NATURAL KILLER–CELL THERAPY

# Emerging Frontiers in Immunotherapy: The Promise of NK-Cell Therapies

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**THE LANDSCAPE OF** cancer therapy has been witnessing a paradigm shift with the advent of immunotherapy treatments, especially for patients with hematologic malignancies. Immunotherapies such as immune checkpoint inhibitors and autologous chimeric antigen receptor (CAR) T-cell therapies have led to considerable improvements in survival yet still come with efficacy limitations, manufacturing challenges, financial toxicity, and significant safety risks. Switching to an allogeneic approach could help overcome such limitations and allow for treatment of more patients with fewer donors.

Natural killer (NK)–cell therapies are increasingly being explored as an alternative and promising approach to immunotherapy. **Katy Rezvani, MD, PhD**, said NK-cell therapies are of interest as a possible "faster, cheaper, safer" alternative to CAR T-cell therapy.

"These cells have a lot of promise…[and they give] me a lot of hope that CAR NK cells could add to the armamentarium of what we have available for cancer immunotherapy," said Rezvani, a professor of medicine in the Department of Stem Cell Transplantation at The University of Texas MD Anderson Cancer Center in Houston, in an interview with *Targeted Therapies in Oncology*.

## The Charm of NK Cells

NK cells are part of the innate immune system and can target cancer cells that downregulate HLA class I molecules.[1] These cytotoxic lymphocytes are tasked with the surveillance of stressed cells,[2-4] making them "our first line of defense to virally infected cells and abnormal cells," Rezvani explained.

NK cells are of interest because they do not require full HLA matching, reducing the risk of graft-vs-host disease (GVHD) as well as lengthy manufacturing times for unique products.[1-5] This allows NK-cell therapies to be a more "off-the-shelf," readily accessible treatment compared with first-generation CAR T-cell therapy.

CAR T-cell treatments are also associated with other significant safety concerns, such as cytokine release syndrome (CRS) and immune effector cell–associated neurotoxicity syndrome (ICANS) toxicity, which are reduced with NK-cell therapies.[4,5]

NK-cell therapies are viewed as a possibility for overcoming the manufacturing times of CAR T-cell therapies and the large price tag associated with the treatment as well as the safety risks.[5] On the other hand, NK cells have a limited life span after transfusion.[1,4] They can be modified with genetic engineering to allow for greater efficacy.[4,5]

Sources for NK-cell production include cell lines, peripheral blood cells, umbilical cord blood, and induced pluripotent stem cells (iPSCs)—an emerging origin source.[4,6] Current research focuses most on cord blood–derived NK cells, which require donation and expansion. iPSC-derived NK cells do not require collection of cells from a donor.[6]

Clinical trials are beginning to show promise for CAR-NK-cell therapies, based on the benefits seen with CAR T-cell therapies.

