escape: as TAAs are not required for tumor cell survival, loss of TAA expression is the major cause of development of resistance to CAR-T therapies. A strategy to circumvent tumor resistance/escape is to target multiple TAAs simultaneously by constructing bivalent- or multivalent-specific CAR-T cells, such that only tumor cells that lack expression of all target molecules would escape CAR-T therapy. Other approaches are also explored with some success.

Third, solid tumors: although some satisfactory efficacy results were observed in some leukemic patients, especially with CD19 CAR-T therapy for B-cell lymphoma and BCMA CAR-T for myeloid myeloma, comparable efficacy in solid tumors has not yet been achieved. These disappointing results can be attributed to several factors, including the inability of the CAR T cell to infiltrate into solid tumors, immunosuppressive molecules, and cells in the tumor microenvironment (TME), heterogenous expression of target antigens in tumor cells, and intrinsic T cell dysfunction and exhaustion. Regional or intratumor injection instead of systemic administration has been shown to be superior at least in animal model studies.

Combination of CAR-T with modulators of the TME, such as checkpoint inhibitors, have shown efficacy in some cancers. Other strategies include (a) depleting Tregs and myeloid-derived suppressor cells with blocking antibodies, (b) engineering CAR T cells to secrete extracellular matrix-degrading enzymes that degrade cancer-associated fibroblasts, (c) altering CAR-T cell metabolic profiles to enhance their function in hostile TME, and (d) constructing bivalent/multivalent CAR-T cells. Some of these approaches are now undergoing clinical evaluation.

Fourth, manufacturing: all the above CAR T optimizations are biological challenges, and the manufacturing of CAR-T cells is another challenge. Most CAR-T cells are derived from patients own peripheral blood mononuclear cells (PBMCs), which requires a bespoke manufacturing process for every patient after leukapheresis. The cost of the process, as well as potential manufacturing failure and delay (~3 weeks), renders the affordability and availability less favorable. Furthermore, individual to individual variations make the CAR-T therapeutics inconsistent and could result in reduced efficacy in some patients owing to their T cell dysfunction. By simultaneous introduction of CAR and disruption of T-cell receptor (TCR) and CD52 in T cells, Qasim et al reported the generation of allogenic CAR-T cells, which are able to evade host immunity and avoid graft vs host disease (GVHD) for the treatment of unmatched patients. The allogenic off-the-shelf CAR T cells from healthy donors is an alternative strategy to address the complexities of manufacturing and high costs of individualized CAR-T cell products, but there is still the risk of GVHD caused by less than even 1% of TCR+ cells in TCR knock-out CAR-T cell preparation, and early-phase clinical trials have met some hurdles. On July 6, 2020, the FDA issued a clinical hold for the phase I trial of a universal CAR-T product, UCARTCS1A (ClinicalTrials.gov Identifier: NCT04142619) developed by Cellectis, due to the death of one participant caused by cardiac arrest. To realize the therapeutic potential of engineered immune effector cells, other types of cells, including dendritic cells, macrophages, red blood cells, mesenchymal stem cells, and endothelial progenitor cells, have been explored; however, one type of immune cells—the natural killer (NK) cell—has been considered the most promising of cells for immunotherapy.

2 | NK CELLS AS AN IDEAL ACT PRODUCT

2.1 | NK cells as allogenic therapeutics

NK cells are a type of cytotoxic lymphocyte of the innate immune system and are the gatekeeper of the immune system, with the ability to
recognize and kill targets without HLA restriction or prior sensitization, allowing for a much faster immune reaction.\textsuperscript{22} NK cell cytotoxicity is regulated by the balance of activating and inhibitory receptors, which can efficiently kill abnormal cells in case activating ligands are present and signals for inhibitory receptors are absent. Different from T cells, NK cells are capable of killing cells that are missing “self” markers of MHC I antigens, whereas harmful cells missing MHC I markers cannot be detected and destroyed by T cells.\textsuperscript{22} Most importantly, adoptive NK cell transfer does not require strict HLA matching and lacks the potential to cause GvHD, a critical risk imposed by any T cell immunotherapy.\textsuperscript{23} Therefore, NK cells can be off-the-shelf allogeneic therapeutics.

