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Review article

The NK-92 cell line—30 years later: its impact on natural killer cell research and treatment of cancer

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ABSTRACT

The NK-92 cell line, established in 1992, mirrors all the characteristics of highly active blood natural killer (NK) cells but with much broader and greater cytotoxicity. The cell line was established from the blood cells of a patient with lymphoma and has been made widely available for research since it was deposited into the American Type Culture Collection in 1998. The worldwide distribution of NK-92 cells has led to a plethora of scientific discoveries that have greatly increased the understanding of NK-cell biology. NK-92 cells also have been developed for clinical use, overcoming the challenges of obtaining and expanding NK cells from donor or patient blood. More than 100 patients with cancer have now been treated all over the world with unmodified or genetically engineered NK-92 cells. Modified cells include high-affinity Fc-receptor expressing NK-92 cells (haNK^{R}) and various chimeric antigen receptor targeted haNK cells (t-haNK^{TM}). Infusions of either unmodified or modified NK-92 cells have been reported to be safe and efficacious, leading in some cases to disease remission even in patients who had failed multiple previous lines of therapy. It is the purpose of this review to distill the plethora of scientific data on NK-92 cells and its genetic variants, highlighting relevant experimental findings that have contributed to a better understanding of NK cell biology and summarize the therapeutic potential of these cells for treatment of cancer and infections.

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Introduction

Cellular therapy for cancer is receiving some attention with the emergence of the ability to engineer T and natural killer (NK) cells to recognize cancer-specific surface molecules. Providing patients' own T cells with a chimeric antigen receptor (CAR) against CD19 expressed on lymphocytes has resulted in some long-lasting remissions of patients with lymphoma [1]. However, the process of generating patient-specific CAR/T cells is quite involved, requiring preparation time, carries the risk of the cytokine release syndrome (CRS; and immune effector cell−associated neurotoxicity syndrome [ICANS]) and also has some significant financial implications. Increasingly, blood NK cells are considered as targeted effector cells as they have fewer major histocompatibility complex restrictions and also express the Fc-receptor that can mediate antibody-dependent cellular cytotoxicity (ADCC) [2−4]. However, blood-derived NK cells have challenges to overcome to make them truly off-the-shelf cellular therapeutics. The continuously growing NK-92 cell line overcomes some of those challenges and has the additional benefit that it can be effectively engineered with CARs. This review summarizes the characteristics of this unique cell line, which has been available to the research community for almost 3 decades. The multitude of scientific research projects with NK-92 has advanced the knowledge on NK cell biology. Furthermore, NK-92 and its genetically modified variants have been given to patients with cancer, in some cases with remarkable responses.

Challenges of Using Blood NK Cells as Cellular Effectors in Cancer Treatment

Over the last few decades, there has been significant advancement of our knowledge concerning the biology of NK cells, which comprise about 10% of the circulating blood lymphocyte pool. A more comprehensive characterization, including gene and surface molecule expression, as well as proteomics, recently has become available [2−4]. It is now well accepted that NK cells are an important component of the rapid cellular immune response that can inhibit the cell-to-cell spread of malignant or infected cells. Not only can they execute immediate ("spontaneous") killing upon contact with those cells (without the need to be "primed" like T-cells), but they also produce a host of cytokines that support the expansion and function of other immune cells in the blood circulation and the tumor microenvironment.

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Through their FcγIII (CD16) receptor, NK cells are major effector cells of ADCC, which is the main effector arm for immunoglobulin G (IgG) 1 and IgG3 monoclonal antibodies (mAbs). Notably, NK cells that express the high-affinity (158V) variant of the CD16 receptor are more effective for mAb-mediated targeted cell killing, although only approximately 10% of the human population is homozygous for this variant [5].

The effector molecules that execute rapid NK cell−mediated target cell killing are perforin and granzymes and to a lesser extent FasL and tumor necrosis factor−related apoptosis inducing ligand (TRAIL) [6]. The former are released on contact with transformed cells and act within minutes, with perforin "punching holes" into the membrane of target cells so that granzymes can enter the cell and destroy it by degrading their genetic material. FasL and tumor necrosis factor−related apoptosis inducing ligand seem to have a more delayed cytotoxic effect through activation of the death receptor pathways on target cells [6].

