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REVIEW

Engineered iPSC-derived natural killer cells: recent innovations in translational innate anti-cancer immunotherapy

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Abstract

Natural killer (NK) cells are increasingly recognised as potent tumoricidal agents that can be utilised for cancer immunotherapy. Their innate cytotoxicity against tumor cells, and reduced risk of causing transplantation or toxicity issues in patients, makes them a valuable option for exploration in allogeneic adoptive cell immunotherapies. However, sourcing NK cells from peripheral blood poses challenges in terms of scalability, consistency and variability. Induced pluripotent stem cells (iPSCs) are emerging as a platform to create specific cells with highly controlled processes, allowing for a common cell source for cell therapies and offering a promising inexhaustible source of genetically modifiable NK cells. This review highlights recent developments in the field of generating iPSC-derived NK cells in defined culture systems, and advancements in genetic modification to improve iPSC-NK cell therapy. We further discuss the development of iPSC banks and examine the potential of these cells in next-generation immunotherapies. Finally, we summarise the improvements in cancer targeting, expansion, persistence and cytotoxic functionality of iPSC-derived NK (iNK) cells both *in vitro* and *in vivo*, achieved through genetic modification of iPSCs, as well as recent related clinical trials.

Keywords: adoptive cell therapies, gene editing, NK cells, pluripotent stem cells, tumoricidal immune responses, universal cell sources

INTRODUCTION

Natural killer (NK) cells are innate immune cells, making up approximately 5–10% of the total lymphocytes that are in peripheral blood. As innate cytotoxic lymphocytes, NK cells are essential to host immunity, identifying and eliminating altered cells with reduced expression of MHC class I and increased expression of stress markers that bind activating receptors (such as NKG2D). This process is frequently observed in cancer cells.[1] The immunotherapy field has recognised the unique and advantageous characteristics of NK cells. As a result, allogeneic NK cell therapies and approaches to activate NK cells with various reagents, including antibodies, to enhance their tumor-killing potential have advanced rapidly over the past decade.

Currently, NK cell therapies that are using NK cells from primary sources encounter significant

manufacturing challenges, especially in expansion and storage. NK cells make up only a small percentage of lymphocytes in peripheral blood and cord blood, making it challenging to obtain large cell numbers from these sources. Additionally, the yield and quality of NK cells from leukapheresis products are variable between donors. By contrast, NK cells derived from induced pluripotent stem cells (iPSCs) offer a scalable, off-the-shelf alternative that can potentially overcome these limitations. Evidence suggests that iPSC-NK cells can display *in vitro* cytotoxicity comparable to, or even exceeding, that of primary NK cells derived from peripheral blood mononuclear cells (PBMCs) against numerous tumor cell lines. In this review, we summarise and explore the advancements of iPSC-NK (iNK) cells for immunotherapy, highlighting recent progress in their application for cancer treatment.

DEVELOPMENT OF IPSC

iPSC generation

iPSCs are regarded as a revolutionary breakthrough in regenerative medicine and stem cell research. First introduced in 2006 by Professor Shinya Yamanaka (2012 Nobel Prize) and their team,[2] iPSCs can be derived from somatic cells that are reprogrammed from a stable state to a pluripotent state through the introduction of four stem cell transcription factors that include SRY-box transcription factor 2 (Sox2), Octamer-binding transcription factor 4 (Oct4), Kruppel-like transcription factor 4 (Klf4) and bHLH transcription factor (c-Myc). All together their initials form 'OSKM', and they were named 'Yamanaka factors'.[3–5] The techniques for various reprogramming methods include viral vectors, episomal vectors and mRNA-based methods to introduce the reprogramming factors.[3,6] Furthermore, iPSCs are characterised by the stem cell markers POU class 5 homeobox 1 (Oct3/4), Sox2, Nanog Homeobox (Nanog), T-cell receptor alpha locus (Tra 1–60 and Tra 1–81), stage-specific embryonic antigen-4 (SSEA4) and differentiation ability to the three embryonic germ layers, mesoderm, ectoderm and endoderm.[7] This means that iPSCs possess similar characteristics to embryonic stem cells with the ability to renew and become any cell type in the body. Embryonic stem cell research is limited by specific restrictions and regulations,[8,9] unlike embryonic stem cells, iPSCs are reprogrammed from body cells and do not require access and destruction of embryos, making them a powerful and ethically favorable alternative. The potential of iPSCs is undeniable, as they offer a reliable, renewable and autologous cell source able to differentiate and support the development of human *in vitro* models and cell therapies while avoiding ethical concerns.

iPSC culture systems

There are two defined conditioned culture systems well recognised to maintain iPSCs, which are used by most researchers worldwide. Both culture systems have been used to maintain hundreds of iPSC lines, supporting the expression of key pluripotency markers. These iPSC lines have been cultured for over 50 passages while maintaining a normal karyotype and retaining trilineage differentiation potential into all three germ layer lineages.[10,11] The iPSCs can also be adapted between two culture systems by gradual medium changes. An overview of these two culture systems has been summarised in Table 1.