## 2.2 | CAR-NK vs CAR-T

Similar to T cells, NK cells have been engineered with CAR expressed on the surface to target a specific antigen, enhancing their natural cytotoxicity with specificity.\textsuperscript{24,25} Recently, Liu et al\textsuperscript{26} reported the interim results of a phase I/II trial using umbilical cord blood (UCB)-derived, CD19-targeted CAR-NK therapy in patients with relapsed or refractory CD19\textsuperscript{+} cancers. Eight of 11 patients had an objective response to treatment without development of major toxic effect, demonstrating the safety and efficacy of CAR-NK against hematopoietic malignancies.\textsuperscript{26} Compared with CAR-T cells, CAR-NK cells have the following advantages\textsuperscript{27} (Table 1): (a) Unlike CAR-T cells, CAR-NK cells retain an intrinsic capacity to recognize and target abnormal cells through their native receptors, making the escape of abnormal cells through downregulation of CAR targeted antigens less likely; (b) CAR-NK cells do not undergo clonal expansion and cause CRS, neurotoxicity and other side effects that have been observed in many CAR-T clinical trials; (c) CAR-NK cells do not need to be patient matched, therefore cells from healthy donor PBMC or UCB can be used for the manufacture of allogeneic CAR-NK cells, which render it more affordable and feasible; (d) By default, NK cells have been shown to preferentially target and kill cancer stem cells as they are quiescent and express low levels of MHC I.\textsuperscript{28,29} This is advantageous because cancer stem cells are resistant to both traditional chemotherapy and radiotherapy, as well as immunotherapy, which leads to relapse and metastasis.

## 2.3 | Donor-based CAR-NK: Challenges

The main sources of donor NK cells are peripheral blood (PB) and UCB, and their concentration of $1 \times 10^6$ cells/L comprises $1-2\%$ of total WBCs, or 0.01-0.02\% of all cells in the PB.\textsuperscript{30} Although in vitro exponential expansion with/without artificial antigen presenting cells (aAPC) from a single donor will be able to provide enough cells for a single or limited number of patients, the doses are still limited from a single-donor phlebotomy.\textsuperscript{31-33} which is far less for an ideal off-the-shelf allogeneic commercial therapeutic product. Functional and expansion variations among different donors add another burden for batch to batch validation as well as manufacture delay, which lead to inconsistent clinical outcome. Furthermore, transfection of NK cells with viral CAR-constructs was associated with low or variable levels of transgene expression and unfavorable effects on cell viability, limiting other genetic engineering to improve the efficacy of CAR-NK cells.\textsuperscript{24} NK cell lines, such as NK9-2, have been pursued for clinical applications. These cells are cancerous in nature and need to be irradiated before infusion into patients, compromising their cancer killing capabilities.\textsuperscript{34}

As an ideal cellular immunotherapy product, cells should possess high specificity on targets with minimized off-target toxicities; be prepared from sustainable sources so every patient receives the same cell product; be able to be stored and ready for patient administration whenever needed, not be prepared for each individual patient; and be centrally manufactured in a current good manufacturing practice (cGMP) facility and be able to distribute worldwide. CAR-NK cells from donor sources fulfill most of above criteria but miss the most important property for an allogeneic off-the-shelf cell product—sustainability. One potential source of CAR-NK cells is pluripotent stem cells.
(PSC) including human embryonic stem cells (hESC) and induced pluripotent stem cells (iPSC), which may circumvent the challenges associated with donor-derived CAR-NK cells.