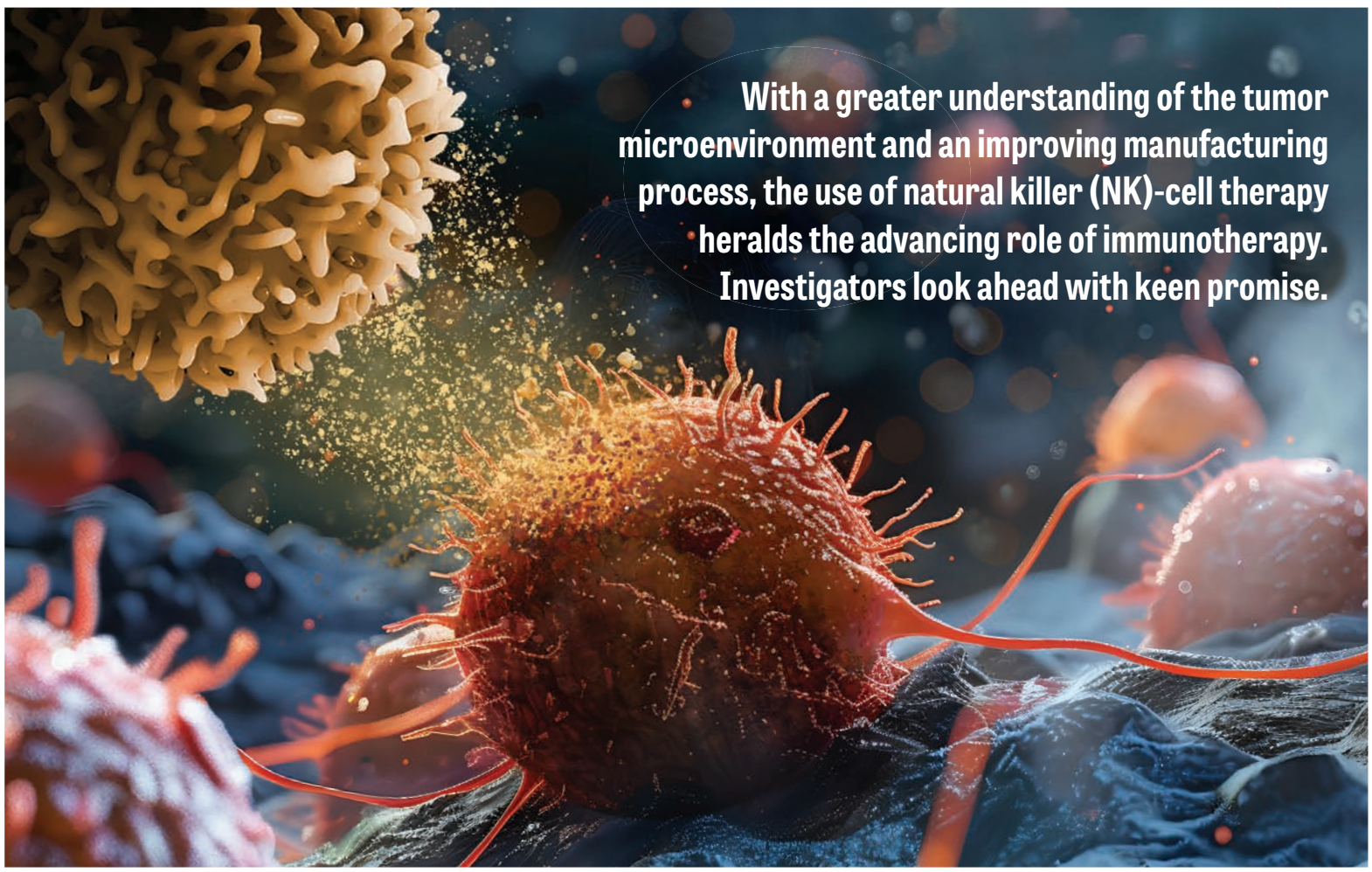
## NK Cells in Action

### *Anti-CD19 CAR-NK*

Although still in the early stages of discovery and study, NK-cell therapies are beginning to show promise in clinical trials for their safety and convenience, as well as for efficacy on par with current CAR T-cell therapies.[1] At the recent 65th American Society of Hematology (ASH) Annual Meeting and Exposition, findings were presented from early-stage studies of CAR NK-cell therapies showing their potential. Rezvani, the recipient of the E. Donnall Thomas Lecture and Prize, presented the latest research on this cellular therapy.

Rezvani highlighted studies of an anti-CD19 CAR-NK agent.[2] Following the success and approval of autologous anti-CD19 CAR T-cell products, a CAR NK-cell therapy derived from cord blood was created that was directed against CD19 for patients with CD19-positive malignancies; CAR NK cells did not require HLA matching with the recipient.[1,2]

In the phase 1/2 trial (NCT03056339), 11 patients with relapsed or refractory CD19-positive hematologic malignancies—5 with chronic lymphocytic



**With a greater understanding of the tumor microenvironment and an improving manufacturing process, the use of natural killer (NK)-cell therapy heralds the advancing role of immunotherapy. Investigators look ahead with keen promise.**

k_e_n (NK cell) - stock.adobe.com; background image by Jennifer Kralyevich/MJH Life Sciences using AI

lymphoma, 2 with diffuse large B-cell lymphoma, and 4 with follicular lymphoma, 3 of which were transformed—received the therapy in a single treatment. Objective responses were seen in 8 of 11 patients (73%) within a month of treatment, and all but 1 were complete responses (CRs). CAR NK cells were also detectable for up to a year after infusion.