Differentiation of iPSCs to NK cells

Primary NK cells have made significant strides in immunotherapy, especially for cancer treatment. However, their therapeutic potential has been hindered by challenges in obtaining adequate quantities and consistency from donor sources. The development of iPSCs as an NK cell source provides a transformative solution to these challenges. Published protocols for differentiating iPSCs to NK cells usually include two main steps. Firstly, iPSCs are differentiated to $CD34^+$ haematopoietic stem cell (HSC)-like cells using cytokines and small molecules or by co-culturing with stromal cells that have been irradiated. Secondly, HSC-like cells are then directed to form NK cells through the addition of specific cytokines, such as IL-3 (Interleukin-3), Flt3 (fms-like tyrosine kinase 3), SCF (stem cell factor), IL-7 (Interleukin-7) and IL-15 (Interleukin-15).[12–14] There are several methods to derive NK cells from iPSCs, with unique approaches and optimisation steps. The differentiation methods can be performed in three-dimensional (3D) and two-dimensional (2D) culture systems.[15] However, a concern using $CD34^+$ HSCs from cord blood or iPSCs differentiated in 2D *in vitro* is that they may

Table 1. Comparison of two major culture systems for iPSC cells

Name	Overview	Composition	Advantages	Simplicity	Cell growth	Cell stability	Cost	Flexibility	References
Essential 8 with vitronectin coating	Defined, xeno-free	Chemically defined components with growth factors	Defined formulation, Stable pluripotency, Cost-effective	Simpler formulation	Normal proliferation	Stable	Low	Low	92–95
mTeSR plus with ES-Matrigel coating	Defined, xeno-free, high pluripotency, long-time culture stability	Chemically defined components with higher concentration of growth factors, such as fibroblast growth factor 2 (FGF-2)	Defined formulation, Higher cell growth rate, Improved stress tolerance, Stable and long-term maintenance, Minimal medium changes	Richer formulation	Faster proliferation and better cell survival	Excellent stability in long-time cultures	Higher, but minimal medium changes	High	92,94,96

not consistently produce fully mature NK cells.[12,15,16] Therefore, current methods for iPSC differentiation into NK cells are based on either 3D systems or a combination of 2D and 3D systems. A summary of the primary methods used to derive NK cells from iPSCs is listed in Table 2.

Another key aspect of iPSC-NK (iNK) cells is the differentiation approach that can be controlled, allowing for tailored phenotypes and functionality in the final product. For example, enhancing Wnt signalling through the GSK3b inhibitor CHIR99021 promotes definitive haematopoiesis,[17,18] NK cells developed under these conditions show an increased production of inflammatory cytokines. In contrast, primary fetal NK cells, which are Wnt-independent, exhibit higher cytotoxicity.[19] This flexibility in differentiation supports the potential to generate resident or organ-specific NK cell phenotypes.[20] iNK cells can be further expanded with cytokines including IL-2 and/or IL-15, or by using K562 (human erythroleukaemia cell line) cells engineered to express IL-15, IL-21 (interleukin 21) and 4-1BB ligand.[21,22] These iNK cells exhibit many essential features of primary NK cells, including the expression of key markers of NK cells, killer immunoglobulin-like receptors (KIRs), CD94, CD16, NKG2D, CD56, NKp44 and NKp46, and display strong killing capacity against various solid tumors and haematologic diseases.[23–25] To date, iNK cells represent a promising and highly adaptable platform for immunotherapy, addressing key limitations of donor-derived NK cells by enabling controlled differentiation, tailored phenotypes and scalable production to meet the demands of modern cancer treatments.

iPSC platform for NK cell therapy

iPSCs can potentially differentiate into all cell types, including NK cells, while being expanded rapidly. This makes iPSCs an ideal starting point for producing large amounts of NK cells for therapeutic use. Generation of iPSCs can easily be derived from readily available sources such as human skin and peripheral blood. Once reprogrammed and characterised, these cells can expand robustly *in vitro* while maintaining their pluripotency.[26] Current research focusing on improving efficiency of human iPSC derivation using safer, non-integrating methods is also progressing towards clinical translation.[27] Hence, iPSCs are a good starting point to produce large

Table 2. Summary of current methodologies for differentiating iPSCs into NK cells

Culture system	Methods	Key steps	Cytokines used in NK differentiation	Advantages	Disadvantages	References
2D System	Stromal cell co-culture	iPSCs are co-cultured with stromal cells (S17, OP9 or AFT024), which provide necessary growth factors for haematopoiesis. Over time, haematopoietic progenitors emerge, which are then further cultured in media containing cytokines to promote differentiation to NK cells	IL-15, IL-7, IL-3 and SCF	Use natural growth factors for haematopoietic progenitor production	Variability because of stromal cells Risk of contamination with mouse cells	16,23,30,75
	Monolayer differentiation with defined cytokines	The method skips the stromal cells and relies on a stepwise addition of defined cytokines to guide the differentiation of iPSCs in NK cells	IL-15, IL-7, SCF and Flt3-L	Suitable for GMP-compliant production	Optimising the cytokine cocktail is complex and may require extensive fine-tuning	97
3D System	Feeder-free embryoid body (EB) formation	iPSCs are aggregated to form EBs	IL-15, IL-3, IL-7, SCF and Flt3-L in first week only	More direct and scalable compared to co-culture. Suitable for clinical scale production	Requires careful control of aggregation and differentiation Variable efficiencies	21,28,98–101
	OP9- DLL4 stromal cell co-culture with EB	iPSCs are aggregated for form EBs and are co-cultured with stromal cells (OP9-DLL4) on Day 14	SCF, IL-15, IL-7, SCF and Flt3-L	Increased cytotoxicity toward cancer cell lines compared to feeder-free produced iPSC-NK cells	Risk of contamination with genetic modified stromal cells Long-term feeder condition	102,103
	Organoid formation	EB-free, organoid aggregate method for NK cell generation from iPSCs	Flt3, SCF, EGF, BMP4, IGF-1, TPO, VEGF, FGF for LPM based differentiation	Lateral plate mesoderm (LPM) cell -based iNK differentiation, higher iNK production	Risk of contamination with feeder cells Complex reagents (small molecules and cytokines) for cell differentiation	104

