CELL & GENE THERAPY INSIGHTS

CELLULAR IMMUNO-ONCOLOGY 4.0

SPOTLIGHT

EXPERT INSIGHT

Natural killer cells may be scaled and engineered as a next generation, off-the-shelf cell therapy for cancer

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The advent of immunotherapies for cancer, and more recently of cellular immunotherapies, has substantially altered the treatment landscape. In hematological malignancies, complete response rates to CAR-T cells can exceed 80%. These responses are often durable in nature and have attracted considerable excitement and investment in the development of cell therapies for an expanded range of indications, particularly in solid tumors. The continued progress of cell therapies depends on overcoming key obstacles that include a lengthy and costly manufacturing process, a high degree of product variability, risks of severe adverse events, and limited available targets. Natural killer (NK) cells have the potential to overcome these limitations. NK cells are highly potent lymphocytes that target cancer through multiple broadly expressed activating ligands; they can be used allogeneically without posing a risk of graft-versus-host disease (GvHD). In recent years, technologies have been developed that allow their efficient expansion and engineering. We will describe the current status of development of NK cell therapies as targeted, off-the-shelf, allogeneic cell therapies for cancer, highlighting the different approaches that have been taken for their effective exploitation, and will outline remaining obstacles to the advancement of the field.

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ADVANTAGES & LIMITATIONS OF CAR-T CELL THERAPIES

Cancer immunotherapy has assumed a prominent position in therapeutic development. The monoclonal CTLA-4 antagonist ipilimumab was the first immmuno-oncology agent to show an overall survival benefit in advanced melanoma, followed closely thereafter by the PD-1 antagonist nivolumab. Antagonists of the PD-1/PD-L1 signaling axis have since made major inroads in the treatment of melanoma, non-small cell lung cancer, renal cell carcinoma, and hepatocellular carcinoma among other indications [1]. Though impressive, overall response rates to these agents have varied widely, and as single agents are rarely greater than 40% and typically closer to 20%. More recently, results obtained in B-cell malignancies using CD19 and BCMA-directed chimeric antigen receptor (CAR) T cells have been dramatic, with overall response rates of 80% being observed in some settings [2]. While these results have galvanized the field, the challenges associated with CAR-T cell therapies are also daunting [3,4]. Toxicities associated with CAR-T cells can be serious and even fatal. The severity of these adverse events has in some cases limited the clinical sites at which they can be safely administered to those which have the training and infrastructure in place to recognize and respond to emerging symptoms of cytokine release syndrome and neurotoxicity, whose onset and progression can be rapid. Just as widely understood is the cost associated with the manufacture of these products, which to date have required expansion and engineering of autologous T cells drawn from the patient. The autologous nature of the products also means that it can typically take several weeks before a patient's product is available for infusion, with attendant risk of progression during that time frame. Just as important, the quality of the product can vary considerably between patients [5], and manufacturing failure rates of up to 13.2% have been reported [6]. All these factors may limit the widespread adoption of autologous CAR-T cell therapies. There is thus an increasing demand for the development of allogeneic therapies which can be produced at large scale and made available off-the-shelf. Natural killer (NK) cells are well suited to this approach. As we will discuss below, NK cells are potent and safe in allogeneic settings; they can be engineered efficiently and produced at large scale. While the biology of these cells is generally well characterized, production methodologies for clinical application are still maturing, and new methods continue to emerge.

BIOLOGY OF NK CELLS & APPLICATION TO CELL THERAPY

NK cells are uniquely well suited for development as cancer therapeutics (Figure 1). NK cells discriminate naturally between healthy cells and virally infected or transformed cells [7,8]. They recognize a variety of inhibitory signals expressed in healthy cells. One of these signals is mediated by expression of HLA class I molecules in most cells. HLA class I subtypes, responsible for presentation of peptides to CD8+ T cells, are recognized by a polymorphic family of killer-cell immunoglobulin-like