Enriching NK cells from blood (peripheral or cord) either for research or clinical applications usually is done by negative selection employing a number of monoclonal antibodies that can deplete and eliminate non-NK cells (reviewed in Kundo *et al.* [7]). NK cells also may be enriched from blood, based on their characteristic CD56 (neuronal cell adhesion molecule) surface expression. One of the concerns is, however, that the bound antibody may affect the NK cell function and/or can interfere with any further manipulation (engineering) of the cells. Another limitation of such enrichment is the unpredictable variability of yield, particularly for NK cells from patients with cancer whose NK cells often are also dysfunctional due to circulating suppressive serum factors or following chemotherapy. A further challenge is that the NK-cell donors must be connected to a leukapheresis device for several hours to collect their white blood cells, of which only about 10% are NK cells. To achieve sufficient NK cells for therapeutic infusion, NK cells need to be expanded on a cell line feeder layer, which may be genetically altered. In addition, cytokines need to be included to support expansion (reviewed in Saito *et al.* [8]).

As an alternative to using a patient's own (autologous) NK cells, allogeneic NK cells can be collected from the blood of a healthy related or unrelated person. Those allogeneic NK-cell collections need to be further treated to remove T lymphocytes, as those can cause the potentially life-threatening graft-versus-host reactions after infusion. This additional purification step adds to the cost of the cell preparation and also results in a further loss of NK cells. Regardless of the source, blood NK cells need to be expanded to have sufficient numbers of cells available for therapeutic infusion [9]. Another challenge with blood-derived NK cells is the fact that those NK cells are more difficult to manipulate or engineer, requiring more complex, often virus-based gene transduction methods, which come with unpredictable and inconsistent efficacy [8].

Considering those drawbacks of blood-derived NK cells, including the costs of obtaining a consistent NK-cell population from blood for research and clinical applications, a continuously growing NK cell line is an appealing alternative. Although several human NK cell lines have been established [10], only the NK-92 cell line has shown consistently high cytotoxicity against a broad spectrum of cancer cells, and can easily be expanded *ex vivo* with a short doubling time of 24−36 h [11,12]. Importantly, NK-92 can be genetically engineered by plasmid electroporation to express a high-affinity Fc-receptor, CAR, or molecules that can modulate the tumor microenvironment [13,14].

Brief History About the Discovery of NK-92

The NK-92 cell line, named after the year of discovery (1992), was established in the author's laboratory at the British Columbia Cancer Agency in Vancouver, Canada, from mononuclear blood cells from a patient who had been diagnosed with an aggressive NK-cell lymphoma [11]. The patient had a relatively high number of circulating NK cells that displayed characteristics of early NK cells. To establish the cell line, various culture media were tested that were available at that time at the Terry Fox Laboratory in Vancouver, Canada, whose major scientific focus is on stem cell biology. The initial culture medium consisted of Minimum Essential Medium Alpha with 12.5% horse serum and 12.5% fetal calf serum in addition to other additives. The medium is commercially available under the name Myelocult (Stemcell Technologies, Vancouver, Canada). NK-92 cells are grown in suspension, forming clumps with individual cells being 10−12 micron in diameter.

Since animal-based media are unsuitable for any clinical development, efforts were made in the ensuing years to identify an animal-free medium that would maintain both optimal growth/expansion and functionality of NK-92. The X-Vivo 10 medium (Lonza Bioscience, Basel, Switzerland) supplemented with 5% human serum was found to meet those requirements. Of note, the medium in which the cells are maintained can affect the expression of surface molecules and the functionality of NK-92 cells. Since the American Type Culture Collection recommends—on their website—the use of the original culture medium containing horse and fetal calf serum, many researchers continue to use that particular medium. An example for the effect of the culture medium used is reflected in the surface molecule expression of NKp44, which is only expressed when NK-92 cells are maintained in culture medium that contains animal serum (Boissel, L. (2016)). Figure 1 summarizes the surface antigen profile of clinical-grade NK-92 when maintained in X-Vivo 10 with 5% human serum.

NK-92 cells express the full spectrum of activating receptors, with CD94/NKG2A and LIR1 being the only known inhibitory receptor expressed [14]. Most importantly, the cells are largely negative for killer cell immunoglobulin-like receptors (KIR), except for KIR2DL4 [14]. Since KIR receptors are known to inhibit cytotoxic activity when engaged with human leukocyte antigen (HLA), this may explain, to some extent, why NK-92 cells show only moderate immunogenicity in patients even after repeated infusions [15].