BMP4, bone morphogenetic protein 4; EGF, epidermal growth factor; FGF, fibroblast growth factors; IGF-1, insulin-like growth factor 1; TPO, thrombopoietin; VEGF, vascular endothelial growth factor.

NK cell numbers for cell immunotherapy.[21] Something to consider is how iNK cells compare to primary NK cells regarding receptor expression and functional potential. Although there is potential to further refine protocols for iNK cell differentiation, current studies suggest that iPSC-NK cells are highly functional.[21,28,29] iNK cells have been shown to effectively kill target cells regardless of HLA expression levels, such as the cancer cell lines K562, SKOV-3 (human ovarian cancer cell line) and SW480 (human colon cancer cell line). Furthermore, they demonstrate higher cytotoxicity versus primary NK cells against cancer cell lines, except against K562, where they exhibit similar killing efficiency.[30] Similarly, *in vivo* studies have shown that iNK cells exhibit enhanced cytotoxicity against ovarian cell lines (MA148 and A1847) compared to peripheral blood-NK (PB-NK) cells.[29] By banking iPSC lines that have been genetically engineered, it is possible to create 'off-the-shelf' NK immunotherapies. These therapies could be rapidly given to patients, by passing the need for patient-specific cell sourcing and manufacturing.

Genetic engineering of iNK cells

iNK cells have become a favorable option for immunotherapy because of their renewable source, standardisable production and the potential for allogeneic use. Strategies involving genetically engineering cells aim to further improve functional properties of iNK cells for improved therapeutic efficacy.[31] The discovery of genetically modified iPSCs has opened new opportunities for human-specific drug screening and enhancement of iNK cell cytotoxicity in immunotherapy and cancer research. The high proliferation capacity of iPSCs allows them to be genetically engineered for an 'off-the-shelf' iNK cell bank for treating various cancers.[3] However, assessing genome-wide off-target effects when multiple transgenes are introduced into NK cells remains a significant challenge. Using engineered iPSCs to derive NK cells allows efficient addition of multiple genetic modifications and the identification of unwanted genomic alterations by sequencing which can be used to enhance NK cell killing capacity.[32–35] Techniques such as lentiviral transduction and transposon systems enable the effective addition of transgenes with stable expression. For precise gene editing, TALENs and CRISPR/Cas9 are valuable tools for targeted gene insertion or deletion. Additionally, technology involving zinc finger nuclease (ZFN) is used to insert chimeric antigen receptor (CAR) genes into the safe harbour loci such as the adeno-associated virus integration site 1 (AAVS1), providing controlled copy number and robust expression in iPSCs. Once engineered, these genetically modified, undifferentiated iPSCs can be frozen and stored, supporting the subsequent production of NK cells with uniform phenotypes.

Strategies to enhance iNK cell effector functions

Genetic engineering allows iPSCs to be modified to improve the function of the iPSC-derived NK cells. For example, researchers can enhance NK cell cytotoxicity or increase their persistence in the body through targeted gene insertion or deletion. A notable example is the knockout (KO) of cytokine-inducible SH2-containing protein (CISH), a negative regulator of IL-15 signalling. CISH-knockout (CISH-KO) has been developed using an iPSC-derived NK cell platform. Engineered CISH-KO iNK cells exhibit enhanced Janus kinase-signal transducer and activator of transcription (JAK–STAT) signalling mediated by IL-15, leading to increased cell proliferation and cytotoxic activity. In a leukaemia xenograft model, these engineered iNK cells demonstrated prolonged *in vivo* persistence and significantly improved inhibition of tumor progression.[36] Similarly, the deletion of the inhibitory receptor NKG2A was also recently employed, generating iNK cells with higher cytotoxicity against HLA-E-expressing glioblastoma or other leukaemia cells.[37] In addition, a key effector mechanism of NK cells is through antibody-dependent cellular cytotoxicity (ADCC), facilitated through the NK cell Fc receptor CD16a, which binds the Fc portion of IgG antibodies (Abs). Knock-in (KI) of the high-affinity noncleavable variant of CD16a (hnCD16) into iNK cells combined with monoclonal antibodies (mAbs) administration are therapeutic against solid tumors and haematologic diseases.[34,38,39] Other examples of genome editing to improve NK functions include HLA-E KI and EGFR KI to iPSC-NK cells,[40] a triple-gene (hnCD16a KI, IL-15/IL-15R KI and CD38 KO-edited iPSC-NK cells),[41] and an antibody-armed iPSC-NK expressing Fc receptors such as CD64 or CD16A,[32] which allow these cells to also perform ADCC.