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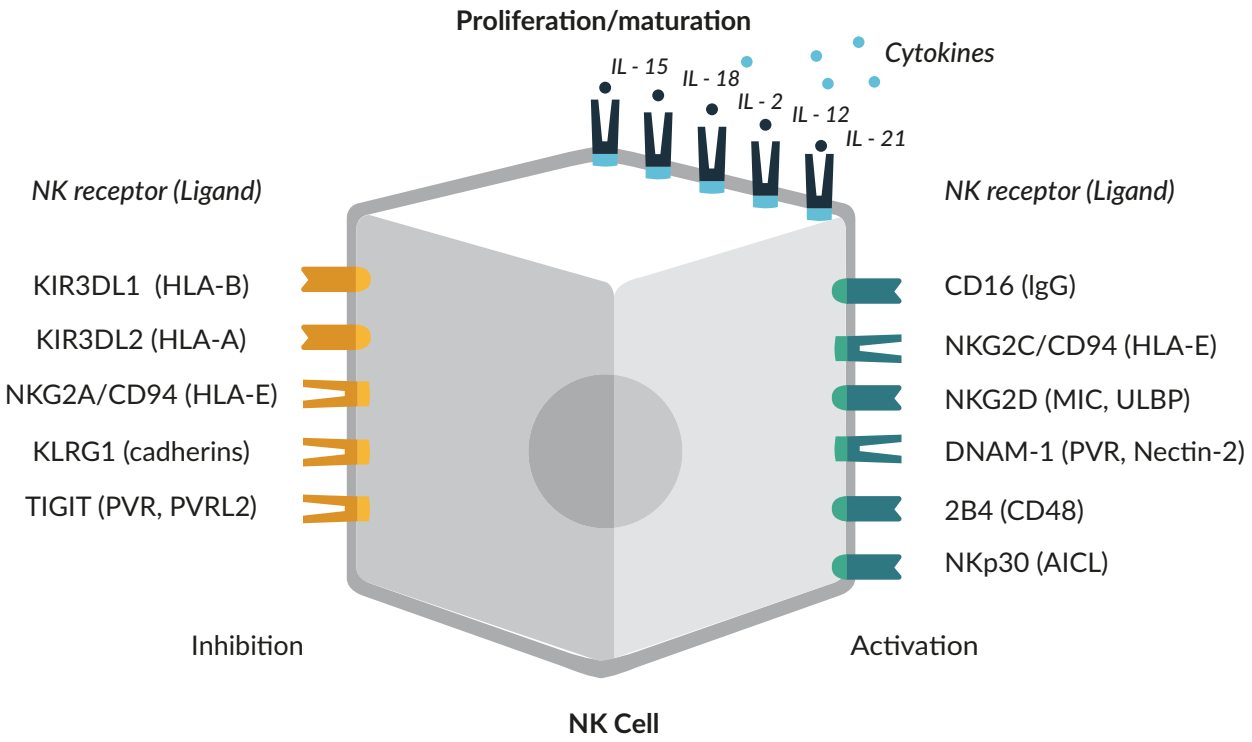
FIGURE 1

NK cells recognize and respond to a wide variety of activating and inhibiting ligands that allow them to distinguish between healthy and diseased cells.



Shown here are receptor (ligand) pairs of particular relevance in determining NK responses to tumor cells.

receptors (KIRs) on NK cells, and can act to suppress their cytotoxicity [7]. Loss of class I expression, or 'Missing self' has been recognized as a driver of NK cell recognition of impaired cells [9]. Loss of class I expression is frequent in tumor cells, abetting their evasion of T-cell recognition at the cost of allowing their targeting by NK cells [10].

NK cell activation is also driven by their expression of receptors capable of binding a variety of ligands frequently upregulated in cells undergoing aberrant DNA replication or protein misfolding, a common characteristic of cancer cells [11,12]. Multiple activating ligands may be expressed in any given tumor cell, giving NK cells multiple opportunities for target recognition [13]. This feature may be particularly important in solid tumor settings, where antigens recognized by T cells or monoclonal antibodies may have incomplete penetrance.

Moreover, unlike T cells whose recognition of tumor cells is restricted by the HLA class system, activating ligands of NK cells are broadly shared between individuals. The NK cells of any given donor can recognize the ligands expressed in a recipient's tumor. Indeed, NK cell activity is greater in allogeneic settings, as matched HLA class I acts as an NK inhibitory signal through the KIR receptor system discussed above.