No cases of CRS, neurotoxicity, tumor lysis syndrome, hemophagocytic lymphohistiocytosis, or GVHD were observed in any of the patients, according to investigators. Observed grade 3/4 adverse events (AEs) were predominantly hematologic.

The final result of the phase 1/2 study of this agent in 37 patients with relapsed/refractory CD19-positive B-cell malignancies showed an objective response rate (ORR) of 48.6% at both day 30 and day 100. CRs were seen in 29.7% of patients by day 100 and 37.8% by 1 year. Responses were seen at a median of 30 days and were durable for 9 of the 10 patients who had a CR.[2,7]

"The responses that we observed were pretty similar to what you would get with [autologous CD19 CAR] T cells," Rezvani noted.

Neither neurotoxicity nor GVHD was reported in any of the patients, but 1 case of CRS was observed.[7]

Rezvani stressed that donor selection was vital in this study and for future studies of cord blood–derived NK cells. Optimal cord blood was determined to be units that were frozen within 24 hours of collection and had a nucleated red blood cell count of less than 80 million.[6]

"[We found that] the most important determinants of who's going to respond or not was the quality of the cord blood that was used for the manufacturing of CAR NK cells," Rezvani said. "The impact that we ended up seeing was huge."

The rate of overall survival (OS) at 1 year was 94% in patients who received cord blood from optimal donors and 48% from those who received cells from suboptimal donors. The progression-free survival rates at 1 year were 69% and 5% for patients who received cord blood from optimal and suboptimal donors, respectively.[2]

Rezvani added that optimal cord blood could maintain long-term cytotoxicity and had greater in vitro proliferation compared with suboptimal cord blood.

Further, optimal cords had greater polyfunctionality vs suboptimal cords, which were characterized by a signature associated with hypoxia and exhaustion.[7]

### *Anti-NKG2D CAR-NK*

A phase 1 study (NCT04623944) presented at ASH showed promise for another CAR NK-cell therapy, NKX101, in patients with acute myeloid leukemia (AML).

The agent is composed of NK cells derived from healthy donors that were engineered to express a natural killer group 2D (NKG2D)–directed CAR and IL-15.[8]

The first cohort included 6 patients with relapsed/refractory AML who had received 3 doses of NKX101 per treatment cycle; 83% had poor-risk factors. Early responses were seen, with 67% of patients achieving a CR or CR with incomplete hematologic recovery (CRi). Two patients achieved minimal residual disease negativity after ▸

only 1 treatment cycle. Three patients were continuing treatment.

All 6 patients reported treatment-emergent AEs of grade 3 or higher, with myelosuppression and infection being most common.

Researchers reported no cases of CRS, ICANS, or GVHD of any grade were reported in the cohort. One case of a grade 5 AE was observed but was considered not related to treatment.

***NK Cell Engager***

A phase 1/2 study presented at ASH showed first-in-human data for the CD123 NK-cell engager SAR443579 in patients with relapsed/refractory AML, B-cell acute lymphoblastic leukemia, or high-risk myelodysplasia (NCT05086315). SAR443579 is a trifunctional anti-CD123 NK-cell engager that targets the CD123 antigen as well as engaging NKp46 and CD16a.[9]

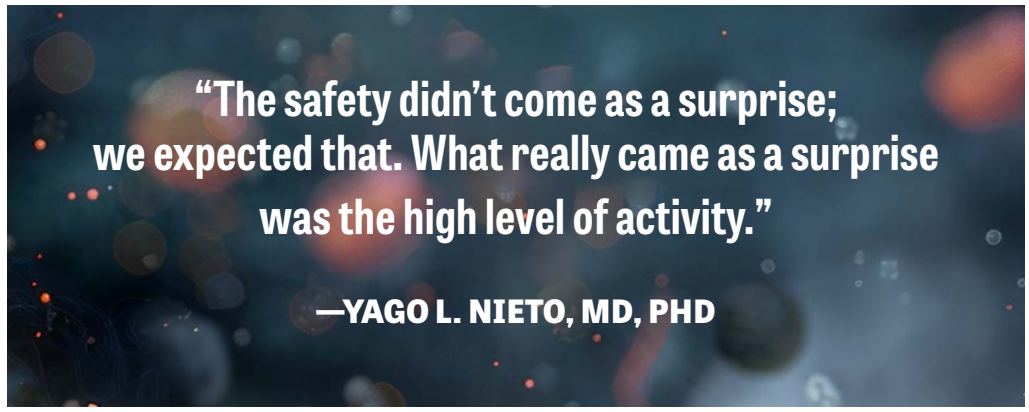
In the dose-escalation portion of the study, cytokine release syndrome (CRS) was reported in 33% of patients with AML who were treated with up to a maximum dose of 1000 µg/kg/infusion. Treatment was ongoing in 3 patients who achieved a CR.