CAR construct integration

NK cells that specifically target tumor antigens can be generated by inserting CAR constructs into iPSCs, like CAR-T cells. However, current CAR constructs are not optimised for NK cell signalling as they are designed for T cells. Li, Ye *et al*. tested nine CAR constructs designed for NK cell activity in the NK cell line, NK-92, to test their ability to kill mesothelin positive (meso^{+}) cells. The results show that NK-92 cells expressing CAR4, CAR7 and CAR9 exhibited the greatest cytotoxicity against meso^{high} targets.[28] Additionally, a novel immuno-engineering approach has been reported showing that iPSCs can be engineered with dual CAR constructs, consisting of an anti-PD-L1 CAR and an anti-fluorescein (FITC) scFv CAR.[33] These engineered iNK cells possess immunological memory for PD-L1, which can be highly expressed on cancer cells, enhancing their immunotherapeutic efficacy which is further enhanced by administering a bispecific adaptor for FITC-folate. This allows for the programmable anti-FITC CAR to bridge with breast cancer cells expressing folate receptor alpha. Results showed that the iPSC-CAR-NK cells with dual CAR demonstrated significantly improved anti-tumor activity.[33] iPSC-CAR-NK cells have been shown to exhibit a memory-like phenotype and demonstrate enhanced universality, safety, potency and persistence in an antigen-dependent manner.[14,28,42,43]

Evasion of immune suppression

Another popular focus of genetic engineering of NK cells is modifying these cells to evade immune suppression by tumors. This includes knocking out inhibitory receptors or modifying NK cells to resist the immunosuppressive tumor microenvironment.[44] NK cells are susceptible to dysfunction within the glioblastoma microenvironment (GBM), despite their ability to eliminate foreign targets.[45,46] In solid tumors such as GBM, the T-cell immunoreceptor with Ig and ITIM domains (TIGIT) and CD155, glioblastoma-associated antigen, form a highly immunosuppressive complex. The TIGIT-CD155 complex can be taken over by the activation of SynNotch signalling. In this study, SynNotch engineered iNK cells have been shown to mediate anti-GBM responses with respect to TIGIT/CD155 and CD73 co-targeting, representing a potent allogeneic treatment for this hard-to-cure brain cancer.[35] The liver tumor microenvironment contains a high expression of transforming growth factor-beta (TGF-β) that is known to inhibit NK cell anti-mediated immunity.[47–51] Thangaraj *et al*. have recently developed iNK cells with either expression of a dominant negative TGFBR2 combined with a CAR construct targeting either Glypican-3 (GPC3) or Alpha-Fetoprotein (AFP) or TGF-β receptor 2 (TGFβR2) KO. The results show improved anti-HCC activity and resistance to TGF-β inhibition by TGFβR2-dominant negative (TGFβR2-DN) and TGFβR2-KO iNK cells. However, iNK cells expressing anti-HCC_CARs require a TGF-β inhibitor for effective anti-HCC activity.[52] Figure 1 exemplifies approaches used to engineer iNK cells against immunoevasion strategies in tumors.

IPSC BIOBANKS

Master iPSC banks

iPSCs are self-renewing and proliferate rapidly, allowing for the creation of cell banks at multiple stages—reprogrammed as a parental cell bank, or engineered as a genetically modified cell bank for the direct differentiation of therapeutic cells. This process is convenient for developing off-the-shelf cell therapies. The creation of iPSC and engineered iPSC banks is a critical step in developing off-the-shelf cell therapies, which would enable pre-engineered NK cells to be readily available for clinical use. iPSC banks address several key issues, including cell availability, standardisation and affordability.[53] This enables the pre-manufacture of therapeutic doses of iNK cells to be used in various patients, enabling broader and more accessible treatment options.

Since the first report of iPSCs,[54] the quantity of iPSC research and the number of iPSC lines has increased rapidly. The self-renewable capacity of iPSCs makes them ideal for selection of cells with genetic modifications and establishing cell banks. The European Bank for iPSCs (EBiSC) is a non-profit for the banking, storage, quality control and distribution of iPSC lines that are research-grade and have been generated across 35 disease areas, making these lines available to researchers.[55] Other major iPSC banks worldwide are summarised in Table 3. These stem cell banks and registries offer essential data for both fundamental research and clinical applications. Furthermore, as technologies for cell line

