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CONCISE REVIEW

STEM CELLS TRANSLATIONAL MEDICINE

Hematopoietic stem cell- and induced pluripotent stem cell-derived CAR-NK cells as reliable cell-based therapy solutions

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Abstract

Hematopoietic stem cell- (HSC) and induced pluripotent stem (iPS) cell-derived natural killer (NK) cells containing engineered functions, such as chimeric antigen receptors (CAR), offer great promise for the treatment of seemingly incurable oncological malignancies. Today, some of the main challenges of CAR cell-based therapeutics are the long manufacturing time and safety of the cell sources used. Additional challenges include avoiding graft vs host disease (GVHD) and cytokine release syndrome (CRS). Here, we show compelling evidence for the use of NK cell therapeutics as a reliable off-the-shelf option, as they address key issues. Furthermore, we highlight how iPS cells and directed differentiation toward HSC and NK cells address industrial scalability and safety.

KEYWORDS
CAR-NK, chimeric antigen receptor (CAR), hematopoietic stem cell (HSC), induced pluripotent stem (iPS) cell, natural killer cells (NK)

1 | INTRODUCTION

Over the last decade, immunotherapy has emerged as one of the areas with the highest pharmaceutical growth potential. It presents increasing opportunities for the treatment of malignancies, whose outcome is limited with traditional radiation or chemotherapy. Among those immunotherapies, cell-based therapeutics are particularly attractive for the remarkable possibility of programming various effector functions to personalize the treatment and even develop autologous therapeutic alternatives with reduced or nonexistent adverse effects. Cell-based immunotherapy has seen a transition from unmodified immune cells or cell lines toward cells expressing novel functions. Instrumental to such developments have been the advent of chimeric antigen receptor (CAR), as it engages an immune cell with a particular cancer cell surface marker. CAR proteins are composed of two main components: First, an antigen recognizing section, mainly a single chain variable fragment (scFv), composed of the fusion of an antibody heavy chain variable fragment (VH) and a light chain variable fragment (VL). Second, endodomains that transform the binding signal from the surface into a signaling cascade that ultimately activates the lytic properties of effector cells toward cancer cells. Traditionally, CAR have been expressed in T cells through viral integration systems, mainly for

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their abundance in peripheral blood and for the simplicity of said transgene integration technology.

Although natural killer (NK) cells have been explored as a therapeutic alternative in clinical settings, mainly NK cell lines such as NK92 and allogeneic NK cell transplantations, their use with CAR engineered functions has gained traction only recently. NK cells are part of the innate immune system and exert their functions against virally infected cells and cancer cells. Today, there are a multitude of NK cell-based clinical trials that use a variety of NK cell sources, including immortalized human NK cells, autologous patient-derived NK cells, allogeneic healthy donor NK cells, allogeneic hematopoietic stem cell (HSC)-derived NK cells, and recently allogeneic induced pluripotent stem (iPS) cell-derived NK cells. iPS cells have the capacity to derive all adult tissues, including immune cells such as HSC, T cells, and NK cells. Currently, there is an assortment of differentiation protocols to derive immune effector cells from iPS cells in a preclinical research setting. However, robust xeno-free and defined protocols to differentiate T and NK cells have only recently been achieved.[1] One of the main intermediary stages in the differentiation of iPS cells toward effector immune cells is the HSC stage. HSC are responsible for the generation of all blood cells in adults, including innate and adaptive immune cells. Conventional sources of HSC include adult bone marrow and umbilical cord of newborns. As the state-of-the-art expansion protocols for adult or umbilical cord HSC are prone to exhaustion and differentiation, protocols that maintain pluripotent properties while providing high expansion yields are pivotal for the use of HSC in immunotherapy. Increasing efforts exist to develop defined and xeno-free expansion media for HSC.[2] Alternative or complementary approaches to achieve a high number of HSC include the use of iPS cells, which have a high scalability because of the robustness of iPS cell culture conditions.[3,4]

In this review, we present how the usage of iPS cells or HSC master stocks and their stepwise differentiation toward NK cells represent the most promising strategy in the current technological context of CAR cell-based immunotherapies. We present the current challenges for the main components and prospective technological solutions.

