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REVIEW

Off-the-shelf cell therapy with induced pluripotent stem cell-derived natural killer cells

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Abstract

Cell therapy is emerging as a very promising therapeutic modality against cancer, spearheaded by the clinical success of chimeric antigen receptor (CAR) modified T cells for B cell malignancies. Currently, FDA-approved CAR-T cell products are based on engineering of autologous T cells harvested from the patient, typically using a central manufacturing facility for gene editing before the product can be delivered to the clinic and infused to the patients. For a broader implementation of advanced cell therapy and to reduce costs, it would be advantageous to use allogeneic "universal" cell therapy products that can be stored in cell banks and provided upon request, in a manner analogous to biopharmaceutical drug products. In this review, we outline a roadmap for development of off-the-shelf cell therapy based on natural killer (NK) cells derived from induced pluripotent stem cells (iPSCs). We discuss strategies to engineer iPSC-derived NK (iPSC-NK) cells for enhanced functional potential, persistence, and homing.

Keywords Induced pluripotent stem cells · Natural killer cells · Off-the-shelf · Cell therapy · Chimeric antigen receptor · Cancer immunotherapy

Natural killer cells—an ideal template for off-the-shelf cell therapy

Immunotherapy has become a cornerstone in cancer therapy that includes a broad array of strategies aiming to unleash, direct, and boost the patients' own immune system through adoptive transfer of expanded naturally circulating or genetically engineered cytotoxic lymphocytes. Despite the recent clinical breakthroughs, the field is still in its infancy and the potential for identifying new and more effective strategies is huge. The first FDA-approved gene-edited T cell products for lymphoma and leukemia came out on the market only in 2017 and the needs for new therapies targeting resistant cases and other diseases, in particular solid tumors, remains high. A key challenge for a wider implementation of cell therapy is to come around the laborious procedures of identifying HLA-matched healthy related or unrelated donors and harvesting their cells for engineering and infusion to one patient. Although such tailored one-donor one-patient strategies are feasible and have proven effective, they have a significant lag-time to patient delivery, are very expensive, and may be less well suited for large-scale implementation of living immunotherapy drugs. Other bottlenecks include difficulties in obtaining large quantities of T cells or natural killer (NK) cells from peripheral blood, inability to uniformly engineer effector cells, and poor function of the cells due to exhaustion [1, 2]. A very attractive solution to these problems would be to expand and genetically engineer cytotoxic lymphocytes (CD8T cells, γδT cells, or NK cells) that can be stored in cell banks and administered across HLA barriers as an off-the-shelf therapy. Off-the-shelf strategies based on T cells require genetic manipulation and knock down of the endogenous T cell receptor to avoid graft-versus-host disease (GVHD). Such strategies are available and discussed in several comprehensive reviews [3–7]. Here, we focus on the prospects

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for using NK cells in off-the-shelf cancer immunotherapy. NK cells do not express rearranged receptors and can be easily transferred across HLA barriers without causing GVHD. In fact, under specific circumstances, transfer across HLA barriers trigger NK cell alloreactivity and has been shown to induce remission in acute myeloid leukemia and myelodysplastic syndrome [8–10].

NK cells have an important role in regulating the defenses against virally infected and tumor cells [11, 12]. NK cells were first described in the 1970s by Kiessling and Herberman for their ability to lyse target cells without prior sensitization [13–16]. Based on patterns of cytotoxicity against panels of tumors cells with different levels of MHC and inspired by the hunt for foreign submarines in the Swedish archipelago [17], Klas K rre identified a key principle behind the functional regulation of NK cells, e.g., the recognition of targets that lacked expression of MHC class I on their cell surface [18–20]. NK cells therefore provide an alternative immune defense mechanism compared to T cells and form a second line of defense in case tumors cells escape T cell recognition by down-modulating MHC [20, 21]. While this may possibly occur spontaneously during immune surveillance, it is certainly of relevance in the era of immune selection and loss of HLA class I imposed by T cell-based immunotherapies [22].