3 | PSCs AS A SOURCE OF ALLOGENIC OFF-THE-SHELF CAR-NK CELLS

3.1 | In vitro differentiation of PSCs toward NK cells

Both hESCs and iPSCs can be propagated and expanded in vitro indefinitely, providing a potentially inexhaustible and donorless source of hematopoietic cells for human therapy. Differentiation of PSCs into hematopoietic cells has been extensively investigated in vitro for the past two decades. Hematopoietic precursors as well as mature, functional progenies representing erythroid, myeloid, macrophage, megakaryocytic, and lymphoid lineages have been identified in the differentiating PSC cultures.\(^{35-40}\) The differentiation of PSCs into lymphoid lineage cells has proven to be more difficult than their differentiation into other lineages. Dan Kaufman’s group has pioneered the differentiation of NK cells from both hESCs and iPSCs. The 2-step differentiation procedure starts with PSCs on feeder cells to generate CD34+ hematopoietic progenitor cells,\(^{38}\) which was later optimized to culture PSCs directly on matrix coated petri dishes as adherent monolayer culture.\(^{39}\) CD34+ cells (5-15% of total cells depend on culture conditions) were then isolated from the culture and transferred onto engineered stroma in medium containing mixture of cytokines for approximately 2 weeks, which generated a highly enriched homogeneous population of CD45+ CD56+ CD94+ NK cells.\(^{38,39}\) Both in vitro and in vivo studies showed that PSC-NK cells were capable of secreting IFNγ in response to cytokine stimulation and harbored potent natural cytotoxicity against multiple types of cancer cells as well as displayed antitumor activity in xenograft mouse models.\(^{38,39,41,42}\) Several groups have also successfully differentiated PSCs into NK cells that are similar in phenotype such as NK-associated receptor phenotype and effector function when compared with PB-NK cells.\(^{43,44}\)

3.2 | CAR-NK cells derived from PSCs: Advantages

PSCs provide a completely novel pathway for the generation of gene engineered cell products such as NK cells. There are several advantages of PSC-CAR-NK compared with donor based CAR-NK cells (Table 1). First, CAR construct can be inserted into undifferentiated PSCs to establish permanent, stable, and clonal PSC-CAR lines, which can be used for unlimited manufacture of homogenous CAR-NK cells, a true off-the-shelf and consistent product for all patients.\(^{41}\) As CAR is engineered at the pluripotent stage of PSCs, there is no need to transfect NK cells with viral vectors, avoiding mass cell death caused by viral infection and uncontrollable variation of gene insertion and expression. Li et al reported iPSC-CAR-NK cells with improved killing of ovarian cancer cells in vitro and in vivo.\(^{45}\) Based on the above technology, several iPSC-CAR-NK cells are currently in clinical trials to evaluate their safety and efficacy for both hematological and solid tumors by Fate Therapeutics. Second, CARs targeting different antigens can be introduced into PSCs to establish PSC-CAR banks, covering targets for a variety of cancers, viral infection, and autoimmune diseases. The master PSC-CAR cell bank will provide inexhaustible cell sources for the manufacturing of truly off-the-shelf CAR-NK cells for all patients. Third, it allows precise genetic engineering at PSC stage to generate fully genetic edited clonal lines, shaping the phenotype and functionality of the resulting products and overcoming the various limitations associated with NK therapies. These manipulations include (a) introduction of mutated versions of high affinity CD16 receptor conferring resistance to enzymatic cleavage with improved antibody-dependent cell-mediated cytotoxicity (ADCC) and cancer cell killing,\(^{45}\) which is undergoing phase I clinical trials in adults with hematologic malignancies by Fate Therapeutics; (b) incorporation of fused cytokine receptor-ligand receptors such as IL15-IL15r fusion receptor (RLI) to improve in vivo persistence and expansion\(^{46}\); and (c) knock-out of checkpoint inhibitors such as CISH gene to improve metabolic fitness and cytotoxicity.\(^{47}\) These advantages offer new solutions for obstacles associated with conventional NK therapies.\(^{46}\)