Just as importantly, NK cells are not promiscuously activated by HLA mismatches with neighboring cells. For this reason, NK cells can be safely administered across HLA barriers without triggering graft-versus-host disease; a recent review of clinical use of NK cells documented no cases of GvHD in 17 different studies, comprising over 300 patients, using allogeneic NK cell in a non-transplant setting [14]. Thus, NK cells are extremely well suited to allogeneic application. Unlike T cells, which require extensive modification for safe allogeneic administration, NK cells can be safely administered to haplo-matched or completely unmatched recipients without serious toxicity – in fact the lack of KIR-mediated inhibition may enhance their potency in these settings [15].

Tumors suppress immune responses by multiple mechanisms that include secretion of inhibitory factors such as TGFβ, a hypoxic tumor microenvironment, infiltration by regulatory T cells, M2 macrophages, and myeloid-derived suppressor cells, and others [16]. The immunosuppressive tumor microenvironment has been an obstacle to successful therapy with either immune checkpoint inhibitors or CAR-T cells. Overcoming this barrier will likely require combinatorial approaches, and this creates an opening for applications of NK cells that may be synergistic with current standards of care. NK ligand expression is frequently sensitive to cancer therapeutics of all classes: radiation, chemotherapies, hypomethylating agents, and various targeted therapies (including proteasome inhibitors, histone deacetylase inhibitors, and tyrosine kinase inhibitors) have all been shown to upregulate activating NK cell ligands [17,18], creating many potentially synergistic opportunities for combining NK cells with existing standards of care. Moreover, NK cells will combine quite naturally with many marketed monoclonal antibodies, which frequently operate through antibody-dependent cellular cytotoxicity (ADCC), which is mediated primarily by NK cells through the low-affinity FcγRIIIA receptor CD16, which triggers potent NK cell activity [19,20]. Many such combinations have been demonstrated *in vitro*; a systematic exploration of potential combinations in animal models and eventually in clinical settings may ultimately be key to unlocking wider success of cellular immunotherapy for cancer.

BRIEF HISTORY OF NK CELL ADOPTIVE TRANSFER

As detailed by Veluchamy *et al.*, over 30 studies incorporating adoptive transfer of allogeneic NK cells have been published, representing well over 500 hundred patients treated [14]. Many of these studies have focused on the use of NK cells to support hematopoietic stem cell transplants (HSCT) in patients with hematological malignancies. In these settings, allogeneic NK cells were typically partially HLA matched, and most frequently derived from related donors (most often the same donor used for transplant). In the transplant setting, infusion of the allogeneic NK cells is provided to support disease suppression as the newly engrafted HSCT matures into a more fully functional immune system. While in general, adoptive transfer of NK cells in this setting has been well tolerated, some instances of GvHD

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have been observed, typically attributed to contaminating T cells in the NK cell infusion or resulting from the HSCT itself [21]. The methods used for production and purity of the NK product varied widely over these studies, and interpretation of both safety and efficacy results is complicated by the concomitant transplant.

In the non-transplant setting, over 300 patients have been treated with NK cells. Acute myelogenous leukemia (AML) and other hematological malignancies were the most frequently treated indications, but patients with various solid tumor diseases have also been included. No published studies have incorporated engineered cells. NK production methods in these studies have encompassed everything from overnight stimulation with IL-2 to several weeks' expansion in the presence of IL-21. Doses in these studies have ranged from under 1 x 10^6 NK cells/kg to over 1 x 10^8 cell/kg for primary NK cells, and up to 1 x 10^{10} cells/m^2 for the NK cell line NK-92 [22]. The wide variety of settings and product characteristics make systematic analysis difficult, but adoptively transferred NKs in the non-transplant setting are very well tolerated with no reported GvHD. Therapeutic responses have been variable in these studies; the largest such study, conducted by Bachanova and colleagues, reported a 30% overall complete response rate in a cohort of 57 patients with relapsed/refractory AML, with a 53.3% CR rate in a 42 patient cohort that received NK cells combined with a diptheria-toxin conjugated IL-2 for depletion of regulatory T cells [23]. A similar complete remission rate was achieved in a study by Romee and colleagues, where four complete responses were observed in nine treated patients with relapsed/refractory AML [24]; this study used a novel method for overnight stimulation of NK cells with IL-12, IL-15 and IL-18, a combination the authors demonstrated to result in differentiation of what the authors term cytokine-induced memory NK cell population (see below).