Because the original NK-92 cell line requires the presence of interleukin (IL)-2 (or IL-15) in the culture medium to support continuous growth and expansion, two IL-2−independent variants of NK-92 were generated by transfecting the gene for IL-2 into the parental cells [16]. The NK-92ci cell line was named after the pCEP4-LTR plasmid vector used to generate the variant, and the NK-92mi cell line was named after the MFG vector construct. Both constructs were transfected into the parental cells using particle mediated gene transfer (Bio-Rad's "gene gun"), a method that generally has a low transfection efficiency. However, since the transfected cells were left to grow in the absence of IL-2 in the culture medium, only those few successfully transfected cells expressing the IL-2 gene grew out and expanded. The selected NK-92ci and NK-92mi variants were able to produce IL-2 in sufficient quantities to maintain their own growth, expansion and cytotoxic activity [16] (Table 1). Of note, the NK-92mi cells produce and secrete significantly greater concentrations of IL-2 than the NK-92ci cells.

All three NK-92 cell lines were deposited into the American Type Culture Collection cell line repository (Manassas, VA, USA) and the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany). Researchers have had access to these cell lines since the mid-1990s. The wide availability of those cells and their use as a research tool have resulted in a plethora of scientific publications that not only have contributed to a better understanding of NK-92 cell biology but also to NK cell biology in general.

For example, the Johnston Space Center (Houston, TX, USA) used NK-92 cells to determine whether long space flights can impair NK-cell function [17]. Blood samples collected from astronauts returning from long space flights were co-incubated with NK-92 to measure whether the body would generate any blood/serum changes that