3.3 | Manufacturing challenge of current PSC-NK cells

The manufacture of safe and effective PSC-NK cells will offer a viable alternative and future replacement of donor-NK sources. As summarized here, significant progress has been made toward this end by genetic manipulating PSCs and driving them toward NK lineage development. Despite many exciting advances with in vitro culture systems, the requirement for initial monolayer or forced embryoid body (EB) formation in specialized 96-well plate and the need to isolate rare CD34+/CD45+ CD56+ CD94+ NK cells,\(^{38,39}\) various challenges have also been encountered. Several groups have successfully differentiated PSCs into NK cells that are similar in phenotype such as NK-associated receptor phenotype and effector function when compared with PB-NK cells.\(^{43,44}\)

3.4 | 3D platform for industrial scale production of PSC-NK cells

We have developed a novel 3D-bioreactor platform to efficiently convert human PSCs into highly pure NK cells continuously in a single bioreactor (Figure 1).\(^{48}\) This differentiation system uses a defined serum-free medium and eliminates the use of any feeder cells. First, human PSCs were adapted from 2D monolayer culture to 3D spheres and expanded in a bioreactor, then 3D PSC-spheres were induced toward mesoderm lineage differentiation, which convert 60-70% PSCs into hemogenic endothelial progenitors (CD31+ CD144+ CD34+) in a week. Hemogenic endothelial cells were then switched to hematopoietic and NK cell differentiation conditions for up to 35-45 days. Starting from approximately day 15, NK cells were
released from these 3D-spheres and lasted for ~4 weeks, and the released NK cells were harvested by simple centrifugation (Figure 1). The released PSC-NK cells display a distinctively homogenous morphology and express gene signatures characteristic to typical NK cells. Over 95% of collected cells express CD56 and activating receptors of NKG2D, NKp44 and NKp46 (Figure 2). These CD56+ PSC-NK cells do not express T cell markers of TCR and CD3, nor B cell marker CD19; whereas more than 60% CD56+ PSC-NK cells also express CD8α (Figure 2) as compared with ≈30% of PB CD56+ NK cells, the increase of which is associated with disease regression in AIDS patients. We have reproducibly generated ~10^10 pure NK cells with a 300 mL bioreactor and this process has been repeated with multiple hESC and iPSC lines with/without gene editing or CAR insertion.

In vitro assays showed that PSC-NK cells produced by 3D-bioreactors were capable of secreting IFNγ, expressing CD107a (degranulation) in response to stimulation, and also displayed potent natural cytotoxicity against multiple tumor cells including leukemia cells as well as pancreatic, ovarian, colorectal and lung cancer cells. We also demonstrated that PSC-NK cells harbored anti-virus activity, and specifically killed normal cells infected with influenza A, Dengue, Zika, and HIV viruses, but not uninfected normal cells.

This 3D PSC-NK manufacture platform uses defined materials without serum or feeder cells, and is scalable and reproducible for industrial application.

**FIGURE 1**  Sequential differentiation of pluripotent stem cells (PSC) toward hemogenic endothelial (HE), hematopoietic progenitors and NK cells in 3D bioreactor. A, Scheme shows approximately times and stepwise strategy used for 3D-PSC differentiation into hemogenic endothelium, hematopoietic progenitors, and NK cells. B, Morphology and specific gene expression at different stages. SLT, secondary lymphoid tissues

**FIGURE 2**  Flow cytometry analysis of iPSC-NK cells. A, morphology of iPSC-NK cells collected from 3D-bioreactors without purification. B, Representative forward and side scatter plots of iPSC-NK cells. C-J, Representative flow cytometry plots of iPSC-NK cells for (C) CD56 (y) and TCR(x); (D) CD56(y) and CD3(x); (E) CD19 (y) and PE conjugated antibody control (x); (F) CD8(y) and CD4(x); (G) CD56(y) and NKG2D (x); (H) CD56(y) and NKp44(x); (I) CD56(y) and NKp46(x); and (J) CD56(y) and KIR2DL1/DS1(x)
generation of homogenous functional NK cells. With the establishment of permanent master PSC-CAR cell lines, this novel technology platform will provide inexhaustible cell sources for the generation of off-the-shelf CAR-NK cells suitable for treatment of a large numbers of patients, which can revolutionize the field of cellular immunotherapy.