Immune suppression is required prior to either HSCT or adoptive transfer of allogeneic NK cells, and this has typically been conducted using a non-myeloablative conditioning regimen consisting of cyclophosphamide (Cy) and fludarabine (Flu); early studies showed that this chemotherapy conditioning was required for engraftment, and associated with transient elevation of IL-15 in patient plasma [25]. In addition to initial engraftment, key questions for allogeneic therapies surround the persistence of the NK cell product in the face of the host versus graft immune response, and the degree of persistence required to mediate a long-term benefit. Persistence can be measured by a variety of means, including flow cytometry using antibodies directed to HLA- or KIR-mismatched surface molecules, or more sensitively by qPCR to detect SNP or microsattelite chimerism. By either measurement, peak chimerism in peripheral blood usually occurs within 7 to 14 days following infusion [23,24,26,27]. Measurements at other anatomical sites, including bone marrow or lymph nodes, have been more sporadic; the general kinetic of peak chimerism seems to be similar [24,28]. The kinetics of NK cell engraftment correlate with suppression of the host immune system. While timing can vary,

recovery from lymphodepletion typically corresponds with loss of NK cell chimerism [23,27], as the transferred NK cells are presumably recognized and killed by the patient's immune system. In the study cited above, Bachanova *et al.* found that early persistence of NK cells at 7 days correlated well with AML clearance [23]. This finding is promising, especially for use of NK cells in hematological settings.

Overall, the safety and clinical responses rates achieved using allogeneic NK cells have encouraged further exploration of the therapeutic application of these cells. The methods used for production of the cells has been quite variable, often limiting the cell dose that could be administered, and perhaps the quality of the cell product delivered. The resultant variability in observed clinical outcomes has spurred research into optimizing techniques for both expansion and engineering of NK cells. As discussed below, these methods should permit delivery of multiple doses of cells engineered for greater activity and durability.

NK EXPANSION APPROACHES

At this point, several hundred cancer patients have been treated with adoptively transferred NK cells. A wide diversity of sources and methods have been used for derivation and expansion of NK cells for clinical use (Figure 2). While most methods start with allogeneic cells from a healthy adult donor, NK cells have also been expanded from cord blood [29], from differentiated induced pluripotent stem cells (iPSC) or hematopoietic stem cells (HSC) [26,30], or from autologous cells. NK-92, a cell line derived from a NK cell malignancy, has also been used in several studies [31].

Each of these sources introduces different opportunities and challenges to the manufacture and use of NK cell products. Cell lines like NK-92 are perhaps the most readily scaled starting material. Klingemann and colleagues pioneered the implementation of clinical scale GMP manufacturing processes for these cells [31]. Development has continued to advance, now incorporating both expansion and genetic engineering of the cell line, [32]. The ability to grow these cells in suspension to virtually any scale, using a well-characterized GMP master cell bank as starting material, represents an attractive option for development of an off-the-shelf product. Multiple trials using NK-92 cells engineered to express CARs are underway. As a transformed cell line, however, NK-92 must be irradiated prior to administration. In previous clinical studies, this has perhaps been responsible for the limited persistence observed for these cells; doses of up to 1 x 10^{10}/m^2 are detectable in patients' blood for only 48 hours [22]. Whether this will be sufficient to enable durable clinical responses remains an open question.

The use of stem cells as starting material also represents a powerful avenue towards development of scalable processes. Kaufman and colleagues demonstrated in 2005 that NK cells could be successfully differentiated from human embryonic stem cells [33]; his group later showed that NK cells can also be differentiated from iPSC in a multistep induction process [34] involving timed use of appropriate growth factors and cytokines. Clinical-scale