Although NK cells share many characteristics with other lymphocytes in the adaptive immune system, NK cells are classified as part of the innate immune system due to their lack of receptors for antigen specificity as a result of gene rearrangement which is seen in B and T cells [23–25]. Instead, the activation of NK cells is determined by the integration of signals from an array of germline encoded activating and inhibitory receptors [26]. Many of these receptors, including the MHC-binding killer cell immunoglobulin-like receptors (KIR), are acquired during NK cell differentiation and contribute to the functional calibration of the cell during interaction with host cells in a process termed NK cell education [27]. Unlike T cells, NK cells circulate in a primed state and express high levels effector molecules, including perforin and granzyme B [27]. NK cells are therefore ideal cellular templates for gene editing approaches aiming at directing their potent cytotoxic potential against a desired target. To harness the full potential of NK cells, one needs to consider the cellular mechanisms involved in acquisition of effector function during terminal differentiation and the subsequent fine tuning through education. This is particularly relevant when using induced pluripotent stem cells (iPSCs) or cord blood-derived $CD34^+$ hematopoietic stem cells as a source for NK cells since in vitro differentiation may not always lead to fully mature NK cells. This review discusses several issues that need to be considered in order to develop efficient platforms for iPSC-derived off-the-shelf NK cell therapy, including specificity, in vivo persistence, homing, resistance to immune suppression, and modulation of effector function (Fig. 1).

iPSC-derived NK cells

In 2006, Takahashi and Yamanaka first produced induced pluripotent stem cells by simultaneously introducing four genes into mouse fibroblasts. These reprogramming factors effectively convert mouse fibroblasts to pluripotent stem cells with properties similar to embryonic stem cells (ESCs) [28]. A year later, a similar reprogramming method was used to generate iPSCs from human fibroblasts [28, 29]. While ESCs and iPSCs have similar morphology, growth characteristics, and developmental potential, global gene expression analysis of ESCs and iPSCs have shown some persistent donor gene expression in iPSCs, indicative of a potential difference between iPSCs and ESC [30, 31]. Likewise, microarrays of human iPSC and ESC lines revealed differentially expressed genes [32]. In addition, several studies showed differences in DNA methylation between ESC and iPSC lines as well as an epigenetic imprint of donor cells in human iPSCs [33–37]. However, other studies have attributed the differences in gene expressions to different culture conditions in vitro [38, 39].

Human embryonic stem cells (hESCs) were initially used to produce human NK cells able to kill diverse tumor cells in vitro [40]. These hESC-derived NK cells were shown to be better than NK cells derived from umbilical cord blood (UCB-NK cells) in killing K562 cells in a murine xenograft model [41]. Therefore, while hESCs also serve as an effective source of off-the-shelf NK cells for allogeneic immunotherapy, iPSCs are easier to obtain and provide potentially greater donor diversity which may be of particular interest for examining influence of KIR haplotypes on adoptive NK cell immunotherapy.

iPSCs can now be routinely generated from a variety of easily obtainable sources such as skin and peripheral blood (PB) and once reprogrammed, iPSCs are able to undergo essentially unlimited expansion in vitro without losing pluripotency [42]. Therefore, iPSCs can serve as a resource to produce unlimited numbers of NK cells for cell therapy [43]. One concern regarding immune effector cells derived from iPSCs is how iPSC-derived NK cells compare to their natural counterparts in terms of receptor expression and functional potential. Parallel phenotypic assessment of iPSC-derived NK (iPSC-NK) and PB-NK cells revealed highly similar phenotypes, except for higher expression of NKG2A in iPSC-NK cells [43]. NKG2A is typically expressed on more immature NK cells and has been shown to restrict targeting of tumor cells that express high levels of HLA-E [44]. Moreover, NKG2A is emerging as a major checkpoint in cancer immunotherapy so it may be important to develop strategies to limit its expression on iPSC-derived NK cells. iPSC-NK cells also express less KIR, reflecting the fact that KIR acquisition is a relatively late event during NK cell differentiation and that it is challenging to achieve terminal differentiation of iPSC-NK cells in vitro [45].