4  |  THE FUTURE PERSPECTIVE

Human PSCs promise to provide a potentially inexhaustible source of CAR-NK cells for immunotherapy. However, before PSC-CAR-NK cells can be used in the clinic, it is important to understand the risks and challenges associated with these therapeutic products.

One of the most important characteristics of undifferentiated PSCs is teratoma formation. This characteristic is a serious concern, because if undifferentiated or under-differentiated PSCs remain in the final CAR-NK product, tumor formation may occur. Fortunately, several groups have demonstrated that undifferentiated human iPSCs are the target of NK cells both in vitro and in vivo. Specifically, injection of human iPSCs in immune deficient mice failed to form teratoma when these animals were reconstituted with allogenic or autologous PBMC or purified NK cells; whereas teratomas were observed in animals reconstituted with autologous PBMCs depleted off NK cells, confirming NK cells prevent the formation of teratoma. We also observed that coculture of human iPSCs with autologous iPSC-NK cells overnight caused mass iPSC death, demonstrating pluripotent iPSCs, if present, cannot survive the hostile surroundings of a highly concentrated iPSC-NK cell population. Furthermore, various suicide genes have been transduced into PSCs that can eliminate undifferentiated PSCs and prevent teratoma formation in PSC-derived cell therapy products. The approval of multiple iPSC-NK products (Fate Therapeutics) by the FDA clearly paves the way for clinical application of PSC-CAR-NK products.

Due to the ethical concerns, very limited new hESC lines have been derived and most existing hESC lines have not been derived under GMP conditions by utilizing undefined animal products and feeder cells, which could potentially contaminate them with animal pathogens and viruses. Although iPSC lines can be reprogrammed from many types of somatic cells, only a limited number of GMP compliant iPSC lines are currently available mainly due to the regulatory requirements for donors and absence of qualified materials and reagents for the generation of iPSCs. Therefore, in order to comply with government regulations and fulfill the promise of PSC-CAR-NK therapeutics, new hESC and iPSC lines will need to be produced under GMP conditions using defined materials that can be traced back to the origin or using xeno-free products in manufacturing PSC-CAR-NK cell products for clinical application.

Successful clinical application of cell products derived from PSCs will require efficient and controlled differentiation of PSCs toward a specific cell type with the generation of a homogeneous cell population. Our 3D PSC-NK technology provides a scalable, consistent, efficient, and cost-effective manufacturing platform for the generation of homogenous functional NK cells. As illustrated in Figure 3, GMP grade PSC lines can be genetically manipulated at the pluripotent stage to enhance the persistence and cytotoxicity of final NK cell products. Gene edited PSC lines can be further engineered with CARs targeting different cancer antigens and viral antigens or autoreactive T cells as well as chimeric autoantibody receptor (CAAR) against autoreactive B cells. The establishment of multiple master PSC-CAR cell banks targeting different specific antigens will provide inexhaustible cell sources for the manufacture of truly allogenic off-the-shelf CAR-NK cells, which can be rapidly deployed worldwide for patients of cancer, infection and autoimmune diseases. Future work aims toward developing larger (>5 L) bioreactors and logistics, with ultimate goal of making CAR-NK products affordable and available for ordinary patients and as easy to handle as conventional drugs.

CONFLICT OF INTEREST
S.J.L. is the co-founder and chief executive officer and Q.F. is the co-founder and chief scientific officer of HebeCell Corporation. Both S.J.L. and Q.F. declare employment and stock ownership at HebeCell Corporation.
AUTHOR CONTRIBUTIONS
S.J.L and Q.F.: conception/design and manuscript writing.

DATA AVAILABILITY STATEMENT
Data sharing is not applicable to this article as no new data were created or analyzed in this study.