Significance statement

Cell-based therapeutics, particularly hematopoietic stem cell (HSC) and induced pluripotent stem (iPS) cell-derived natural killer (NK) cells, constitute an ideal programmable vehicle for emerging therapeutic functions. NK cells with cancer cell-engaging proteins as chimeric antigen receptor (CAR) are prone to revolutionize immunotherapy. This is due to intrinsic advantages of NK cells over T cells, which include (a) wider immune activating pathways and cytotoxicity mechanisms, (b) allogeneic and autologous effector functions independent of MHC-presentation through "missing-self" activation, and (c) innate activity without the need for antigen priming. Hence, CAR-NK cells with safer cell sources and genetic engineering approaches can solve many of the challenges faced by conventional CAR-T-based therapeutics.

2 | SOURCE OF THE THERAPEUTIC CELLS

Conventional CAR cell therapy relies on the usage of autologous patient T lymphocytes for the expression of engineered functions.[5,6] When considering the cell source for therapeutic applications, the use of autologous cells presents various challenges. A first challenge is that not all patients are capable of effectively mobilizing functional cells in numbers suitable for the therapeutic process,[7-9] especially those who have relapsed and are refractory following cycles of traditional treatment. This limitation is common to any type of mature effector cell, including T and NK cells. A second challenge is that patient-derived immune cells are normally accompanied with a certain degree of functional impairment, senescence, or exhaustion, which leads to a reduction in the functionality of products derived from them. A third challenge is that the use of patient autologous cells in the current practice setting is time intensive and hence excludes patients with time constraints; therefore, it is not accessible to everyone. The exception to this corresponds to either autologous or allogeneic HSC obtained from stored bone marrow or stored umbilical cord blood. However, the use of autologous bone marrow poses the risk of contamination with cancer cells, particularly in hematological malignancies. This risk is absent in stored autologous umbilical cord blood tissues, as the onset of the pathology is both chronologically and anatomically distant. Recent reports have highlighted the risks associated with the use of autologous cell sources for CAR manufacturing. It is now accepted that cancer cells can be unintentionally copurified with therapeutic T cells in the context of hematological malignancies.[10] Unintended copurification has resulted in CAR-containing cancer cells with both self-activating and self-masking of the antigen, hindering recognition of effector immune cells.[10] Such observations should drive us to pursue prevalidated master stocks as the therapeutic cell source for either autologous or allogeneic options. Such prevalidated master stocks should be either iPS cells or HSC, as they possess unlimited expansion capacity and can bypass the unavailability of autologous cell material. As opposed to patient-originated mutations in the cell source, one could prevent the risk of in vitro-generated mutations in off-the-shelf settings by continuously controlling for genetic instability, particularly of known in vitro-induced mutations.[11] HSC derived from cord blood or bone marrow are currently being evaluated to manufacture CAR-HSC, which can in turn be differentiated into effector cells, including CAR-T and CAR-NK cells. However, a major challenge faced by the usage of HSC cell sources is the limited expansion capacity of primary pools while preserving their stemness properties. The generation of novel approaches to expand undifferentiated pools of HSC is a promising gateway to foster the usage of HSC. Recent studies have pursued the expansion of HSC in

defined culture conditions.[2] Optimally, iPS cells and HSC sources could be derived, expanded and stored ahead of therapeutic need, thus allowing additional validation and quality control steps. Furthermore, human iPS cells, and recently HSC, can be subjected to several iterations of genome editing for patient-tailored CAR therapies, providing unparallel advantages to autologous effector cells. The expansion yield of human iPS cells has been greatly enhanced with the emergence of defined extracellular matrices such as vitronectin,[3] laminin 521,[4,12] and laminin 511.[13] These matrices enabled the transition of an otherwise complex culture system to automated approaches comparable to standard cell line culture systems. Furthermore, the usage of coated microcarriers[14] or hollow fiber[15] reactors has allowed expansion of up to 20-fold and yields of up to 7×10^9 cells in closed automated culture systems. Such expansion capacity facilitates the initiation of HSC and NK differentiation protocols. The differentiation of HSC from iPS cells has reported efficiencies of up to 19% CD45 and CD34 double-positive hematopoietic progenitors.[16] Similarly, NK differentiation has reported efficiencies of up to 72% in defined culture conditions.[1] In parallel, conventional expansion methods of primary HSC are reported to reach up to 899-fold increase,[2] while the differentiation of cord blood HSC-derived NK cells is reported to reach 90% purity.[17]