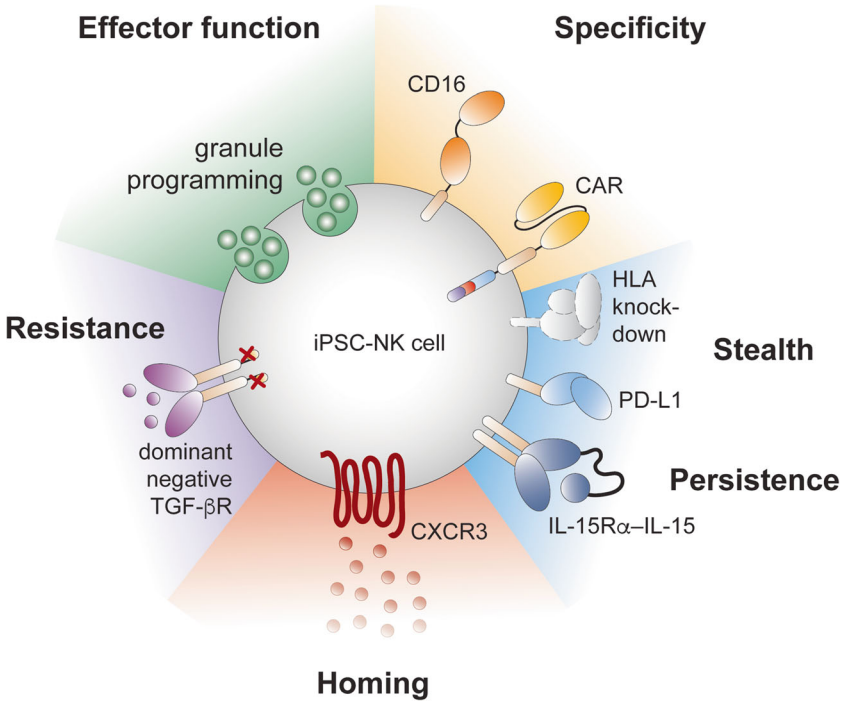

Fig. 1 Engineering of a synthetic killer. The figure illustrates the potential of iPSC-derived NK cells as a template for engineering. Examples of such gene edits include (i) strategies aiming to introduce specificity through cleavage-resistant CD16 for combination with therapeutic antibodies or CAR; (ii) strategies aiming at improving persistence of the infused allogeneic iPSC-NK cells by escaping recognition of host T and NK cells and by making cells self-sufficient in terms of growth factors; (iii) strategies to direct trafficking of iPSC-NK cells to the tumor, here illustrated by overexpression of the chemokine-receptor CXCR3; (iv) strategies to make the cell resistant to immune suppressive factors in the tumor microenvironment, for example by introducing a signaling-dead TGF-β receptor; and (v) strategies to improve effector function, for example by modulating the cytotoxic payload

Whether the lack of KIR expression may be an advantage in terms of less inhibition during the effector phase or constitute a problem due to lack of KIR-mediated education remains unclear. Likewise, it remains unknown how *KIR* genetics, including variation in *KIR* gene content and copy number, influence the iPSC-NK repertoire [46, 47]. Although these phenotypic traits suggest that there may be room to further improve iPSC-NK cell differentiation protocols, current evidence suggests that iPSC-NK cells are highly functional in their native state [43, 48, 49]. iPSC-NK cells show effective killing of both HLA-deficient and HLA-sufficient targets including K562, SKOV-3, and SW480. In fact, the efficiency of iPSC-NK cells was found to be greater than that of PB-NK cells in all lines except K562, where the killing efficiency was similar [50]. In vivo studies further demonstrated that iPSC-NK cells have improved activity compared to PB-NK cells against ovarian cell lines MA148 and A1847 [49]. Future studies investigating the transcriptional regulation of effector function during terminal NK cell differentiation may pave the way for new strategies to promote differentiation and obtain more mature and functional iPSC-NK cells. Likewise, insights into the molecular mechanisms that determine NK cell education may foster new strategies to engineer iPSC-NK cells for increased effector potential.