REFERENCES
1. Sadelain M, Brentjens R, Rivière I. The basic principles of chimeric antigen receptor design. Cancer Discov. 2013;3:388-398.
2. Maude SL, Laetsch TW, Buechner J, et al. Tisagenlecleucel in children and young adults with B-cell lymphoblastic leukemia. N Engl J Med. 2018;378:439-448.
3. Schuster SJ, Bishop MR, Tam CS, et al. Tisagenlecleucel in adult relapsed or refractory diffuse large B-cell lymphoma. N Engl J Med. 2019;380:45-56.
4. Locke FL, Gobradi A, Jacobson CA, et al. Long-term safety and activity of axicabtagene ciloleucel in refractory large B-cell lymphoma (ZUMA-1): a single-arm, multicentre, phase 1/2 trial. Lancet Oncol. 2019;20:31-42.
5. Neelapu SS, Locke FL, Bartlett NL, et al. Axicabtagene ciloleucel CAR T-cell therapy in refractory large B-cell lymphoma. N Engl J Med. 2017;377:2531-2544.
6. MacKay M, Afshinee-ko E, Rub J, et al. The therapeutic landscape for cells engineered with chimeric antigen receptors. Nat Biotechnol. 2020;38:233-244.
7. Larson RC, Maus MV. Recent advances and discoveries in the mechanisms and functions of CAR T cells. Nat Rev Cancer. 2021;21:145-161.
8. Schultz L, Mackall C. Driving CAR T cell translation forward. Sci Transl Med. 2019;11:eaw2217.
9. Srivastava S, Riddell SR. Chimeric antigen receptor T cell therapy: challenges to bench-to-bedside efficacy. J Immunol. 2018;200:459-468.
10. Xie G, Ivica NA, Jia B, et al. Engineered CAR-T cells targeting a neoepitope derived from intracellular NPM1c exhibit potent activity and specificity against acute myeloid leukemia. Nat Biomed Eng. 2020;5:399-413.
11. Zhao J, Song Y, Liu D. Clinical trials of dual-target CAR T cells, donor-derived CAR T cells, and universal CAR T cells for acute lymphoid leukemia. J Hematol Oncol. 2019;12:17.
12. Jones HF, Molvi Z, Klatt MG, Dao T, Scheinberg DA. Empirical and rational design of T cell receptor-based immunotherapies. Front Immunol. 2021;11:585385.
13. Hermann AC, Im JS, Pareek S, et al. A novel T-cell engaging bispecific antibody targeting the leukemia antigen PR1/HLA-A2. Front Immunol. 2019;9:3153.
14. Tchou J, Zhao Y, Levine BL, et al. Safety and efficacy of intratumoral injections of chimeric antigen receptor (CAR) T cells in metastatic breast cancer. Cancer Immunol Res. 2017;5:1152-1161.
15. Martinez M, Moon EK. CAR T cells for solid tumors: new strategies for finding, infiltrating, and surviving in the tumor microenvironment. Front Immunol. 2019;10:128.
16. Hong M, Clubb JD, Chen YY. Engineering CAR-T cells for next-generation cancer therapy. Cancer Cell. 2020;38:473-488.
17. Qasim W, Zhan H, Samarasinghe S, et al. Molecular remission of infant B-ALL after infusion of universal TALEN gene-edited CAR T cells. Sci Transl Med. 2017;9:eaaJ138.
18. Morgan MA, Bünning H, Sauer M, Schambach A. Use of cell and genome modification technologies to generate improved “off-the-shelf” CAR T and CAR NK cells. Front Immunol. 2020;11:1965.
19. Townsend MH, Bennion K, Robison RA, O’Neill KL. Paving the way towards universal treatment with allogenic T cells. Immunol Res. 2020;68:63-70.
20. Bennett C. Patient death puts trial on hold for universal CAR-T in myeloma. Cancer Therapy Advisor. 2020;20. https://www.cancertherapyadvisor.com/home/cancer-topics/multiple-myeloma/universal-cart-therapy-multiple-myeloma-patient-death-trial/