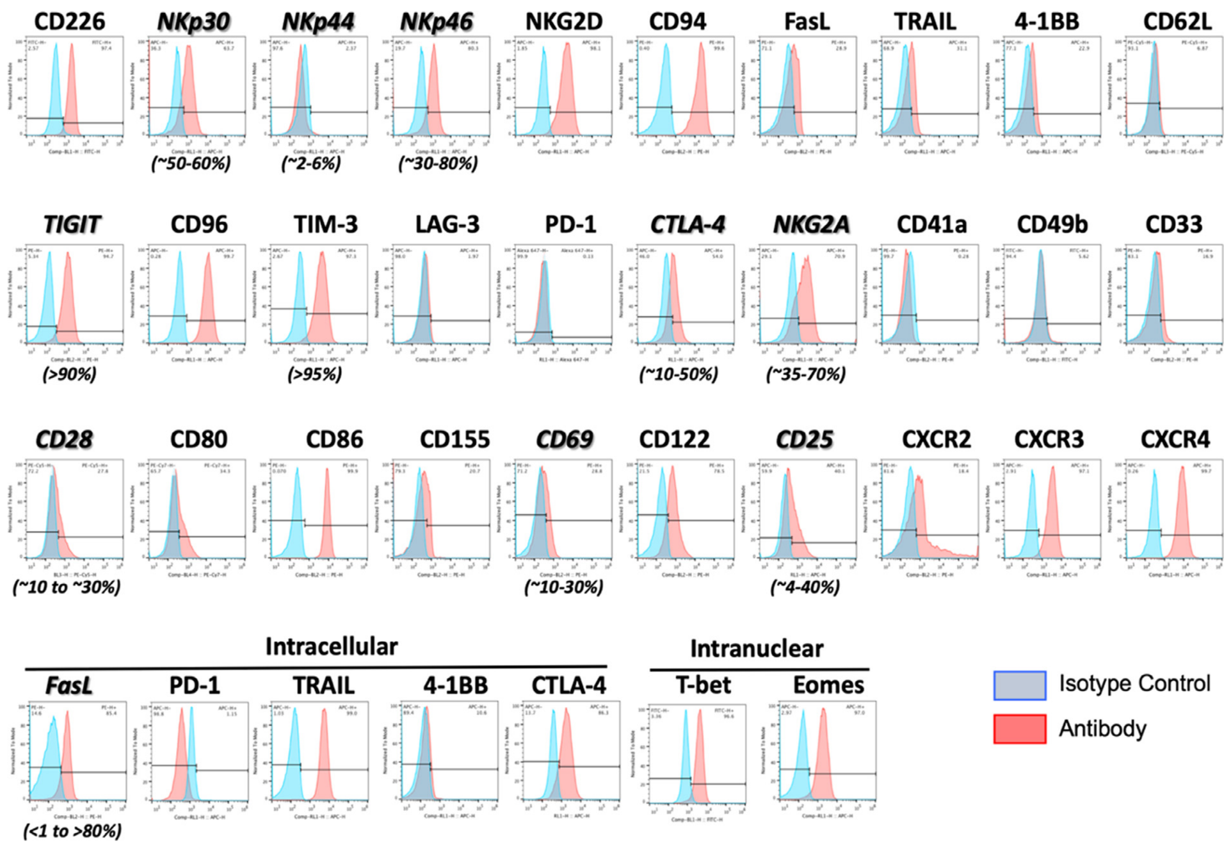

Fig. 1. Flow cytometric profile of NK-92 cells grown in X Vivo 10 medium with 5% human serum. The top three rows represent surface receptor profile. Intracellular and intranuclear protein expression is shown in the bottom row.

could negatively affect the function of NK cells. The study confirmed that this was not the case.

NK-92 cells show consistently high cytotoxic activity against malignant cells even in comparison with enriched and optimally activated blood-derived NK cells. There are several reasons for this broad and consistently high cytolytic activity: (i) NK-92 cells have a greater content of granzymes than blood-derived IL-2−dependent NK cells [15,16], hence the cytotoxic effect with NK-92 occurs at a much lower effector-to-target ratio compared with blood NK cells; (ii) NK-92 cells express only one of the inhibitory KIR family of receptors that silence NK cell function when they encounter unrelated (allogeneic) cells [13]; (iii) NK-92 express a number of activating receptors, like NKG2D as well as adhesion receptors (leukocyte function-associated antigen-1, intercellular adhesion molecule) that are relevant for engaging with tumor targets [14,16]; (iv) NK-92 cells do not display a loss of activity in a hypoxic tumor tissue microenvironment [18], and

Table 1
Secretion of human IL-2 by the parental NK-92 cells and two IL-2-transfected variants, NK-92ci and NK-92 mi.

	Duration of culture, h		
	24	48	72
Cell line	IL-2, pg/mL		
NK-92	1.7 ± 3.8	1.7 ± 2.1	2 ± 1.7
NK-92ci	9.3 ± 3.2	15.7 ± 1.5	21 ± 2.6
NK-92mi	550 ± 34	1260 ± 252	2248 ± 21

One million cells of each variant were cultured in 8 mL of IL-2 free medium and harvested 24, 48 and 72 h later. IL-2 levels were determined by enzyme-linked immunosorbent assay (average concentration ± standard deviation of three independent experiments) [16].
IL-2, interleukin-2.

(v) NK-92 can perform serial killing of target cells to a much greater extent that blood NK cells [19].

It was recognized early on that NK-92 cells could potentially be developed for the treatment of diseases, in particular cancer. Numerous studies *in vitro* and *in vivo* had confirmed that parental, nonengineered NK-92 effectively kill human cancer cell lines as well as primary cancer cells [20−24].

For successful development of the cells for patient treatment, two major issues had to be addressed: (i) the risk for lymphoma developing in recipients due to NK-92 cells originating from a patient with lymphoma and (ii) the issue of its potential rejection due to the allogeneic nature of NK-92. To address the first concern, *in vitro* studies were performed that confirmed that 1000 cGy of radiation was sufficient to completely arrest the proliferation of NK-92 cells [14,25]. When immuno-compromised NSG mice were injected subcutaneously with increasing numbers of NK-92 cells that had been irradiated with at least 500 cGy, no tumor outgrowth was seen, leading to the conclusion that 1000 cGy of radiation is sufficient and safe to prevent proliferation in patients. Importantly, the irradiated cells maintained full cytotoxicity for at least 16−24 h, including cytokine production. Hence, in all clinical studies conducted so far with the parental NK-92 cells or its engineered variants, the cells have been subjected to 1000-1500 cGy of irradiation before infusion.