3 | TYPE OF THERAPEUTIC IMMUNE CELL

On the other hand, when considering the identity of effector therapeutic cells, there are currently two main alternatives: T cells or NK cells. Some of the drawbacks associated with CAR-T cells include cytokine release syndrome (CRS),[18-20] immune effector cell-associated neurotoxicity syndrome (ICANS),[21,22] and composed burden of organ toxicities.[23] The composition of autologous T-cell lymphocytes obtained by leukapheresis varies greatly from patient to patient and from patients to healthy individuals.[8] This results in a non-homogeneous starting material for CAR-T manufacturing, as T cells are not selected for a beneficial ratio of CD8 to CD4 positive cells,[22] which seems to have an important role in the therapeutic outcome of CAR-T cells. The use of prestored autologous or superdonor allogeneic iPS cell-derived T cells could solve the issues of heterogeneity, as the ratio of CD8 and CD4 positive cells can be tuned during manufacturing.[24] Additionally, the use of T-cell receptor (TCR) and MHC-matched superdonor collections as starting material for differentiation enable the manufacturing of a set of master pools that can cover a large fraction of the population and provide immune compatibility.[25,26] Engineering CAR on iPS cells prior to their differentiation toward any immune cell, including T and NK cells, would then avoid the need to collect and genetically modify heterogenous autologous patient cells, which might have underlying genomic integrity issues. This offers a gateway to reach higher immune compatibility for both T cell and NK cell options. In vitro differentiation of iPS cells toward T cells poses the challenge of mimicking positive and negative selection for recombined TCR. This aspect has been addressed by using T-cell-derived iPS cells with characterized recombined *TCR* gene.[24] Similarly, introducing a transgene, such as CAR, on the TCR loci renders cells into TCR knockout[25] and eliminates potential TCR-mediated immune incompatibility. Exogenously expressed CAR on primary T cells presents the disadvantage of parallel signaling though both TCR and CAR, which might amplify autoimmune T-cell pools. The usage of allogeneic T-cell lymphocytes bearing TCR does indeed present the disadvantage of GVHD because of incompatibilities, including HLA, major histocompatibility complex region, minor histocompatibility targets of allorecognition, and overall genetic variation that affects immune responses.[27,28] On the other hand, donor NK cells convey absent or limited induction of GVHD.[29] Remarkably, recent studies evidence that NK cells and CAR-NK cells do not induce CRS nor ICANS nor GVHD.[30] Such studies can operate in a yet unreached maximum tolerated dose, with upper tested levels of up to 1×10^7 cells per kg.[30] The ideal therapeutic CAR-expressing identity is likely that of a NK cell, which is either derived from an iPS cell or from a suitable HSC source. This is because differentiation protocols from both iPS cells and HSC toward NK cells have matured considerably,[1,17] achieving a reproducible defined status.[2] Additionally, as described above, iPS cells and recently HSC present promising scalability of cellular populations while maintaining their respective identity. Furthermore, such sources of NK cells are exempt from patient-to-patient variation, cellular exhaustion or senescence and free from patient-related viral and cancer cell contamination. Finally, these cell sources are receptive to various layers of manufacturing quality controls, compatible with off-the-shelf production, and suitable for superdonor libraries. In Table 1, we compare the main areas of contrast between T cell and NK cell-hosted CAR therapy.