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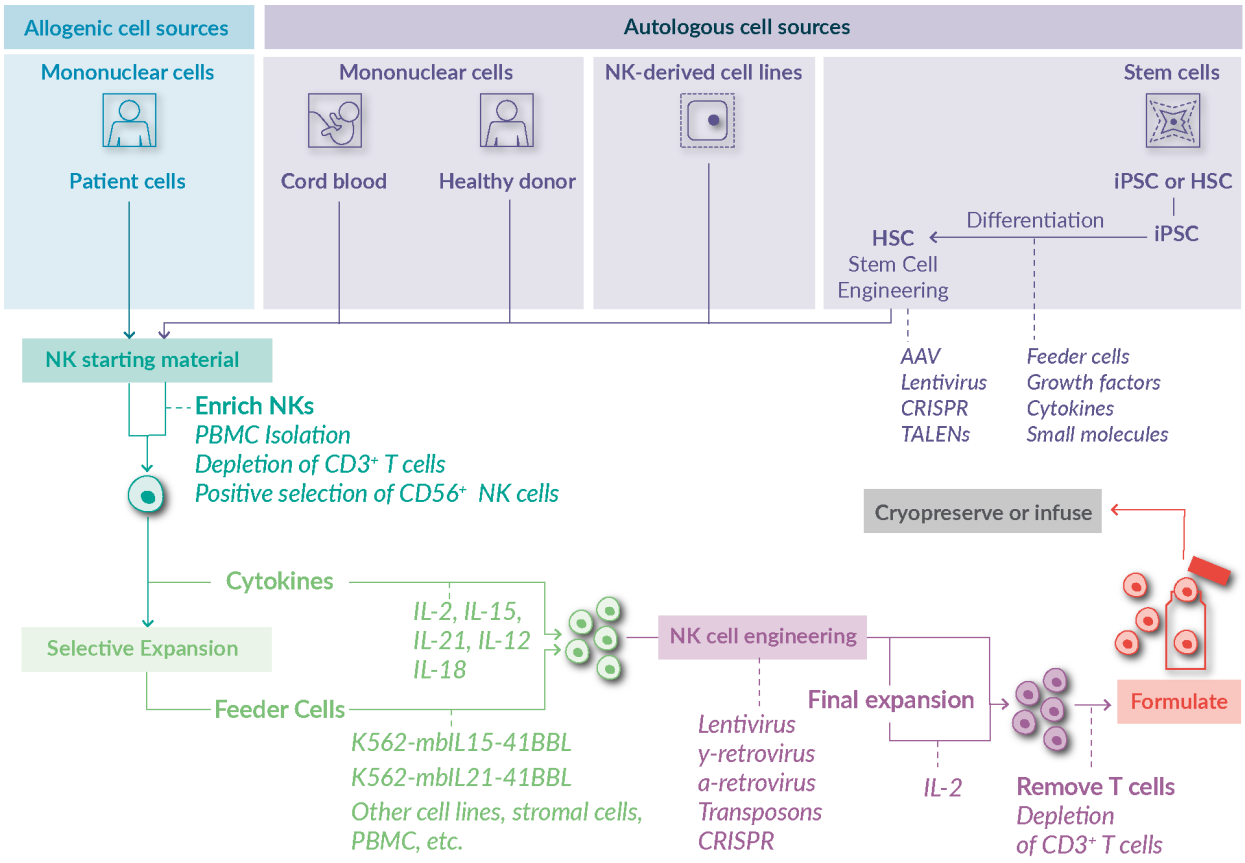
FIGURE 2

The divergent paths to CAR NK therapeutics.



NK cells are expanded and engineered using a wide range of processes; cells source, expansion methodologies, and engineering techniques have not been standardized.

methods for derivation of NK cells from iPSCs have been developed [35], as have methods for engineering iPSC prior to differentiation for production of CAR NK cells [36]. iPSC can be grown at scale in suspension with cytokine support, engineered with multiple modalities, and clonally isolated for GMP master cell bank production; they represent a powerful starting platform for production of engineered NK cell products [30]. It should be noted that, notwithstanding the evident scalability of iPSC production, NK cells must still be differentiated and expanded from the source material. The scalability and reproducibility of those process steps remains a challenge; further, prior to clinical assessment, the potential teratogenicity of residual iPSC in the NK final product will inevitably remain a concern. Initial clinical studies using iPSC-derived NK cells are now underway.