iPSC-NK cell specificity

Whereas unmanipulated iPSC-NK cells provide great promise to treatment of diverse tumor types and particularly against HLA loss variants [22], there is even more exciting potential to use iPSC-NK cells as a template for genetic modifications that will further improve their anti-tumor activity (Fig. 1) [48, 51, 52]. The success of chimeric antigen receptors (CARs) that redirect T cell specificity in treatment of hematological cancers has spurred interest in the development of CARs in NK cells [53–55]. CARs are synthetic receptors composed of three parts: ectodomain, transmembrane domain, and endodomain. The ectodomain is composed of the single-chain variable fragment (scFv) derived from a monoclonal antibody or an antigen-binding fragment (Fab) which directs antigen recognition specificity either targeting antigens that are overexpressed or antigens that are tumor specific [56]. The scFv can be fused to numerous intracellular signaling domains. Connecting the ectodomain to the transmembrane domain is the hinge region. The intracellular domain or endodomain directs the functionality of the CAR. Since their discovery, CARs have undergone extensive development and there are currently four generations of CARs. In the first generation, the CAR signaling domain is composed of the ζ chain of the T cell receptor complex CD3 as a single activation

domain [52]. In second generation CARs, a co-stimulatory signaling domain and an activation domain together act as a dual-signaling domain to increase T cell activation and proliferation. The addition of such a dual-signaling domain has also shown to increase persistence in vivo [56–58]. The signaling domain of third generation CARs contains two co-stimulatory domains in addition to the activation domain in order to increase persistence and cytolytic function beyond what was achieved with second generation CARs [59–62]. Finally, the fourth generation CARs, or so called armored CARs, carry a payload in the form of a cytokine or even a checkpoint inhibitor, secreted into the tumor environment [63].

We recently examined the impact of NK subset diversity on CAR function in vitro [64]. We found that the intrinsic potential of NK cells, determined both by differentiation and education by self KIR, had an impact on the functional potential after CAR-engineering. So called adaptive NK cells, also referred to as memory NK cells [65], having increased cytotoxic payload and potent effector function [66, 67], displayed the strongest functional responses following engineering with a third generation CD19-specific CAR. Moreover, CAR engineering did not affect the NK receptor repertoire and CAR-NK cells retained their intrinsic capacity to recognize and lyse tumor cells independent of the presence of the CAR target antigen. Thus, CAR-NK cells could possibly dampen tumor growth even in the event of antigen loss variants, for example downregulation of CD19 during CAR T cell therapy [68].

In another set of recent studies, we designed a novel set of NK cell-specific CARs expressed in iPSC-derived NK cells [48]. Here, we targeted ovarian cancer cells in vivo using an anti-mesothelin (meso) CARs. After testing ten different CAR constructs with different transmembrane (TM) and signaling domains, including a "conventional" third generation T cell CAR (41BB-CD28-ζ), we found a CAR with the NKG2D TM domain, 2B4 co-stimulatory domain and the CD3ζ signaling domain was optimal. This "CAR4" construct was shown to stimulate key NK cell signaling pathways including DAP10 activation, increased Syk activity, phosphorylation of PLC-γ1 and PCL-γ2, as well as activation of the NFκB pathway. These NK-CAR-iPSC NK cells demonstrated improved killing of human ovarian cancer cells in a murine xenograft model, including prolonged survival and expansion of the CAR-NK cells in vivo. Interestingly, direct comparison of NK-CAR-iPSC NK cells to "conventional" anti-meso CAR-T cells found that the CAR-NK cells mediated less toxicity and improved survival.

In addition to CAR-mediated specificity, CD16 expression can be used as a universal targeting approach when combined with therapeutic monoclonal antibodies such as Herceptin, Erbitux, and Rituxan. Through the mAb-mediated targeting, CD16 elicits antibody-directed cellular cytotoxicity (ADCC) that can effectively eliminate the targeted cells. Because CD16 sheds upon activation, modifications have been made to prevent the stimulation-mediated shedding to continue signaling and improve function [69]. Taken together, these findings suggest that one may achieve greater clinical efficacy by combining strategies to enhance the intrinsic functional potential of iPSC-NK cells with careful CAR or CD16 designs using optimized signaling motifs that tap into specific NK cell signaling pathways.