To address the immunogenicity question, the UCLA Immunogenetics Center conducted experiments exposing allogeneic donor lymphocytes (separated into CD4 and CD8 cells) to NK-92. No significantly increased proliferation of T cells was noted and INF-γ production by T cells as a second readout for allogeneic stimulation was not significantly increased [unpublished data]. A relatively moderate allogeneic response was also seen in phase I studies in Toronto [15] and Frankfurt [26]. Despite repeated infusion, only six of 12 patients

in the Toronto study developed HLA antibodies against NK-92, and the mixed lymphocyte culture with patient lymphocytes as responder cells was negative in all patient samples. Similar results were reported in the Frankfurt study.

Largely because of its predictable fast growth characteristics (doubling time of 24−36 h) and ease of expansion, NK-92 cells have become “popular” effector NK cells for genetic engineering, particularly for introducing CARs. The groups of Dr. Winfried Wels and Dr. Torsten Tonn in Frankfurt, Germany, were the first to describe CAR-engineered clinical grade NK-92 cells using HER-2 as the CAR [27]. Some of the research with CAR-engineered NK-92 cells has been summarized in recent review papers [13,28-30]. Investigators used first- and second-generation CAR constructs generally in lentiviral or retroviral constructs. Although those studies confirmed the efficacy of CAR-modified NK-92, viral vectors are not ideally suited for clinical applications for safety and regulatory reasons. Consequently, current clinical trials with CAR engineered NK-92 cells by ImmunityBio Inc., which holds the worldwide rights to NK-92 and its variants—use non-viral, plasmid-based CAR gene constructs, transfected by simple electroporation.

Early Clinical Studies with NK-92

In 1997, the license and rights for NK-92 were transferred from the University of British Columbia to Rush Medical Center in Chicago, where in 2001 a phase I trial with NK-92 in patients with advanced cancer was conducted [31]. Due to the responses seen with LAK cell infusions in patients with renal cell cancer and melanoma [32], those two diseases were selected as initial indications for NK-92 infusions.

In that study, NK-92 cells were infused on days 1, 3 and 5 with the rationale that it usually takes a few days to trigger a T-cell−mediated allogeneic immune response that could potentially lead to rejection of the infused NK-92 cells. In this phase I dose-escalation study, 12 patients were enrolled and received a starting dose of 1×10^9 NK-92 cells/m^2. The greatest dose administered was 5×10^9 NK-92 cells/m^2 cells. None of the patients experienced grade 3 or 4 side effects, and only a few patients developed a mild fever or a rash. Although phase I studies are not designed to assess efficacy, it is noteworthy that the majority of the patients with renal cell cancer experienced a prolonged disease-free survival and overall survival compared with historical controls. The single patient with melanoma in that study had a partial response with significant tumor reduction after the third infusion. In contrast to CAR/T-cell therapy that generally includes some form of immunosuppressive chemotherapy before infusion, NK-92 cells in those and all other studies (including with CAR modified NK-92) were given without any preparative chemotherapy.

Contemporaneous with the aforementioned study, Dr. Torsten Tonn, who had previously performed post-doctoral work in the author’s laboratory, initiated a phase I trial in Frankfurt, Germany, in pediatric and adult patients with advanced, mostly solid cancers [26]. The schedule consisted of two infusions, given on day 1 and 3. The dose of 1×10^{10} cells/m^2 was considered dose-limiting, but not because of side effects, but rather because of logistical challenges of expanding larger numbers of NK-92 cells at that time.

Another phase I trial was conducted under the guidance of Dr. Armand Keating, at Ontario Cancer Center in Toronto [15]. Among the 12 patients with advanced hematologic malignancies enrolled in the study, two patients (with myeloma and Hodgkin disease) experienced long-lasting clinical remissions. Patients received multiple infusions over time (planned six monthly cycles). Only one-half of the patients developed HLA antibodies, and none had a positive mixed lymphocyte culture when NK-92 cells were used as stimulators.

In another phase I trial conducted at the University of Pittsburgh, seven patients with treatment-resistant acute myeloid leukemia were enrolled and received NK-92 [33]. Although none of the patients experienced serious adverse effects, no significant clinical responses were noted, prompting the group to investigate possible reasons for the lack of response. In a subsequent publication, they reported that acute myeloid leukemia cell−derived exosomes collected pre-therapy from all seven patients had the ability to inhibit the cytotoxic anti-leukemia effects of NK-92 in co-incubation assays [34].