HSCs present many of the same opportunities as iPSCs. CD34+ human stem and progenitor cells (HSPCs) from umbilical cord blood have been successfully differentiated to NK cells and expanded in GMP-compliant clinical processes [26,37]. Stem cells are first expanded in culture using a mix of cytokine and growth factors; differentiation to NK cells can be initiated by addition of IL-2 and IL-15. The derived cells have phenotypic and functional characteristics typical of peripheral

blood NK cells [38]. To date, these cells have been clinically applied in HLA haplomatched patients [26]; scalability of the process has thus not been put to the test. Few reports have described engineering of HSCs prior to NK cell differentiation [39], and none to date have described clinical results.

Whether derived from allogeneic donors or autologously from patients, the earliest protocols for NK cell expansion used only IL-2 for cytokine stimulation in a suspension culture [40]. As an understanding of the basic biology of NK cells has grown, other cytokines have been added to either improve expansion or confer desirable biological properties on final product cells. form IL-15 has been used most frequently [41]. IL-15 is a key NK cell growth factor and is relatively selective in its support of NK cell expansion [42]. NK cells have been expanded in suspension using cytokine support alone [43], but since Campana and colleagues demonstrated robust NK cell expansion from peripheral blood mononuclear cells (PBMC) using a K562 cell line engineered to express 4-1BB ligand and a membrane-bound form of IL-15 [44], the use of stimulatory cell lines has become more common. Stimulatory support cells have either been sourced autologously [45], or more commonly are derived from engineered cell lines used in conjunction with soluble cytokines [44,46–48]. A membrane-bound form of IL-21 has frequently been substituted for IL-15 in engineered K562 cells [49]. Engineered K562 cells can be grown to large scale under GMP conditions and banked [50]; working cell banks are irradiated prior to use to ensure that no replicating stimulatory cells will be carried over to the patient. Alternately, engineered K562 cells expressing 4-1BBL and membrane-bound IL-21 have been converted to microparticles and used to successfully stimulate NK cell expansion from PBMC; use of the microparticles has been proposed both as a means to expand NK cells *ex vivo* and to maintain their expansion and persistence *in vivo* [51].

Several of the methods outlined above can be used to achieve expansion of NK cells by two logs or more in 7–9 days; 21-day expansion of NK cells of over 40,000-fold was reported by Denman and colleagues using stimulator cells expressing membrane-bound IL-21 [49], though expansion of this magnitude has not yet been reported at scale. Maturation of NK cell culture methods are bringing greater attention to the demands of developing scalable, GMP-compliant processes using closed systems and commercially available components, with the goal of producing an off-the-shelf cell product with the potential of longer term persistence *in vivo*. Several such methods have been reported; one closed and largely automated system for NK cell expansion from cord blood represents a promising avenue, though scalability remains to be determined [48]. Where scalable production of NKs has been reported, it has typically relied on initial expansion and differentiation of either hematopoietic [37] or more primitive pluripotent stem cells [35]. While these systems provide an avenue towards large scale NK production, they are inherently complex; mastering the control of such systems to limit lot-to-lot variability will continue to present challenges.

Final product cryopreservation will also be required to deliver a scalable, off-the-shelf NK cell product.

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NK cells are relatively large, granular cells, and have historically fared poorly following cryopreservation, with low recovery and hampered activity [52,53]; cellular function could be recovered following overnight re-stimulation with IL-2, suggesting that improved cryopreservation techniques might address the issues observed. More recent studies have reported more success through attention to freezing parameters [54] and cryopreservation media formulation [55], raising the promise that reproducible and reliable freezing methods may be achievable.