4 | METHODS OF INTEGRATION FOR ENGINEERED FUNCTIONS

Therapeutic cells bearing a CAR require sustained and long-lasting expression of the CAR gene to exert therapeutic function. The gene integration methods most widely used in CAR clinical practice to date include retroviruses such as lentiviruses. The use of such systems relies on well-defined viral packaging methodologies. Viral integration systems have been characterized, and it is now known that integration takes place randomly in euchromatic regions, with certain hotspots identified. It has not been addressed to date how such random integration affects the clinical outcome in patients. It is known that transgenes carried by such systems can integrate into oncogenes and tumor suppressors. This constitutes one of its main limitations, as the risk of transformation during genetic engineering cannot be avoided. Additionally, the copy number of integrated CAR per cell of manufactured cells cannot be adequately predefined with viral systems. This results in a choice between transgenic cellular yield (Gaussian gene density in the population) vs controlled genetic dose (Poisson gene density in the population). Furthermore, lentiviral systems are known to be subjected to chromatin-dependent silencing, where the expression level of the transgene drops over time. Such a silencing process can impact reversion and mediate relapse. In light of the

TABLE 1 Comparison of sources and effector identity between CAR-T and CAR-NK therapy

Feature	T cells - CAR-T	NK cells - CAR-NK
Primary autologous effector cell source	Conditional to patient apheresis count	
Primary allogeneic effector cell source	Not permissive, causative of GVHD[27]	Absent GVHD[29,30]
Allogeneic off-the-shelf effector cell option	Not permissive, causative of GVHD[27,31,32]	Permissive (ie, NK92)
Graft versus host disease (GVHD)	Yes	Absent GVHD[29,30]
Cytokine release syndrome (CRS)	Yes	Absent CRS[30]
Immune effector cell-associated neurotoxicity syndrome (ICANS)	Yes	Absent ICANS[30]
Required TCR knock out	Yes	Not necessary
One step autologous iPS cell differentiation	No. Negative selection required or T cell derived iPS source or TCR knock out clones	Yes
One step autologous HSC differentiation	Possibly	Yes
Primary autologous cord blood HSC source	Potential autoimmune clone enrichment	Permissive
Primary allogeneic cord blood HSC source	Not permissive for high risk GVHD	Permissive

limitations of viral systems, locus-specific integration of transgenes mediated by designer nucleases offers great promise for the manufacturing of effector cells with a defined CAR copy number and the expression of consistent and stable gene levels. This assertion identifies two key manufacturing components: first, the designer nuclease to use and second, the genome loci for integration.

4.1 | Nucleases

Over the last decade, an assortment of precise genome engineering tools has emerged, including zinc finger nucleases, TALE nucleases, and CRISPR nucleases. Regarding the nuclease, three factors control the choice: simplicity to manufacture the nuclease, on-target efficiency for transgene integration or knockout, and reliability to avoid off-targets. Regarding the simplicity of nuclease manufacturing, both zinc finger and TALE nuclease synthesis are cumbersome.[33] On the other hand, CRISPR nucleases are technologically simple to program, as they require only an oligonucleotide component for specific engagement with target DNA.[34] Regarding the on-target specificity and prevention for off-targets, the three types of nucleases present similar performance. In fact, they can specifically engineer a line without introducing off-target mutations.[35,36] Overall, because of its swiftness, the use of CRISPR nucleases is preferred for preliminary mapping of suitable integration loci and validation experiments, while the use of zinc finger and TALE nucleases is more suited for validated loci and known integration performance.

4.2 | Loci of integration

As outlined above, the random integration of a transgene can result in gene silencing and disruption of essential genes. Safe harbor loci are genomic regions with permissive epigenetic signatures that allow stable transgene expression across lineages or within a specific cell type. Classical safe harbor loci include the *CCR5* gene locus, adeno-associated virus site 1 (AAVS1) locus, and the human ortholog of the mouse ROSA26 locus.[37,38] Recent studies have shown sustained expression of CAR proteins from the AAVS1 safe harbor loci.[39] As expected from preclinical research applications, AAVS1 allowed sustained expression of CAR on T cells.[39] Alternatively, lineage-specific loci known to be in euchromatic state represent a powerful alternative to all-lineage safe harbor loci. The *TCR* gene in T cells is one such example.[25] Mapping of DNAse sensitivity tracks together with epigenetic marks of gene-body activation, such as H3K36 methylation,[40] across immune cells will further unlock safe loci for sustained expression of therapeutic genes such as CAR. In this regard, iPS cells and HSC with well-characterized precise genetic engineering tools and methodologies have emerged as suitable cell sources with greater potential for CAR therapies. The rationale outlined above supports precise genome engineering methodologies of safe harbor loci as a promising approach for the integration of CAR genes. Engineering such loci in iPS cells and HSC, which allow robust genetic manipulation, combined with cell type-specific differentiation toward NK cells would offer a highly attractive alternative for off-the-shelf therapy development.