Pooled response data from all 4 phase I studies reveal that approximately 36% of the treated patients showed some tumor reduction, ranging from tumor shrinkage to partial remission. Most importantly, repeated infusions with NK-92 cells, even at high cell numbers, did not induce any side effects greater than grade 2. In all trials the NK-92 cells were expanded in flasks, bags or G-Rex bioreactors using X-Vivo 10 medium with 5% human serum. For currently ongoing trials with engineered NK-92, the same medium is used but production is scaled up by use of large bioreactors.

The Next Generation of Engineered NK-92: haNK, taNK, t-haNK, qt-haNK

High-affinity fc-receptor expressing NK-92 (haNK)

Largely as the result of predictable rapid proliferation (doubling time of 24−36 h) and ease of expansion, several genetically engineered variants of NK-92 cells have been generated, including a line expressing a high-affinity Fc-receptor, haNK. NK cells are the main effector cells for mAbs of IgG1 or IgG3 type such as trastuzumab, rituximab or avelumab that engage the CD16 Fc-receptor on NK cells for ADCC. The Fc-receptor on NK cells can have low, intermediate or high affinity for IgG. However, only approximately 10% of the general population expresses the high-affinity Fc-receptor for mAbs on their NK cells, with the majority of people expressing a low- or intermediate-affinity Fc-receptor [5]. This also implies that the majority of the population lacks the most relevant effector mechanism for mAb-mediated cytotoxicity. Moreover, the Fc-receptor on blood NK cells is sensitive to the enzymatic cleavage by the ADAM17 enzyme, which can result in reduced efficacy of mAbs [35].

With these considerations in mind, haNK cells were generated from NK-92 that express a high-affinity Fc-receptor (CD16A, 158V) genetically linked to endoplasmatic reticulum IL-2 [35]. The endoplasmatic reticulum−linked IL-2 guarantees that only low amounts of IL-2 are secreted by haNK cells, but the intra-cellular concentration is sufficient enough to maintain cell viability, expansion and cytotoxicity. Importantly, the Fc-receptor on haNK cells is resistant to ADAM17-mediated degradation [35]. Moreover, studies from the National Cancer Institute have shown that haNK cells do not lose cytotoxicity under low oxygen (hypoxic) conditions, which is in contrast to blood derived NK cells [18]. Hypoxia is one of the significant immunosuppressive factors in the tumor microenvironment.

In a phase II clinical trial (NCT03853317) at the University of Washington, haNK cells were given in combination with Avelumab (anti-programmed death-ligand 1 [PD-L1] mAb) to patients with refractory Merkel cell cancer [36]. The haNK infusions were well tolerated, and despite the very advanced disease state of these patients, objective responses were seen in two of seven patients, including reversal of programmed cell death protein 1 (PD-1) refractoriness in one patient.

NK-92 cells have been further engineered to express a recombinant receptor containing the extracellular portion of the high-affinity Fc-receptor CD64 with the transmembrane and intracellular region of CD16A (referred to as CD64/16A). According to preliminary *in vitro* studies, ADCC is further improved over NK cells that express the CD16A variant [37].

The main mechanism of action for most clinically effective mAbs is through ADCC with direct cytotoxicity and complement-mediated cytotoxicity playing a less important role. For that reason, the high-

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affinity FcR expressing NK-92 cells continue to be used by numerous biotech companies and research laboratories for development and testing of mAbs (available from www.BrinkBiologics.com).

Targeted NK-92 (taNK) cells expressing CARs

The features of NK-92 cells have stipulated the generation of CAR-expressing variants. Table 2 lists the variants of NK-92 cells, including NK-92ci, NK-92mi, haNK, taNK, t-haNK and qt-haNK. Much of the research with CAR-engineered NK-92 cells has been summarized in review papers [38−40]. Investigators have used first-and second-generation CAR constructs generally delivered by lentiviral or retroviral constructs. Although several studies confirmed the efficacy of CAR-modified NK-92, ImmunityBio, Inc. (which now holds worldwide rights to NK-92 variants) developed CAR-modified NK-92 cells by use of plasmid-based CAR gene constructs and transfected by simple electroporation to generate the NK-92 variants currently used in clinical studies [38,41].