Past the ability to produce NK cells at large scale, questions have arisen around the selection or differentiation of the appropriate sub-set of NK cells for clinical application. Newer analytic methods such as CyTOF have shown that the diversity of phenotypically distinct NK cell sub-populations is complex, and at this point not well understood [56]. Moreover, many lines of research in both animal models [57,58] and in humans [59] have indicated the existence of NK cell populations with adaptive or memory properties. These cells are defined by their ability to respond more vigorously to repeat challenge with the appropriate antigen, or virus. Studies in mice showed that development of memory NK cells depended on key inflammatory cytokines, and that memory-like cells could be induced *in vitro* with appropriate cytokine stimulation [60], which included use of IL-15, IL-12 and IL-18. Culture with these cytokines also induces greater proliferation, persistence and IFNγ secretion in human NK cells; as described above, these memory-like cells could mediate impressive clinical responses [24]. It is safe to say that the phenomenon of NK cell memory is still incompletely understood in humans; various terms have been used to describe potentially related sub-sets of NK cells, including memory, adaptive, and memory-like NK cells, and phenotypic characterization of the cells has varied, though the maturation marker CD57 and the activating receptor NKG2C are frequently used for identification of cells with enhanced properties [61]. The attractive functional features of these cells has encouraged novel approaches to the selective expansion of NK cell sub-sets *ex vivo*. In addition to approaches using cytokines, small molecules may also be used to influence NK cell differentiation in culture. Cichocki and colleagues, for example, have shown that altering NK cell metabolism with inhibitors of Glycogen Synthase Kinase 3 allows expansion of NK cells with a more mature (CD57+) phenotype and improved anti-cancer activity [62]. Alternately, rather than biasing culture conditions to favor outgrowth of a given NK cell phenotype, cell isolation methodologies have also used at the outset of culture to select an NK population expressing low levels of KIR receptors to enhance NK cell function [63]. As the field is able to characterize the functional properties of these sub-populations more completely, it is inevitable that novel methods will continue to be developed to select for those cell types that possess desirable attributes.

Regardless of cell source, development of analytical methods to better characterize both source material and final product will also be essential for ensuring production of NK cells with consistent properties. Recent advances in mass cytometry have shown the complexity of the NK cell compartment [64];

multi-parametric analyses such as these may not be suitable for routine analytical purposes, but they may facilitate the identification of better-defined phenotypic attributes for donor selection, in-process analysis, and final release of NK cell products.

NK CELL ENGINEERING

As noted above the safety of allogeneic NK cells has been demonstrated in multiple clinical studies, durable complete response rates have been modest [14]. This has led to a more intense focus on engineering NK cells for increased potency, migration, or resistance to the tumor microenvironment. Viral transduction techniques using lentiviral or retroviral vectors developed for T cells initially proved of limited utility in NK cells, resulting in very low transduction efficiencies even at small scale [65]. Understanding has continued to advance of the determinants of efficient gene transfer to NK cells, including the importance of transfer vector and viral envelope, as well as the purity and ongoing proliferation of the NK cells. Under the correct conditions NK cells can be transduced using standard γ-retroviruses at efficiencies similar to CAR-T cells and recent studies have demonstrated transduction and transgene expression levels suitable for clinical use [66]. Engineering of NK cells at large scale remains a challenge for consistent manufacturing (as it does for T cells as well). Viral vectors are typically the most expensive raw material in the manufacturing process, and their production and performance can be inconsistent; moreover, the vectors used for gene deliver to NK cells have limited packaging capacity, limiting the complexity of genetic alterations that might be affected. New viral vector systems hold considerable promise for achieving more efficient and cost-effective genetic transgene delivery [67]. Successful transposon-based transgene delivery to NK cells has also been reported [68]; rapid advances in gene editing techniques will undoubtedly be rapidly applied to NK cells to expand the range of engineering possibilities for these cells.

NK cells were initially engineered over a decade ago with a first-generation chimeric antigen receptor [65]. Since then, multiple studies have shown that NK cell activity can be enhanced or redirected through expression of suitable transgenes, primarily chimeric antigen receptors [69]. Most of these transgenes have borrowed designs used in CARs designed for use in T cells, using the CD28 or 4-1BB costimulatory domains. While in some instances, costimulatory domains more characteristic of NK cell have been used, little systematic evaluation has been conducted to determine whether those domains that confer optimal function in T cells are identical to those that perform best in NK cells. One recent study by Li and colleagues compared the use of different transmembrane and costimulatory domains in NK cells derived from iPSCs [36], and showed that 'conventional' domains drawn from those used most frequently in T cells may not be best in NK cells. Undoubtedly there is a great deal of further exploration to be conducted before we fully understand how best to engineer chimeric receptors for use in NK cells.

In addition to conventional CARs, several groups have attempted to augment native signaling axes in NK cells by enhancing or altering the expression of the receptors

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that dominate NK cell responses. antibody-dependent cell-mediated cytotoxicity (ADCC) has been enhanced through forced expression of the naturally occurring low-affinity CD16 receptor, which binds IgG and triggers potent activity [70,71]. In the same vein, groups have focused on enhancing signaling through NKG2D, a receptor which binds 8 different ligands that are frequently upregulated in tumor cells [72,73]. Chimeric NKG2D receptors have been shown to broadly improve NK cells recognition of tumor cell lines; the low level of expression of ligands for this receptor in healthy tissues and their broad expression across both hematological and solid tumor malignancies makes this a promising avenue for NK cell clinical application.