5 | CAR STRUCTURES USED

Manufacturing more effective and safer CAR cell-based therapeutics can be accomplished by modulating the structure of the CAR protein. Understanding how the modifications of CAR functional domains impact downstream signaling pathways, transcriptional output, and cell identity is essential for the development of next-generation cell-based therapeutics. CAR protein modules can be classified into two main sections: first, endodomains that mediate signal transduction

TABLE 2 Clinical trials involving primary NK cells, NK cell lines, HSC and iPS cell-derived NK cells and CAR-NK cells

Primary NK cells and NK cell lines					
Modification	**Cell source, therapy**	**Target**	**Malignancy**	**Phase**	**Clinical trial**
Anti-CD22 CAR	Allogeneic NK cells	CD22	Refractory B-cell lymphoma	Early phase 1	NCT03692767
Anti-CD19 CAR	Allogeneic NK cells	CD19	Refractory B-cell lymphoma	Early phase 1	NCT03690310
Anti-CD19 CAR (CD28 4-1BB CD3z)	Allogeneic NK cells (NK92)	CD19	Acute Lymphoblastic Leukemia (ALL) Chronic Lymphoblastic Leukemia (CLL) Follicular lymphoma Mantle cell lymphoma B-cell prolymphocytic leukemia Diffuse large cell lymphoma	Phase 2	NCT02892695
Anti-BCMA CAR	Allogeneic NK cells (NK92)	BCMA	Multiple myeloma	Phase 2	NCT03940833
Anti-CD19/CD22 CAR	Allogeneic NK cells	CD19 CD22	Refractory B-cell lymphoma	Early phase 1	NCT03824964
Anti-CD33 CAR	Allogeneic NK cells	CD33	Acute myeloid leukemia (AML) AML with maturation AML without maturation Acute non-lymphoblastic leukemia	Phase 2	NCT02944162
Anti-mesothelin CAR	Allogeneic NK cells	Mesothelin	Epithelial ovarian cancer	Early phase 1	NCT03692637
Anti-PSMA CAR	Allogeneic NK cells	PSMA	Castration-resistant prostate cancer	Early phase 1	NCT03692663
mRNA anti-NKG2DL CAR	Allogeneic NK cells	NKG2DL	Solid tumor	Phase 1	NCT03415100
Anti-ROBO1 CAR	Allogeneic NK cells	ROBO1	Solid tumor	Phase 2	NCT03940820
ROBO1 BiCAR	Allogeneic NK cells	ROBO1	Pancreatic cancer	Phase 2	NCT03941457
ROBO1 BiCAR	Allogeneic NK cells and T cells	ROBO1	Malignant tumor	Phase 2	NCT03931720
Absent CAR, ex vivo activation	PB NK cells Cetuximab Trastuzumab	Undefined	HER2-positive gastric cancer Colorectal cancer Head and neck squamous cell carcinoma EGFR-positive solid tumor HER2-positive breast cancer Hepatocellular carcinoma Non-small cell lung cancer (NSCLC) Renal cell carcinoma Pancreatic cancer Melanoma	Phase 1	NCT03319459
Absent CAR, Ex vivo activation	PB NK cells, IL-2	Undefined	Epithelial ovarian cancer Fallopian tube cancer Primary peritoneal cancer	Phase 1	NCT03213964
Absent CAR, Ex vivo activation	PB NK cells, IL-2	Undefined	Refractory AML Relapsed AML	Phase 1	NCT03081780
Absent CAR	Haploidentical allogeneic CIML NK cells IL-15 superagonist (N-803) Ipilimumab	Undefined	Head and neck squamous cell carcinoma	Phase 1	NCT04290546
Absent CAR	CIML NK cells IL-15 superagonist (N-803) IL-2	Undefined	AML Myelodysplastic syndrome	Phase 1 Phase 2	NCT01898793