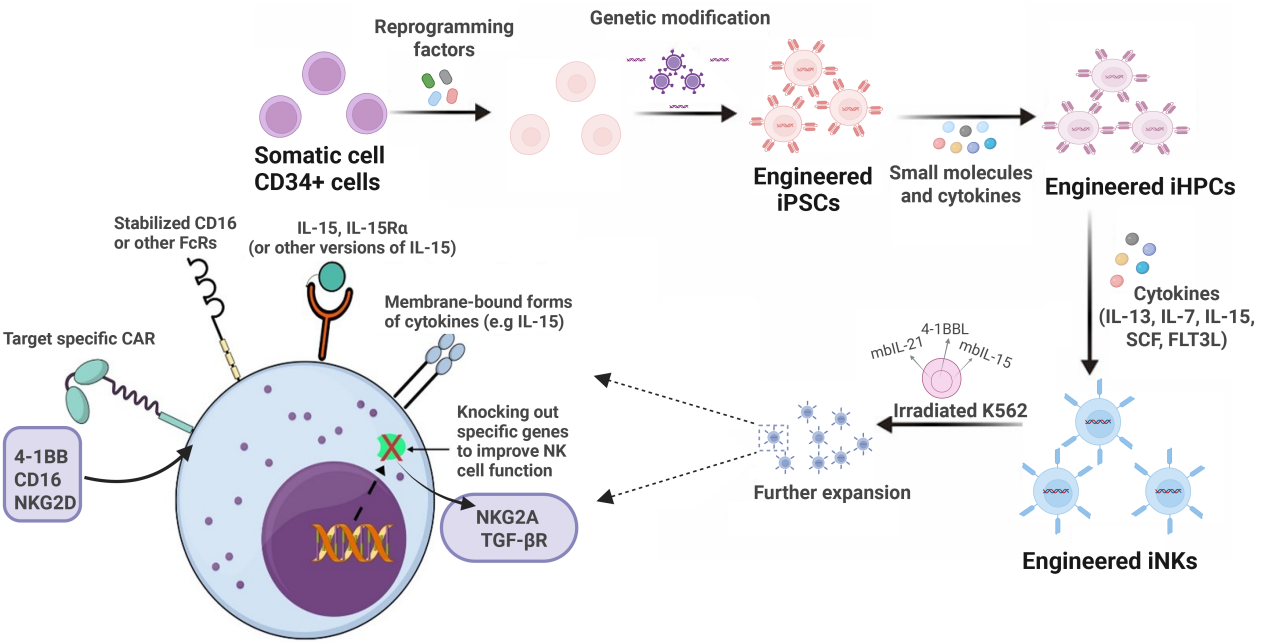

Figure 1. Schematic representation of genetic enhancements in induced pluripotent stem cells (iPSC)-derived natural killer (NK) cells. Somatic cells are reprogrammed into iPSCs, which are then genetically engineered to produce NK cells with enhanced therapeutic functions. Large-scale production of these engineered iPSC-derived NK (iNK) cells is facilitated by feeder cell-based systems, such as irradiated K562 cells expressing membrane-bound IL-15 (mbIL-15), mbIL-21 and 4-1BBL, along with other bioproduction strategies. Modifications to iNK cells include the introduction of target-specific chimeric antigen receptors (CARs), stabilised Fc receptors (e.g. CD16) and survival/persistence transgenes (e.g. IL-15/IL-15Rα complex or mbIL-15). Further optimisation is achieved through the knockout of inhibitory checkpoint molecules such as CISH and NKG2A, enhancing cytotoxic activity and persistence. The figure was created with biorender.com.

characterisation move forward, the inclusion of new quality control has led to increasingly more complex and varied data sets across registries and stem cell banks.[53] These banked cells are playing prominent roles in bringing more relevant cell models to the laboratory. Development of iPSC-NK cells from a clonal master iPSC line from cell banks allows for the mass production of iNK cells which are more consistent, with increased quality standards, and the ability to be cryopreserved for long-term storage.[56] A novel haplobanking approach for iPSCs can be utilised, in which cells are separated and stored according to different HLA haplotypes, enabling the production of disease-specific, patient-specific and immune-matched allogeneic cell therapies.[57,58] iPSC banking also ensures that the quality and functional characteristics of iPSC, engineered iPSC and iNK cells remain consistent across different batches, making it easier to scale production and meet regulatory standards.

The clinical application of iPSCs is accompanied by several critical safety concerns that must be addressed in the establishment and maintenance of iPSC banks. Key risk factors included as follows. (1) *Genomic instability*: During both the reprogramming of somatic cells into iPSCs and their prolonged *in vitro* expansion, cells may acquire genetic and epigenetic alterations, including chromosomal aberrations, copy number variations and point mutations. These changes can negatively impact the safety, differentiation potential and increase tumorigenic risk of derived cellular products.[59] To mitigate these risks, non-integrating reprogramming methods such as episomal vectors, Sendai virus (SeV) and mRNA have been developed. Schlaeger *et al*. evaluated these methodologies and their implications for genomic stability. The Cytotune®-SeV reprogramming kit has demonstrated high efficiency in reprogramming skin fibroblasts and blood cells and is widely recommended for use in research laboratories.[60] The cGMP-grade CTS™ Cytotune®-SeV reprogramming kit is currently being utilised in clinical and translational research.[61,62] Notably, routine genomic screening,