Grade 3 or higher treatment-emergent AEs were reported in 60.5% of patients across dose levels and grade 5 events were seen in 11.6%, although all were considered not related to treatment with SAR443579. The most common events observed were infusion-related reactions (67.4%) and constipation (25.6%). CRS was reported in 2 patients but no cases of ICANS were observed. In June 2023, the agent received an FDA fast track designation for the treatment of patients with hematologic malignancies.[10]

***NK Cells in Combination***

During the ASH meeting, Yago L. Nieto, MD, PhD, presented findings from a phase 1/2 study of AFM13 (Acimtamig), a tetravalent bispecific antibody construct with CD30 and CD16a, in combination with cord blood–derived, cytokine-induced, memory-like expanded NK cells in patients with relapsed/refractory CD30-positive lymphomas (NCT04074746).[11]

A total of 42 heavily pretreated patients who were double refractory to brentuximab vedotin (Adcetris) and checkpoint inhibitors, most with Hodgkin lymphoma (88%), received NK cells 15 days before treatment with AFM13. The ORR was 93% and the CR rate was 67%. At the recommended phase 2 dose level (108 NK/kg), the ORR was 94% and the CR rate was 72%. Responses were reported in 97% of patients with classical Hodgkin lymphoma (n = 32) and CRs in 78%.




The OS rate in patients who received 2 cycles of treatment was 85% at 6 and 12 months. In patients who received 4 cycles, the OS rate was 87% at 6 months and 85% at 12 months.

In patients with Hodgkin lymphoma, the OS rate was 92% at 6 and 12 months for those who received 2 cycles of treatment and 85% and 82%, respectively, for those who received 4 cycles.

No cases of CRS, neurotoxicity, or GVHD were reported in the study; even infusion-related reactions were considered infrequent. Moderate neutropenia and thrombocytopenia were seen with the lymphodepleting chemotherapy.

“The safety didn’t come as a surprise; we expected that. What really came as a surprise was the high level of activity in the heavily pretreated patients with refractory tumors we treated,” said Nieto, a professor in the Department of Stem Cell Transplantation at The University of Texas MD Anderson Cancer Center in Houston during an interview with *Targeted Therapies in Oncology*.

He added that 6 of 7 patients who had a response subsequently consolidated with a stem cell transplant remained in CR at more than 18 months, making it an effective bridging therapy.

In September 2023, AFM13 in combination with allogeneic NK cells (AB-101) received an FDA fast track designation for the treatment of patients with relapsed/refractory Hodgkin lymphoma.[12]

Going forward, AFM13 is being explored in combination with AB-101 in patients with relapsed/refractory Hodgkin lymphoma and CD30-positive peripheral T-cell lymphoma in a phase 2 trial (LuminICE-203; NCT05883449). The combination is expected to augment the innate immunity of AFM13 alone to boost the antitumor cytotoxicity in patients with CD30-positive tumors.[12,13]

Nieto also explained that the AFM13 and NK-cell therapy model can be extrapolated to treat other malignancies by choosing an alternate bispecific antibody for the tumor type.

***iPSC-Derived, CAR-BCMA NK Cells***

An ongoing phase 1 trial (NCT05182073) of FT576, a multiplex-engineered, BCMA-targeted CAR NK-cell therapy, in patients with relapsed/refractory multiple myeloma has shown early promise and safety for the iPSC-derived agent as a monotherapy and in combination with daratumumab (Darzalex) (NCT05182073).[14]

No cases of CRS, ICANS, or GVHD were reported in the trial at any dose level with or without added daratumumab. Additionally, no dose-limiting toxicities or serious treatment-related AEs were observed.