iPSC-NK cell stealth and persistence

One key area of interest for the development of successful off-the-shelf cell therapy is to identify strategies to improve persistence of allogeneic cell therapy products. Adoptively transferred PB-NK cells typically expand and persists for about 1–3 weeks following conditioning chemotherapy such as cyclophosphamide and fludarabine, and systemic treatment with interleukin (IL)-2 [70–72]. Heroic efforts are currently being made to create a bank of clinical grade iPSC lines that can be used to treat a variety of disorders in a large number of patients. Donors with homozygous HLA types allow for HLA matching for a majority of recipients [73]. It has been proposed that as few as 50 appropriately selected HLA homozygous donors would provide HLA-matched tissue for over 90% of the Japanese population [74]. An alternative strategy is to create universal iPSCs by knocking down HLA class I to avoid recognition by T cells. However, this strategy is unlikely to be successful since HLA class negative cells are rejected by host NK cells. Possible workarounds involve expression of immune-suppressive ligands, for example single chains of β2m linked to the non-classical HLA-E in complex with carefully selected leader peptides to shut down NKG2A expressing NK cells and yet avoid T cell recognition of HLA-E [75]. However, expression of HLA-E would only protect the cells from the subset of NK cells that actually express NKG2A, which is roughly 50% of the NK cell population [76]. Moreover, knock down of HLA leads to a rather rapid loss of function in NK cells due to disarming [77]. Alternative strategies are needed, perhaps acting at multiple levels to generate universal cells that combine three critical features (i) escape from T cells, (ii) escape from NK cells, and (iii) maintained function.

In addition to avoiding immediate rejection, persistence may also be improved by exogenous or endogenous cytokines. Cytokines play a crucial role in NK cell development, differentiation, and homeostasis. Therefore, one of the hurdles to be overcome in NK cell immunotherapy is sustaining in vivo proliferation and persistence of NK cells post infusion. Interleukin 2 (IL-2) has been one of the most commonly studied cytokine in clinical settings, as it was the first growth factor and activation factor described for NK cells. Systemic administration of IL-2 is associated with high toxicity and also with the expansion of T regulatory cells (Tregs), which

downregulates NK cell function [78–80]. IL-15 is a pleiotropic cytokine that plays a crucial role during development and maintenance of NK cells [80, 81]. To be fully effective, IL-15 must be trans-presented to IL-15 receptor alpha (IL-15Rα). In order to harness the effects of IL-15 on NK cell expansion and survival, ALT-803, a fusion protein that contains a mutated high-affinity IL-15 linked to IL15Rα sushi domain and fused with an Fc domain, was created and has entered clinical trials for solid tumors and relapsed hematological cancers [82, 83]. The first results demonstrate increased expansion of NK cells with less toxicity for 19% of patients that had relapsed after hematopoietic stem cell transplantation [83]. Cytokines are also able to change the chemokine receptor repertoire and boost NK cell migration toward sites of inflammation [84]. Retroviral transduction of NK cells to produce IL-2 and IL-15 has been shown to increase in vivo persistence and activation of NK cells in tumor-bearing mice [85]. Similarly, retroviral transduction of a CD19 CAR and IL15 in ex vivo expanded NK cells showed a significant increase in the in vivo persistence and activation of CAR-NK cells in a mouse leukemia model [51, 86]. Apart from increased in vivo expansion and persistence, such self-supported NK cells may attain a higher level of functionality and at the same time avoid the toxicities associated with high doses of systemic IL-15. An interesting question is whether NK cells that carry an IL-15 transgene are rescued from cell death due to cytokine withdrawal associated with adoptive transfer of the in vitro expanded NK cells [87].