Table 3. List of the major worldwide banks for iPSCs

Bank	Location	Number of iPSC lines
California Institute for Regenerative Medicine (CIRM)	USA	1556
Coriell Institute for Medical Research (Coriell)	USA	91
Fujifilm Cellular Dynamics International (FCDI)	USA	N/A
Center for iPS Cell Research and Application (CiRA)	Japan	22
European Bank for Induced Pluripotent Stem Cells (EBiSC)	UK, Germany	897
Human Induced Pluripotent Stem Cell Initiative (HipSci)	UK	835
Human Disease iPSC Consortium Resource Center (Taiwan Human Disease iPSC Consortium)	Taiwan	102
Institute of Physical and Chemical Research (RIKEN)	Japan	4102
Korean National Stem Cell Bank (KSCB)	Korea	147
WiCell Research Institute (WiCell)	USA	1519
Harvard Stem Cell Institute	USA	41
Eagle-i	USA	2415
NINDS Human Cell and Data Repository	USA	162
The New York Stem Cell Foundation (NYSCF)	USA	111
The Stem Cell Bank of Barcelona (BLCB)	Spain	176
Japanese Collection of Research Bioresources (JCRB)	Japan	31

iPSC numbers were retrieved from Huang *et al.*[105] and Chen *et al.*[106] and company websites.
N/A, information not publicly available.

such as karyotyping and whole-genome sequencing is essential to select genetically stable iPSC clones. Additionally, limiting passage numbers and maintaining cells under optimised culture conditions helps reduce the accumulation of mutations. (2) *Teratoma formation*: Because of their pluripotency, iPSCs can form teratomas if undifferentiated cells persist in the final therapeutic product. This risk can be minimised through the use of efficient differentiation protocols, purification techniques and rigorous quality control assays (e.g. teratoma formation assay)[63]. (3) *Mutations from genetic engineering*: Gene editing technologies like CRISPR/Cas9 are commonly used to enhance iPSC-derived cell therapies. However, such techniques carry risks, including off-target mutations, insertional mutagenesis and unintended functional changes.[64] These risks can be reduced by targeting 'safe harbor loci' (e.g. AAVS1) for gene insertion, using inducible systems to regulate gene expression and performing comprehensive off-target analyses with next-generation sequencing.[65] Furthermore, functional and genomic validation of engineered iPSC clones prior to large-scale production is critical. While iPSC offers significant promise for regenerative medicine, addressing the key risks is essential for ensuring the safety, efficacy and regulatory approval of iPSC-derived therapeutics.

Banking of iNK cells for clinical cell therapy

There are only a few therapeutic companies worldwide using iPSC-NK cells for preclinical and clinical cell therapy (e.g. Fate Therapeutics), as exemplified in Table 4. These companies maintain their own master banks of iPSCs, engineered iPSCs and iPSC-NK cells exclusively for clinical use, rather than for public research. The information regarding these cell banks is considered proprietary. These companies implement rigorous quality control and standardisation processes in accordance with the Food and Drug Administration (FDA) or European Medicines Agency (EMA) guidelines[66] for their cell banks. All clinical products are manufactured in GMP-certified facilities.

Comparison of NK cell sources

NK cells utilised for research and clinical applications can be broadly categorised into primary sources [PB-NK, Umbilical Cord Blood (UCB)-NK], cell line sources (NK-92) and engineered or derived sources [iPSC-NK, Cord blood $CD34^+$ HSC-derived NK cells (HSC-NK)]. A comparative summary of these sources is presented in Table 5.

PB-NK cells are considered a standard source, characterised by variable expression of killer immunoglobulin-like receptors (KIRs), high expression of CD16 and low expression of NKG2A. In contrast, UCB-NK cells represent a more immature population, typically exhibiting lower expression of CD16 and KIRs, and higher levels of NKG2A. UCB-NK cells are more amenable to expansion than PB-NK cells but require additional time to reach full functional maturation. A key limitation of both primary NK cell sources is that their efficacy is highly donor-dependent, and they cannot be derived from a single, renewable source. Additionally, primary NK cells are inherently resistant to genetic modification, which

Table 4. Therapeutic companies using iPSC-NK for clinical immunotherapy

Company name	Location	Website	References
Fate Therapeutics	USA	https://www.fatetherapeutics.com/	107
Cytovia Therapeutics	England	https://www.cytoviatx.com/ink-carink	108
Century Therapeutics	USA	https://www.centurytx.com/	66,109
Shoreline Biosciences	USA	https://shorelinebio.com/	110
HebeCell	USA	https://hebecellcorp.com/	N/A
Cartherics	Australia	https://cartherics.com/technology/	N/A
Nuwacell	China	https://en.nuwacell.com/	N/A

Table 5. Comparison of NK cell sources

Source	Receptor repertoire (KIRs, NKG2A, CD16)	Functional maturity	Licensing/ education	Cytotoxicity	Clinical persistence	Translational potential	Comments
PB-NK	Mature repertoire: variable KIRs, high CD16, moderate NKG2A	Fully mature	Licensed (based on self-HLA)	High (based on high purity)	Moderate	High	Standard source, donor variability affects consistency
UCB-NK	Immature: low KIRs, high NKG2A, moderate CD16	Immature	Poorly licensed	Low –moderate	Short-lived	Moderate	Easier to collect, high expansion potential
iPSC-NK	Engineered or variable, often designed for high CD16	Varies by protocol	Can be enhanced by design	Moderate –high	Engineered for persistence	Very high	Ideal for off-the-shelf, engineered therapies
NK Cell Lines (e.g. NK-92)	Limited: lacks CD16, lacks KIRs, expresses NKG2A	Immortalised, semi-mature	Non-licensed	Moderate	Minimal (irradiated before use)	Limited (non-persistent)	Easy to expand, used mainly in preclinical studies
$CD34^+$ HSC-derived NK	Developing: low NKG2A, low KIR/ CD16	Intermediate	Incomplete	Moderate	Short–moderate	Moderate –high	Requires extensive culture and maturation

poses a challenge for enhancing their anti-tumor activity.[67–70]