21. Motais B, Sandra Charvatova S, Hrdinka M, et al. A bird’s-eye view of cell sources for cell-based therapies in blood cancers. Cancers. 2020;12:1333.
22. Yokoyama WM, Kim S, French AR. The dynamic life of natural killer cells. Annu Rev Immunol. 2004;22:405-429.
23. Ruggeri L, Capanni M, Urbani E, et al. Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants. Science. 2002;295:2097-2100.
24. Yilmaz A, Cui H, Calliguri MA, Yu J. Chimeric antigen receptor-engineered natural killer cells for cancer immunotherapy. J Hematol Oncol. 2020;13:168.
25. Liu S, Galat V, Galat Y, et al. NK cell-based cancer immunotherapy: from basic biology to clinical development. J Hematol Oncol. 2021;14:7.
26. Liu E, Marin D, Banerjee P, et al. Use of CAR-transduced natural killer cells in CD19-positive lymphoid tumors. N Engl J Med. 2020;382:545-553.
27. Xie G, Dong H, Liang Y, Ham JD, Rizwan R, Chen J. CAR-NK cells: a promising cellular immunotherapy for cancer. EBioMedicine. 2020;59:102975.
28. Grossenbacher SK, Canter RJ, Murphy WJ. Natural killer cell immunotherapy to target stem-like tumor cells. J Immunotherapy Cancer. 2016;4:19.
29. Ames E, Canter RJ, Grossenbacher SK, et al. NK cells preferentially target tumor cells with a cancer stem cell phenotype. J Immunol. 2015;195:4010-4019.
30. Alberts B, Johnson A, Lewis J, et al. Histology: The Lives and Deaths of Cells in Tissues (Table 22-1) In Molecular Biology of the Cell. New York, NY: Garland Science; 2002.
31. Liu E, Ang SOS, Kerbauy L, et al. GMP-compliant universal antigen presenting cells (uAPC) promote the metabolic fitness and anti-tumor activity of armored cord blood CAR-NK cells. Front Immunol. 2021;12:626098.
32. Yang Y, Badeti S, Tseng HC, et al. Superior expansion and cytotoxicity of human primary NK and CAR-NK cells from various sources via enriched metabolic pathways. Mol Therapy. 2020;18:428-445.
33. Quintarelli C, Sivori S, Caruso S, et al. Efficacy of third-party chimeric antigen receptor modified peripheral blood natural killer cells for adoptive cell therapy of B-cell precursor acute lymphoblastic leukemia. Leukemia. 2020;34:1102-1115.
34. Reighard SD, Cranert SA, Rangel KM, et al. Therapeutic targeting of follicular T cells with chimeric antigen receptor-expressing natural killer cells. Cell Rep Med. 2020;1:100003.
35. Li F, Lu SJ, Vida V, Thomson JA, Honig GR. Bone morphogenetic protein 4 induces efficient hematopoietic differentiation of rhesus monkey embryonic stem cells in vitro. Blood. 2001;98:335-342.
36. Kaufman DS, Hanson ET, Lewis RL, Auerbach R, Thomson JA. Hemangioblasts from human embryonic stem cells. Ann N Y Acad Sci. 2004;22:405-429.
37. Li F, Lu SJ, Vida V, Thomson JA, Honig GR. Bone morphogenetic protein 4 induces efficient hematopoietic differentiation of rhesus monkey embryonic stem cells in vitro. Blood. 2001;98:335-342.
38. Kaufman DS, Hanson ET, Lewis RL, Auerbach R, Thomson JA. Hemangioblasts from human embryonic stem cells. Ann N Y Acad Sci. 2004;22:405-429.
40. Galic Z, Kitchen SG, Kacena A, et al. T lineage differentiation from human embryonic stem cells. Proc Natl Acad Sci USA. 2006;103:11742-11747.