(Continues)

TABLE 2 (Continued)

HSC and iPS cell-derived NK cells and CAR-NK cells					
Modification	**Cell source, therapy**	**Target**	**Malignancy**	**Phase**	**Clinical trial code**
Transduced Anti-CD19 CAR (CD28 CD3z) Ex vivo differentiation from CD34+ cells iCasp9 IL-15	Autologous HSC derived NK cells Rituximab	CD19	CD19 positive mantle cell lymphoma Recurrent diffuse large B-cell lymphoma Recurrent follicular lymphoma Refractory B-cell non-Hodgkin lymphoma Refractory diffuse large B-cell lymphoma Refractory follicular lymphoma	Phase 2	NCT03579927
Transduced Anti-CD19 CAR (CD28 CD3z) Ex vivo differentiation from UCB CD34+ cells iCasp9 IL-15	Allogeneic UCB derived NK cells	CD19	B-lymphoid malignancies ALL CLL Non-Hodgkin lymphoma	Phase 2	NCT03056339[43]
Ex vivo differentiation from CD34+ UCB cells	Allogeneic UCB derived NK cells IL-2	Undefined	AML Refractory AML	Phase 2	NCT04347616
Ex vivo differentiation from CD34+ UCB cells	Allogeneic UCB derived NK cells Chemotherapy	Undefined	Recurrent ovarian carcinoma Recurrent fallopian tube carcinoma Recurrent primary peritoneal carcinoma	Phase 1	NCT03539406
Ex vivo differentiation from CD34+ UCB cells	Allogeneic UCB derived NK cells	Undefined	Advanced gastric cancer Gastroesophageal cancer	Phase 1	NCT04385641
Ex vivo differentiation from CD34+ UCB cells	Allogeneic UCB derived NK cells Chemotherapy	Undefined	Leukemia Myelodysplastic syndromes	Phase 2	NCT00354172
Ex vivo differentiation of iPS toward NK cells	Allogeneic iPS cell derived NK cells	Undefined	Advanced solid tumors Lymphoma Gastric cancer Colorectal cancer Head and neck cancer Squamous cell carcinoma EGFR-positive solid tumor HER2-positive breast cancer Hepatocellular carcinoma Small-cell lung cancer Renal cell carcinoma Pancreas cancer Melanoma NSCLC Urothelial carcinoma Cervical cancer Microsatellite instability Merkel cell carcinoma	Phase 1	NCT03841110
Ex vivo differentiation of iPS toward NK cells Anti-CD19 CAR CD16 IL-15	Allogeneic iPS cell derived NK cells Rituximab Obinutuzumab	CD19	Lymphoma B-cell CLL	Phase 1	NCT04245722
Ex vivo differentiation of iPS toward NK cells	Allogeneic iPS cell derived NK cells	Undefined	Advanced solid tumor Lymphoma Gastric cancer Colorectal cancer Head and neck cancer Squamous cell carcinoma EGFR-positive solid tumor	Undefined	NCT04106167

(Continues)

TABLE 2 (Continued)

HSC and iPS cell-derived NK cells and CAR-NK cells					
Modification	**Cell source, therapy**	**Target**	**Malignancy**	**Phase**	**Clinical trial code**
			HER2-positive Breast cancer Hepatocellular carcinoma Small-cell lung cancer Renal cell carcinoma Pancreas cancer Melanoma NSCLC Urothelial carcinoma Cervical cancer Microsatellite instability Merkel cell carcinoma		
Ex vivo differentiation of iPS toward NK cells	Allogeneic iPS cell derived NK cells Rituximab Obinutuzumab IL-2	Undefined	AML B-cell lymphoma	Phase 1	NCT04023071
Ex vivo differentiation of iPS toward NK cells	Allogeneic iPS cell derived NK cells Avelumab IL-2	Undefined	Solid tumor	Phase 1	NCT04551885

and second, antigen engaging component or binding-mediated transduction component.