The NK-92 cell line, originally derived from a patient with non-Hodgkin lymphoma, offers a consistent and modifiable platform that circumvents several challenges associated with primary NK cells. NK-92 cells are highly amenable to genetic engineering, including the introduction of CARs or CD16 to augment cytotoxic potential, and they can be expanded efficiently *in vitro*. Nevertheless, their clinical utility is limited by poor *in vivo* persistence. To mitigate the risk of tumorigenicity, NK-92 cells must be irradiated prior to administration, a requirement that significantly diminishes their anti-tumor efficacy.[71]

Engineered NK cells derived from iPSCs and HSCs offer renewable, standardised, off-the-shelf sources for cell therapy. iPSC-derived NK cells provide a versatile platform for genetic engineering, enabling the generation of NK cells with enhanced anti-tumor activity and improved *in vivo* persistence.[23,38,72] HSC-NK cells have demonstrated a stronger cytotoxic function than PB-NK cells and hold significant potential for NK cell-based therapies.[67] Overall, engineered iPSC-NK cells represent an ideal source for off-the-shelf NK cell immunotherapy, combining unlimited availability with enhanced functionality.

CLINICAL APPLICATIONS

CAR-NK cells derived from iPSCs are being explored as a potential alternative to CAR-T therapies since they are less toxic and have fewer complications in patients because of the lack of graft-versus-host disease (GvHD).[73] Beyond cancer treatment, there is potential for using genetically modified iNK cells for treating viral infections, since NK cells can recognise and destroy virus-infected cells, most notably Human

Table 6. Clinical trials of iPSC-NK cells

Trial identifier	Phase	Product	Disease settings	Stage	Sponsor
NCT03841110	1	Non-transduced iPSC-derived NK cells (FT500) with checkpoint blockade	Advanced solid cancers	Completed	Fate Therapeutics
NCT04363346	1	FT516 (hnCD16) iPSC-derived NK cells	COVID-19	Completed	Masonic Cancer Center, University of Minnesota
NCT04023071	1	FT516 (hnCD16) iPSC-derived NK cells with obinutuzumab	AML, B-cell lymphoma	Terminated	Fate Therapeutics
NCT04630769	1	FT516 (hnCD16) iPSC-derived NK cells with enoblituzumab and IL-2	Ovarian cancer	Completed	Masonic Cancer Center, University of Minnesota
NCT04551885	1	FT516 (hnCD16) iPSC-derived NK cells with avelumab	Advanced solid cancers	Terminated	Fate Therapeutics
NCT04714372	1	FT538 (hnCD16/*CD38*KO/IL-15RF) iPSC-derived NK cells with daratumumab	AML	Completed	Masonic Cancer Center, University of Minnesota
NCT05069935	1	FT538 (hnCD16/*CD38*KO/IL-15RF) iPSC-derived NK cells with monoclonal antibodies	Advanced solid cancers	Terminated	Fate Therapeutics
NCT04614636	1	FT538 (hnCD16/*CD38*KO/IL-15RF) iPSC-derived NK cells with daratumumab or elotuzumab	AML, multiple myeloma	Terminated	Fate Therapeutics
NCT04555811	1	FT596 (hnCD16/anti-CD19 CAR/IL-15RF) iPSC-derived NK cells with rituximab	NHL, diffuse large B-cell lymphoma, high-grade B-cell lymphoma	Completed	Masonic Cancer Center, University of Minnesota
NCT04245722	1	FT596 (hnCD16/anti-CD19 CAR/IL-15RF) iPSC-derived NK cells with rituximab or obinutuzumab	B-cell lymphoma, CLL	Completed	Fate Therapeutics
NCT05395052	1	FT536 (hnCD16/*CD38*KO/anti-MICA/B CAR/IL-15RF) iPSC-derived NK cells with monoclonal antibodies	Advanced solid cancers	Terminated	Fate Therapeutics
NCT05182073	1	FT576 (IL-15RF/*CD38*KO/anti-BCMA CAR) iPSC-derived NK cells with daratumumab	Multiple myeloma	Active, not recruiting	Fate Therapeutics
NCT05336409	1	CNTY-101 (sIL15/HLA-IKO/HLA-IIKO/HLA-EKI/EGFR switch CAR) iPSC-derived NK cells with anti-CD19	B-cell malignancies	Recruiting	Century Therapeutics, Inc.
NCT06255028	1	CNTY-101, CD19 targeted CAR iPSC derived NK cells	B-cell autoimmune diseases	Recruiting	Century Therapeutics, Inc.
NCT06027853	1	CLL1KI/Anti-CLL1, iPSC-derived NK cells with anti-CLL1	Acute myeloid leukaemia	Recruiting	Zhejiang University
NCT06367673	1	CLL1 or CD33 KI, iPSC-derived NK cells with anti-CLL1 or CD33	Acute myeloid leukaemia	Recruiting	Zhejiang University
NCT06245018	1	iPSC-derived NK cells	Solid cancers	Active, not yet recruiting	Nuwacell Biotechnologies Co., Ltd