41. Li Y, Hermanson DL, Moriarity BS, Kaufman DS. Human iPSC-derived natural killer cells engineered with chimeric antigen receptors enhance anti-tumor activity. Cell Stem Cell. 2018;23:181-192.

42. Cichocki F, Bjordahl R, Gaidarova S, et al. iPSC-derived NK cells maintain high cytotoxicity and enhance in vivo tumor control in concert with T cells and anti-PD-1 therapy. Sci Transl Med. 2020;12:eaaaz5618.

43. Zeng J, Tang SY, Toh LL, Wang S. Generation of “off-the-shelf” natural killer cells from peripheral blood cell-derived induced pluripotent stem cells. Stem Cell Rep. 2017;9:1796-1812.

44. Larbi A, Gombert JM, Auvray C, et al. The HOXB4 homeoprotein promotes the ex vivo enrichment of functional human embryonic stem cell-derived NK cells. PLoS One. 2012;7:e39514.

45. Zhu H, Blum RH, Bjordahl R, et al. Pluripotent stem cell-derived NK cells with high-affinity noncleavable CD16a mediate improved anti-tumor activity. Blood. 2020;135:399-410.

46. Shankar K, Capitini CM, Saha K. Genome engineering of induced pluripotent stem cells to manufacture natural killer cell therapies. Stem Cell Res Therapy. 2020;11:234.

47. Zhu H, Blum RH, Bernareggi D, et al. Metabolic reprogramming via deletion of CISH in human iPSC-derived NK cells promotes in vivo persistence and enhances anti-tumor activity. Cell Stem Cell. 2020;27:224-237.

48. Feng Q, Zhang MY, Lu SJ. Methods and Systems for Manufacturing Hematopoietic Lineage Cells. PCT/US2019/057929, WIPO PCT WO 2020/086889 A1.

49. Ahmad F, Hong HS, Jäckel M, et al. High frequencies of polyfunctional CD8NK cells in chronic HIV-1 infection are associated with slower disease progression. J Virol. 2014;88:12397-12408.

50. Yamanaka S. Pluripotent stem cell-based cell therapy—promise and challenges. Cell Stem Cell. 2020;27:523-531.

51. Dressel R, Nolte J, Elsner L, et al. Pluripotent stem cells are highly susceptible targets for syngeneic, allogeneic, and xenogeneic natural killer cells. FASEB J. 2010;24:2164-2177.

52. Kruse V, Hamann C, Monecke S, et al. Human induced pluripotent stem cells are targets for allogeneic and autologous natural killer (NK) cells and killing is partly mediated by the activating NK receptor DNAM-1. PLoS One. 2015;10:e0125544.

53. Benabdallah B, Desaulniers-Langevin C, Colas C, et al. Natural killer cells prevent the formation of teratomas derived from human induced pluripotent stem cells. Front Immunol. 2019;10:2580.

54. de Rham C, Sollet ZC, Burkhard P, Villard J. Natural killer cell alloreactivity against human induced pluripotent stem cells and their neuronal derivatives into dopaminergic neurons. Stem Cells Dev. 2020;29:853-862.

55. Wu Y, Chang T, Long Y, et al. Using gene editing to establish a safeguard system for pluripotent stem-cell-based therapies. iScience. 2019;22:409-422.

56. Itakura G, Kawabata S, Ando M, et al. Fail-safe system against potential tumorigenicity after transplantation of iPSC derivatives. Stem Cell Rep. 2017;8:673-684.

57. Lim RM, Rong L, Zhen A, Xie J. A universal CAR-NK cell targeting various epitopes of HIV-1 gp160. ACS Chem Biol. 2020;15:2299-2310.

58. Liu D, Tian S, Zhang K, et al. Chimeric antigen receptor (CAR)-modified natural killer cell-based immunotherapy and immunological synapse formation in cancer and HIV. Protein Cell. 2017;8:861-877.

59. Ellebrecht CT, Bhoj VG, Naceet A, et al. Reengineering chimeric antigen receptor T cells for targeted therapy of autoimmune disease. Science. 2016;353:179-184.

60. Tahir A. Is chimeric antigen receptor T-cell therapy the future of autoimmunity management? Cureus. 2018;10:e3407.

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