iPSC-NK cell homing

Throughout development, acquisition of activating and inhibitory receptors increases NK cell effector capability, while the changes in expression of adhesion molecules and chemokine receptors changes NK cell migration patterns [27]. Activation and expansion of NK cells ex vivo alters the chemokine receptor repertoire of NK cells which may affect the ability of NK cells to migrate to tumor sites [84]. NK cell homing to tumor target sites can also be affected by the changes in expression of chemokines in the tumor microenvironment (TME) as tumor cells change its chemokine composition to promote tumor progression, increase vascularization, and recruit immunosuppressive cells. NK cells are generally absent or found only in low numbers in the TME of metastatic disease; one study in solid tumors showed that as little as 0.01–0.04% of adoptively transferred cells could be detected in the TME [88, 89]. Low infiltration likely reflects the poor capability of NK cells to home effectively to tumor target cells [90, 91]. The infiltration of NK cells in certain solid tumor cancers correlates with better prognosis [91–94]. Therefore, the ability of the NK cells to reach the TME could greatly affect the outcome of treatment. Chemokine receptor 3 (CXCR3) promotes NK cell adhesion and migration and has been associated with effective homing of NK cells to tumor sites [95]. The three CXCR3 ligands, namely CXCL9, CXCL10, and CXCL11, are the most important in acting as a chemoattractant to $CXCR3^+$ NK cells [95]. Mouse studies have shown that local injections of Th1-type chemokines, IFN-γ or CXCL10, led to an increase of $CXCR3^+$ NK cells in the TME [95, 96]. Moreover, lymphocytes can be recruited to the TME by inducing secretion of Th1-type chemokines in dendritic cells by locally injecting Sendai virus particles or PD1 blockade to induce secretion of CXCR3 ligand in myeloid cells when combined with adoptive cell transfer [97, 98]. Local radiation treatment has also been shown in increase CXCR3 ligand secretion in an IFN-γ-dependent manner [99]. Other mouse studies have demonstrated a significant increase in NK cell and T cell infiltration to the tumor site after treatment with the epigenetic modulator PRC2 that inhibits DNA methylation which has been found to suppress secretion of CXCL9 and CXCL10 [100, 101]. For the ultimate design of a synthetic killer, engineering unique and tailored homing properties into iPSC-NK cells may allow targeting of solid tumors that are otherwise poorly infiltrated by cytokine-activated NK cells.

Although we commonly think of NK cells in terms of their killing potential, emerging evidence suggest that other functional properties of NK cells, including their directed secretion of cytokines and chemokines, contribute an important bridge to adaptive immunity [102]. NK cells participate in the recruitment and activation of APCs and promote Th1 type responses [103, 104]. Thus, there is rational to combine NK cells with checkpoint blockade (CPB) therapy to convert “cold” tumors to “hot” tumors and thereby improve CPB therapy.

iPSC-NK cell resistance

NK cells that eventually reach the TME are faced with harsh metabolic conditions, including hypoxia, acidic pH, and low glucose levels that all affect NK cell function [105–107]. Within the TME, the immunosuppressive cytokine, transforming growth factor-β (TGF-β), has a dramatic negative impact on NK cell function. Tregs in the TME suppress NK cell function by releasing TGF-β, which partly acts through the C-type lectin receptor CD69 on NK cells [108]. Immunosuppression of NK cells is multifaceted: TGF-β affects metabolic activity as well as proliferative capacity and alters the receptor repertoire of NK cells, leading to a decrease in cytotoxic activity [109, 110]. TGF-β is induced by hypoxia and in turn promotes the proliferation and accumulation of Tregs within the TME [111]. Tumor-derived cytokines convert macrophages into M2 macrophages, which further promote tumor progression by creating a positive feedback loop and a continual production of immunosuppressive factors such as IL-10 and TGF-β [112]. The hypoxic environment

of the TME not only preferentially recruits Tregs into the tumor and induces M2-polarization but also directly impacts the degradation of granzyme B in NK cells via autophagy [107, 113, 114]. This in turn affects the subset of NK cells that are most potent killers, $CD56^{dim}CD16^{+}$ which are targeted for apoptosis [114]. Cancer-associated fibroblasts (CAFs) are another source of TGF-β in the TME. The high levels of TGF-β in the TME drives the differentiation of various resident cells such as fibroblasts, mesenchymal stem cells, endothelial and epithelial cells into CAFs [115, 116]. Although CAFs phenotypically resemble normal myofibroblasts, they also express CAF-specific markers such as fibroblast-associated protein, fibroblast-specific protein-1, and platelet-derived growth factor [117, 118]. Thus, CAFs not only aid in tumor progression by secreting factors that promote vascularization, such as platelet-derived growth factor and vascular endothelial growth factor, but they also aid in tumor progression by suppressing the immune response by secreting TFG-β.