ALL, acute lymphoblastic leukaemia; CLL, chronic lymphocytic leukaemia; NCT, National Clinical Trial number; NHL, non-Hodgkin lymphoma.

immunodeficiency virus (HIV).[74–76] Ni *et al.*[75] elegantly reported that iPSC-NK cells inhibited the infection of CEM-GFP cells with HIV-1 NL4-3 by lysing infected target cells through ADCC and the release of important cytokines and chemokines. iPSC-derived NK cells are showing great promise for the treatment of haematologic cancers, such as lymphoma and leukaemia.[77] While solid cancers are more challenging because of the suppressive tumor microenvironment, engineered iNK cells can potentially overcome these barriers with proper genetic modifications. Clinical trials are underway to determine the efficacy and safety of this approach in a variety of cancers (Table 6). Several clinical trials have been initiated to evaluate the safety and efficacy of iPSC-NK cell therapies in patients with haematologic malignancies and solid tumors. Notably, FT500 developed by The University of Texas and Fate Therapeutics was the first iPSC-derived NK cell

therapy to enter clinical trials, targeting advanced solid tumors. Preliminary results from phase I studies demonstrated that FT500_NCT03841110 was well tolerated, with no dose-limiting toxicities or evidence of GvHD, underscoring the inherent safety of allogeneic iPSC-NK cell therapy.[78] FT596 is a first-in-class, iPSC-derived NK cell therapy engineered with three anti-tumor modalities: a CD19-targeted CAR, hnCD16 Fc receptor and an IL-15 receptor fusion to promote persistence and proliferation. The Phase I trial investigated FT596_NCT04245722 as monotherapy and in combination with rituximab in patients with relapsed/refractory B-cell lymphoma. Primary objectives included evaluating safety, tolerability and determining the maximum tolerated dose. Secondary endpoints focused on efficacy measures such as overall response rate and duration of response. The trial demonstrated that FT596 was well tolerated, with no dose-limiting toxicities observed. Notably, FT596 induced deep and durable responses, including complete remissions, in a subset of patients, highlighting its potential as a potent off-the-shelf therapy for B-cell malignancies.[79] Additionally, other iPSC-NK products engineered with CARs (e.g. FT576_NCT05182073 and CNTY-101_NCT05336409) are recruiting and undergoing evaluation for multiple myeloma, B-cell malignancies/autoimmune diseases.

CHALLENGES AND FUTURE DIRECTIONS

Currently, the 'off-the-shelf' potential iNK cells and their capacity to produce sufficient numbers required for infusion into multiple patients remains a biomanufacturing challenge to be optimised and proven feasible. Efficient differentiation protocols and bioreactor technologies are continuously being explored by companies and academic researchers to improve the scalability of iPSC-NK cell products. Furthermore, genetic modifications of iPSCs can potentially introduce mutations, off-target effects or other unwanted effects.[80–83] Site-specific integration of transgenes into 'safe harbour' sites in the genome using advanced gene technologies,[84–86] or the use of a safety switch such as dox-inducible[87,88] can aid in mitigating these risks. Additionally, comprehensive preclinical studies and thorough safety evaluations are essential before iPSC-based therapies can advance to clinical trials.[14,89–91] To be used in cell therapy, engineered iNK cells need to be generated using a manufacturing process that is robust, reproducible, and with current good manufacturing practice (cGMP) conditions. Stringent testing and quality control are essential to ensure that engineered NK cells are safe.[14] Collaboration is required among researchers, policymaker and regulators to overcome these challenges and to realise the full potential of iPSC-NK cells for cell therapy. In some approaches, the autonomous persistence of iPSC-NK cells using constructs such as IL-15RF obviates the need for cytokine dosing *in vivo* to sustain NK proliferation/survivor, which can significantly reduce treatment costs and avoids unwanted immunoregulatory responses in patients.[41] However, the manufacture of engineered iNK cells is still highly costly, though advances in automation and process optimisation being considered for cost reduction.

CONCLUSION

In summary, this review presents an optimistic outlook on the future of iPSC-derived NK cells in immunotherapy, as they hold immense promise for revolutionising cancer immunotherapy. The ability to engineer and bank iPSCs provides a scalable, flexible and potentially more effective approach for treating various malignancies. Although challenges such as scalability, safety and cost remain, ongoing research and clinical trials suggest that this approach may lead to the development of a new class of 'off-the-shelf' cell therapies. By harnessing the power of genetic engineering and the versatility of iPSCs, there is significant potential to advance NK cell-based therapies, offering hope for improved cancer treatment outcomes and overcoming the current challenges presented by other NK cell sources.

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AUTHOR CONTRIBUTIONS

Jane Sun: Writing – original draft; writing – review and editing. **Melissa Elliott:** Writing – review and editing. **Fernando Souza-Fonseca-Guimaraes:** Supervision; funding acquisition; project administration; writing – original draft; writing – review and editing.

CONFLICT OF INTEREST

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