5.1 | Endodomains

With the emergence of a large array of CAR protein types, it was noted that while some CAR present binding-dependent signaling cascade activation, others are constitutively active. As an example, CD28-based CAR proteins result in constitutive association with LCK and constitutive phosphorylation of the CAR CD3ζ domain.[41] Such constitutive activation has been associated with T-cell exhaustion and reduced clinical performance.[42] The impact of such constitutively active CAR proteins has been made evident in effector immune cells; however, the effect in progenitor cells, such as HSC or iPS cells, prior to differentiation remains to be uncovered. It could be speculated that such pathways might either guide or hinder the differentiation process itself, modulating the differentiation yield and final cell identity. Some studies support this notion, as CAR containing the CD28 domain favors the differentiation of T cells toward effector type, while the 4-1BB domain favors memory T-cell identity,[41] when already within a T-cell type lineage. This can result in remarkably different clinical responses. Therefore, in settings where iPS cells or HSC progenitors are engineered for the expression of CAR proteins, the CAR should count with a clear antigen-induced on-state for its downstream pathways. It remains to be identified which of these endodomain combinations provide proper on-off switch transitions. Alternatively, in cases where there is a leaky off status, which of these endodomains are compatible with the transcriptional networks present in iPS cells or HSC progenitors and their differentiation intermediates. Ultimately, an important preclinical goal is identifying the endodomain combination that maximizes therapeutic activity in HSC and iPS-derived NK cells. Some of the CAR endodomains reported in clinical trials and in preclinical research[18,43-47] are summarized in Supplementary Table 1.

5.2 | Antigen engaging component

Some studies suggest that the binding affinity of the scFv component of CAR plays an important role in reducing side effects and that CAR with low binding affinity are clinically beneficial.[48] This advantage seems to be beneficial only at the effector cell identity level and during clinical usage. However, it is likely that the affinity of scFv should not impact the manufacturing efficiency when using superdonor progenitors as iPS cells or HSC.

6 | CLINICAL TRIALS INVOLVING NK CELLS

To date, there are over 1340 clinical trials involving NK cell therapy. A vast majority of these trials have focused on hematological malignancies, reasonably because of the higher accessibility to tumorigenic cells by therapeutic NK cells (Table 2). Auspiciously, there is an increasingly growing number of CAR-NK cell clinical trials addressing solid malignancies, such as pancreatic, ovarian, and prostate cancer. Most of the

clinical trials utilize allogeneic NK cells, primarily from healthy donors and NK cell lines such as NK92. However, there is a small fraction of clinical trials evaluating NK cells derived from CD34-positive umbilical cord blood cells (Table 2). The latter approach is particularly interesting since it is compatible with off-the-shelf solutions, with superdonor bone marrow banks and superdonor iPS-derived HSC. Although there are clinical trials covering cytokine-induced memory-like (CIML) NK cells, their outcomes in combination with CAR remain to be evaluated. CIML NK cells have recently been described as a constitutive component of circulating blood,[49] and the differentiation of HSC as well as iPS cells to CIML NK cells offers potential synergies. Promisingly, there are a few clinical trials utilizing iPS cells as the source of NK cells, in which some also express CAR (Table 2).

7 | CONCLUSION

Here, we have presented the importance of the cell source for the development of next-generation cellular immunotherapies and how cell sources shielded from variability and risk factors could drive the development of safer therapeutic alternatives. We support that iPS cells and HSC constitute safer cell sources for the production of off-the-shelf NK cells. We also summarize cumulative evidence about the advantages of NK cells as a vehicle for CAR-based therapies. We present the potential role of CAR domains in the differentiation of HSC or iPS cell sources and how the integration of CAR genes in precise genomic loci at defined genetic dose can contribute to circumvent the drawbacks associated with viral integration methods.

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CONFLICT OF INTEREST

J.A. and J.Y. declared stock ownership in Bright SA, Luxembourg. M.V., J.I., and I.N. declared stock ownership in Joincells Nordic AB Sweden.

AUTHOR CONTRIBUTIONS

J.A.: conception and design, financial support, collection and assembly of data, data analysis and interpretation, manuscript writing, final approval of manuscript; J.Y., J.I., I.N.: conception and design, financial support, manuscript writing; M.V.: manuscript writing. All authors wrote and approved the manuscript.

DATA AVAILABILITY STATEMENT

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

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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