Among 9 patients treated as of the interim report, responses were seen in 33% of patients and stable disease in 55%. One patient treated with FT576 monotherapy, who had received 5 prior lines of therapy and was triple refractory, achieved a very good partial response.

## The Path Forward for NK-Cell Therapies

As of now, efficacy with NK-cell therapies is considered similar to that of CAR T-cell therapies, although the studies of these agents is in the early stages. However, current constructs of first-generation CAR NK-cell therapies have limited long-term efficacy due to shorter in vivo persistence and cell exhaustion. To potentially improve the efficacy beyond that of autologous

approaches and even to generate efficacy in solid tumors, newer approaches are being considered, including engaging different targets, transducing T-cell receptor (TCR)–expressing NK cells, multiplexed engineering, and combination regimens.[2,15]

"The potential is huge [for] what we can achieve with these cells…. With our increasing understanding of NK biology, of access to big data, and also the engineering tools that we have available to use—not just CAR transduction, but for instance with TCR into NK cells, with CRISPR [clustered regularly interspaced short palindromic repeats] gene editing of your NK cells to make them more resistant to the impact of the tumor microenvironment, and combination with various drugs—I think the field could see major advances in a relatively short period of time," Rezvani said.

Targeting CD70 is showing promise as it is expressed in primary AML samples. Investigators created a number of second-generation CAR constructs to determine the most optimal one and found that anti-CD70 CAR NK cells with IL-15 allowed for the most superior antitumor activity of the various constructs in aggressive CD70-positive AML models.[16]

The construct is now being used in an ongoing phase 1/2 basket trial in patients with CD70-expressing hematologic malignancies (NCT05092451) and being explored across 3 dose levels.[2]

To date, NK-cell therapies have not seen the same success in solid tumors as in hematologic malignancies. This is believed to be because of the immunosuppressive tumor microenvironment that hampers NK cell activation and function.

Advances in understanding these barriers and developing strategies to overcome them are critical for enhancing the therapeutic potential of NK-cell therapies in solid tumors.[1,2,15]

TROP2 is a target of interest with NK cells to treat patients with solid tumors as it is overexpressed in many epithelial cancers but not in healthy tissues.[2]

The FDA has approved investigational new drug applications for the study of CAR TROP2/IL-15 NK cells delivered intravenously to patients with advanced solid tumors (NCT06066424) and delivered intraperitoneally to patients with ovarian cancer and pancreatic cancer (NCT05922930).

Genetically engineered New York esophageal squamous cell carcinoma 1 (NY-ESO-1)–targeted, TCR/IL-15–expressing cord blood–derived NK cells are being investigated in a phase 1/1b trial of patients with advanced synovial sarcoma and myxoid/round cell liposarcoma (NCT06083883) as well as in a phase 1 trial for patients with NY-ESO-1–positive relapsed/refractory multiple myeloma or plasma cell leukemia (NCT06066359). NY-ESO-1 is considered highly immunogenic and is expressed in many cancer cells but not in healthy tissue, making it an attractive target.

The phase 1b ADVENT-AML trial is exploring the use of allogeneic NK cells in combination with azacitidine and venetoclax (Venclexta) in patients with newly diagnosed AML (NCT05834244). The synergy of the regimen allows for upregulation of silenced NKG2D ligands, priming of leukemia cells, and reduction of disease burden.[17]

Multiplexed CRISPR gene-edited therapies are being created to design the safest and most effective products for patients. By employing CRISPR/Cas9 technology, multiple genes within NK cells can be simultaneously edited to enhance their persistence, cytotoxicity, and ability to navigate immunosuppressive barriers.[2,15]

A phase 1 trial is exploring the treatment of patients with recurrent glioblastoma with multiplex CRISPR gene-edited NK cells with deleted TGFBR2 and NR3C1 (NCT04991870).

With new, innovative approaches and clinical trials quickly emerging, the field of NK-cell therapies is surely one to watch. **TT**

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