Other aspects of NK biology have also been addressed through appropriate engineering. One of the greatest concerns in the clinical application of NK cells is their relatively short half-life when compared to T cells. While a population of adaptive or memory NK cells may exist, the bulk of NK cells have an effective half-life of 1–2 weeks *in vivo*. Limited persistence may have the beneficial effect of preventing long-term toxicities that have been sometimes associated with autologous T cell therapies, such as the prolonged B-cell aplasia observed in some patients treated with CD19-directed CAR-T cells [74]. There is, however, a concern that NKs may become exhausted too quickly during culture and after infusion, forestalling a consistent therapeutic benefit. To address this concern, several groups have engineered NK cells to express membrane-bound or secreted forms of IL-15 [75,76]. Expression of IL-15 extends the persistence of NK cells *in vitro* and *in vivo* and has been shown to improve the potency of the cells in animal models. Examples also exist of NK cells being engineered with dominant negative forms of the inhibitory TGFβ receptor to better resist tumor immune evasion [72,77]. Ongoing work in mouse models continues to reveal potential targets of genetic modulation to fortify NK tumor surveillance, including the suppressor of cytokine signaling 2 (SOCS2) [78] and the cytokine-induced SH2-containing (CIS) proteins [79].

PERSPECTIVES ON FUTURE DEVELOPMENT: PROMISE & CHALLENGES

Obstacles to the optimal development of NK cell therapies remain. Methods for expansion of NK cells are far more diverse than the methods that have been developed for T cells; while production of NK cells for early stage clinical study has become more tractable, it remains unproven that current methods will be suitable for late stage clinical development and eventual commercialization. Attaining truly consistent manufacturing processes and NK cell products will require continuous improvement in our understanding of the complexity of these cells and the dependence of final product potency and persistence on starting material and expansion methods. In particular we will need to further explore the potential for adaptive or 'memory-like' NK cells to control tumor growth and arrive at a more universal understanding of how best to select, characterize, and culture NK cell subsets with the greatest anti-tumor activity [24,63].

NK cells are well situated for allogeneic use. Like all allogeneic cell therapies, the efficacy of allogeneic

NK cells may depend on their ability to evade immune rejection by the patient's immune system. Most previous studies of allogeneic NK cells have been conducted in transplant settings, and have made use of haploidentical NK cells, typically derived from a close relative of the patient. While durable chimerism has been detected in some cases, more commonly NK cells achieve a peak of chimerism within 1–2 weeks following infusion and decline quickly thereafter [14]. Systematic study of the importance of the extent of HLA haplomatching to patient outcomes has not been conducted outside the transplant setting, but the enormous variety of HLA subtypes means that such matching will not be compatible with scaled manufacture of NK cells for off-the-shelf use. Nor is it feasible to lymphodeplete patients indefinitely without severe risk of infection and other serious adverse events. Thus, other approaches will be required to enhance NK cell persistence following infusion. Recent progress in masking T cells to patient immune responses may be applied to NK cells as well; genetic deletion of β-microglobulin, for instance, has been used to suppress HLA class I expression on the surface of embryonic stem cells, limiting one source of potential host versus graft responses [80]. As an alternate approach, molecules such as HLA-G have been overexpressed in to directly suppress immune response [81]. More extensive engineering of NK cells, which express both class 1 and class 2 HLA, may be required to limit immune rejection. Progress in this direction has recently been shown in an animal model of stem cell transplantation [82]; unquestionably the clinical application of allogeneic NK cells would also benefit by such approaches.

NK cells were discovered some 15 years after T cells, and for many years our understanding of the biology of these cells and their potential for therapeutic application has lagged behind that of T cells. That gap has begun to close however, as the pace of discovery in the NK field has steadily increased and several of the roadblocks to clinical development, including expansion, transduction, and effective engineering, have begun to fall. There is no doubt that the next decade of NK cell application will be bright.

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