When CAR/T-cell therapies were being developed and the first clinical trial results were made available, it became clear that the high costs of producing them as well as the CRS and ICANS were sufficient motivation to explore NK cells and particularly NK-92 cells as an alternative source of CAR engineered cytotoxic immune cells.

In collaboration with the German group in Frankfurt, lentivirus-based transduction was used to generate a clinical grade NK-92 variant that expresses a CAR for HER2 (ErbB2) [42]. The group went on to start a clinical trial (NCT03383978) in patients with HER2-positive glioblastoma. Patients are receiving intracranial HER2 CAR expressing NK-92 cells injected into the resection margin during relapse surgery. The single-dose escalation part of the phase I study in nine patients has been completed, and no significant side effects were observed [43]. In the current extension of the study, a more permanent microcatheter is placed into the post-surgical cavity, and patients receive repeated injections of HER2 CAR-engineered NK-92 cells. Since approximately 40% of glioblastoma tumors are positive for HER2 expression, it will be important to see whether this treatment can prolong remissions and maintain quality of life for patients with this devastating brain cancer.

To further dissect why HER2 taNK cells kill their tumor target so effectively, the group at the University in Dresden, Germany, used special confocal microscopy for live imaging of NK-92 cells and its HER2 CAR variant. The purpose of the study was to determine the different steps of lytic granule movement in both cell lines [44]. They found that although unmodified NK-92 cells are able to form conjugates with Her-2−expressing cancer cells, their lytic granules do not polarize toward the synapse with cancer targets. In contrast, CAR-expressing NK-92 cells or haNK cells, expressing a high-affinity Fc-receptor in combination with a HER2 specific antibody, were able to effectively polarize their granules toward the synapse and release them for effective killing of the cancer target.

In addition to systemic infusion of NK-92 cells/variants, intratumoral injection has been studied. Our group has shown in a murine model that intra-tumor injection of CD19 CAR-modified NK-92 cells can induce regression of subcutaneous lymphoma [45]. Remarkably, upon re-challenge of the mice with the same lymphoma cell line, no tumor re-growth occurred. This strongly suggest that intra-tumor injection of NK-92 cells can induce a systemic memory-like immune response. In a murine glioblastoma model, intra-tumor injection of ErbB2 CAR-NK-92 cells the Frankfurt group confirmed that specific IgG antibodies against the tumor had developed in treated mice that together with T cells provided long-term protection against re-challenge with the same tumor [42].

Table 2
Nomenclature for modified/engineered NK-92 cell lines.

Name	Modification
NK-92/aNK	None: NK-92 = aNK
NK-92ci	NK-92 transfected with IL-2 expressing pCEP4 episomal vector
NK-92mi	NK-92 transfected with an IL-2 expressing MFG vector
haNK	NK-92 transfected with high affinity CD16 FcR and erIL-2−expressing plasmid
taNK	NK-92 transfected with CAR-expressing plasmid or lentiviral vector
t-haNK	NK-92 transfected with a CAR and erIL-2−expressing plasmid
qt-haNK	NK-92 transfected with a CAR, erIL-2 and a fourth gene-expressing plasmid

t-haNK (targeted−high-affinity NK-92) cells expressing a high-affinity FcR and a CAR

The t-haNK modification of NK-92 uses a polycistronic plasmid comprising FcR (158V) and erIL-2 as well as a first-generation CAR. In contrast to CAR/T cells, these t-haNK cells not only provide CAR-specific target cell recognition and killing but also have additional target cell killing mechanisms in place including spontaneous NK-mediated cytotoxicity as well as ADCC through their high-affinity FcR [38,41].

A number of clinical-grade t-haNK clones have been generated expressing the CARs for PD-L1, CD19, Her-2 and epidermal growth factor receptor (EGFR) [4,38]. PD-L1 t-haNK cells have been given to a number of patients with solid tumors in a phase I/II study. In some patients with triple-negative breast cancer and pancreatic cancer, some remarkable responses have been observed. Importantly, no significant side effects were noted, in particular no CRS/ICANS (ImmunityBio, internal data from ongoing studies).

The next generation of t-haNK clones introduces a fourth gene coding for a molecule of choice. In the CD19 t-haNK cell line, the gene for the *CCR7* homing receptor for lymphatic tissue was incorporated into such a quadrocistronic construct [46] (qt-haNK). Encouraging preclinical data including a reduction of human lymphoma growth were noted in NOD SCID mice compared with a CD19 t-haNK without CCR7. The quadrocistronic concept also allows to incorporate genes coding for proteins that can positively affect the tumor microenvironment such as a TGF-beta trap or IL-12 (ImmunityBio, internal data).