The challenges that adoptively transferred iPSC-NK cells face in the TME can be overcome either by pharmacological interventions or through gene-editing strategies that confer resistance to the suppressive factors. Genetic modification of the NK cell line NK-92, to express a dominant-negative, signaling-dead TGF-β receptor that is insensitive to TGF-β immunosuppression led to decreased tumor proliferation, increased production of IFN-γ, and increased survival when mice were challenged with Calu-6 lung cancer cells [119]. Another approach to overcome TGF-β suppression is to use a TGF-β receptor kinase inhibitor, LY2157299, which has been shown to enhance cytokine production and control tumor progression in mice. This effect was greater in combination with adoptive NK cell transfer when compared to treatment of TGF-β receptor kinase inhibitor or adoptive NK cells alone [120]. Currently, drug treatment to overcome oxidative stress such as fluorouracil, cyclophosphamide, doxorubicin, celecoxib, and sunitinib effectively target immunosuppressive cells in the TME but act broadly and therefore can lead to negative side effects [121]. Notably, lessons learned from studying subsets of PB-NK cells, where for example the adaptive NK cells are more resistant to immune suppression due to increased expression of ARID5B [122], can be harnessed in the context of designing iPSC-NK cell products for increased endurance in the TME.

Programming effector function—toward a synthetic killer

In order for NK cells to be effective against the tumor, they not only need to home to and survive in the TME, but must also be able to deliver one or more lytic hits, ultimately killing the intended tumor target. One of the key pathways of NK cell-mediated killing is through degranulation, i.e., the release of dense-core secretory lysosomes that contain perforin and granzymes. NK cells are also able to mediate target cell death by means of death receptor pathways TRAIL/TRAIL-R and Fas/FasL and these mechanisms may operate sequentially [123]. The effector program that regulates NK cell killing evolves under the influence of homeostatic cytokines and metabolic cues and is further shaped by receptor-ligand interactions during NK cell education. We have recently found that educated NK cells express higher levels of granzyme B stored in unique pre-converged dense-core secretory lysosomes [124]. Whereas the gradual increase in effector potential during differentiation are under tight transcriptional regulation, the unique functional phenotype of educated NK cells appears independent of transcription and represents a way for the cell to integrate receptor input into a physical, compartmentalized molecular memory. The increased loads of granzyme B in educated NK cells set the stage for their serial killing capacity [125]. Further insights into the cellular mechanisms that defines the development of a molecular memory in NK cells may pave the way for new means to engineer increased effector function into iPSC-NK cells. For example, it may be possible to identify pathways that boost lysosomal biogenesis and reloading of secretory lysosomes to modulate degranulation in NK cells, which could increase their cytotoxic potency and thereby improve efficiency of NK immunotherapy.

Conclusion

Engineered allogeneic NK cell therapy holds great promise as the next-generation immunotherapy for a wide range of cancer types. However, current protocols based on expansion of PB-NK cells are limited by donor variability in expansion efficiency together with a vast phenotypic heterogeneity of the end products. The use of iPSCs as a source for unlimited doses of genetically engineered NK cell products opens up the possibility to create clinical cell banks available to patients on demand. Additionally, repeated dosing with these off-the-shelf NK cells may be more effective, especially for treatment of solid tumors. These products can be geared for detection of HLA class I loss variants, to express CARs or Fc-receptors that recognize tumors expressing a given antigen or coated with therapeutic antibodies. By deepening our insights into the fundamental mechanisms that regulate NK cell function, trafficking, and tumor-host relationships, we can develop a portfolio of iPSC-NK cell products that have greater function, targeted homing capacity, and ability to persist in an allogeneic host and in the tumor microenvironment.

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Compliance with ethical standards

Conflict of interest K.J. Malmberg and D.S. Kaufman serve on the Scientific Advisory Board of Fate Therapeutics and obtain research support. Bahram Valamehr is employed by Fate Therapeutics. The respective relationships have been reviewed and managed by University of San Diego California, Oslo University Hospital, and Karolinska Institutet in accordance with the institutions' conflict of interest policies.

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