NK-92 Cells for Treatment of Infections

Although the majority of *in vitro* studies have tested the cytolytic/cytostatic activity of NK-92 against malignant targets (solid tumors and hematopoietic malignancies), numerous *in vitro* studies have shown that NK-92 cells also have anti-microbial/anti-viral activity. When NK-92 cells were co-incubated with normal donor B-lymphocytes that had been infected with Epstein−Barr virus, they readily killed the infected B cells but not the uninfected ones (Klingemann, unpublished). Similar cytotoxicity data were reported by other investigators for cytomegalovirus [47]. In addition to anti-viral activity, our group and others have shown that NK-92 can slow the growth of fungus such as *Aspergillus* and *Cryptococcus*, which can affect the respiratory system [48]. Further, the group at Taipei University reported killing of mycobacteria by NK-92 [49]. Somewhat surprising was the observation that NK-92 cells are also able to control parasitic infection. Baratin *et al.* [50] reported that NK-92 cells selectively bind to and kill erythrocytes that were infected with plasmodium parasites.

Commercial Development of NK-92 and Its Variants

Since grant funding to support the research and development of NK-92 in the academic setting was sparse to non-existent at that time, ZelleRx Inc. was founded in Chicago in 2002. In contrast to today, immunotherapy—and in particular the use of NK cells—had not garnered significant interest for therapy compared with the level that is seen now and it was challenging to grow the company.

In 2008, the company was renamed Conkwest. The global recession around that time made it difficult to find enough financial support to keep research and development of NK-92 active and productive. Fortunately, pharmaceutical companies continued to have an interest in the Fc-receptor−engineered NK-92 variants as a tool for their mAb development. In 2014, Dr. Patrick Soon-Shiong, who had worked with NK cells earlier in his career as a pancreatic surgeon, recognized the potential of the NK-92 cell line and acquired the company, which was renamed Nantkwest. The parental NK-92 cells were renamed aNK. Nantkwest went public in 2015 and after a reverse merger with ImmunityBio in 2021, became solely ImmunityBio.

Between 2014 and 2021, the NK-92/aNK variants *haNK, taNK* and *t-haNK* were generated in the company's R&D facility in Boston. Clinical-grade cell expansion facilities were established in Torrey Pines and El Segundo, California, which allowed to move treatment with haNK and t-haNK cells from the laboratory to the bedside. More than 500 infusions of NK-92, haNK, HER2-taNK or PD-L1 t-haNK have now been given to patients with cancer. NK-92 and its engineered variants continue to be central to ImmunityBio's cellular portfolio with new t-haNKs (CD19, HER2, epidermal growth factor receptor) entering clinical development.

Current research efforts at ImmunityBio are directed toward eliminating the need for radiation of the cells before infusion into the patients. The goal is to engineer the cells to have an "off switch" that can be triggered, if necessary, to eliminate the cells in the patient. Further, because NK-92 cells are allogeneic by nature, another goal is to also make them major histocompatibility complex "neutral."

Concluding Remarks

With the recent surge in interest in the development of NK cell−based immunotherapies, NK-92 cells and its engineered target-specific variants are well positioned to provide off-the-shelf cellular NK-cell therapies for patients with cancer and infections. Its wide distribution to the scientific community has resulted in a plethora of discoveries not just related to NK-92 but to NK cell biology in general. A further advantage of the NK-92 cells is their potential to generate cancer/target-specific variants by simple electroporation. Genetic manipulation has also allowed to clone NK-92 variants that simultaneously express a Fc-receptor and multiple CARs opening the door to targeted therapy of cancer and infections.

Funding

No funding was received.

Declaration of Competing Interest

Dr. Klingemann is co-founder of ZelleRx, an early-stage company that acquired the rights to further develop NK-92. He is equity holder in ImmunityBio, which is now the sole owner of NK-92 and its variants.

Author Contributions

Conception and design of the study: HK. Acquisition of data: HK. Analysis and interpretation of data: HK. Drafting or revising the manuscript: HK. HK has approved the final article.

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