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TOOLS & TECHNIQUES

INNATE HARMONY

By Lauren Martz, Senior Writer

On the heels of cancer immunotherapy's clinical success, NK cells — a long-known but underappreciated component of the innate immune system — are emerging as the hottest new tool to fight cancer, with the potential to be safer and more broadly effective than their adaptive immune system counterparts.

The excitement centers on the potential of NK cells to solve some of the challenges of chimeric antigen receptor (CAR) T cells, allowing companies to bring together the immune system's innate and adaptive arms in a more comprehensive approach to creating tumor immunotherapies.

"People who aren't in the field would certainly know about T cell CARs, but few people know about natural killer cells and the important role they play in the immune response against tumors. If you're looking for the next big thing to come down the road in cancer immunology, NK cells are certainly it," said Daniel Kaufman, professor of medicine and director of the Cell Therapy Program at the [University of California San Diego](#).

Unlike T cells, which recognize a specific antigen to elicit cytotoxic activity, NK cells can non-specifically find and destroy tumor cells and other abnormal cells. In addition, their mechanism avoids some of the key liabilities of T cell therapies.

Despite producing some dramatic clinical results in blood cancers, CAR T cells are still on shaky ground because their potential to overstimulate the immune system can lead to severe, possibly fatal, toxicities.

That vulnerability was underscored earlier this month when FDA placed a temporary clinical hold on [Juno Therapeutics Inc.](#)'s Phase II trial of its [JCAR015](#) CAR T therapeutic after two patient deaths from cerebral edema were reported. The trial — which restarted after five days, in patients not taking fludarabine — is evaluating [JCAR015](#) for relapsed or refractory acute lymphoblastic leukemia (ALL).

But NK cells don't carry cytokine-related dangers because they don't undergo *in vivo* clonal expansion like T cells. That expansion leads to a rapid increase in release of cytokines that can overwhelm the immune system.

"T cells can secrete cytokines that cause cytokine release syndrome and neurotoxicities, which haven't been associated

with NK cell fusions," said Nicolai Wagtmann, CSO of [Innate Pharma S.A.](#), a company developing bispecific antibodies to increase NK cell activity. He added that early clinical studies on NK cells showed no cytokine-related side effects.

The cells also have a manufacturing advantage over CAR T cells. They can be produced as an off-the-shelf product because they don't cause graft-versus-host disease (GvHD), and donor-sourced cells are actually more effective at killing tumors than patient-derived cells.

"A lot of people have hit upon NK cells as the next big thing because they can be done on a bigger scale without having to go through the individual patient collection required for CAR T cells," said Kaufman.

At least seven companies have NK cell therapies in clinical and preclinical development (see "Natural Killer Cell Therapeutics"). The most advanced product, [CNDO-109](#)-Activated NK cells, are donor NK cells primed with a cancer cell line cell lysate to activate the cells prior to delivery. [Fortress Biotech Inc.](#) has the cells in a Phase I/II trial for acute myelogenous leukemia (AML).

KILLING THEM SOFTLY

NK cells were discovered more than 40 years ago, but interest in them has undergone a resurgence in the last 10 years in oncology following the discovery that patients with impaired or depleted NK cells had a higher incidence of several cancers.

More recently, momentum has gained in the field, prompted in part by researchers looking for alternatives to the problems of using the adaptive immune system against tumors (see "Best of Both Worlds," page 7).

"Even though we and others have been doing NK cell-based therapies for the past 10 years, the excitement around CAR T cells is what has stimulated more interest in the NK cell field now, because they can potentially get around some of the limitations of CAR T therapies," said Kaufman.

NK cells target abnormal or stressed cells, such as cancer cells or pathogens, that meet two criteria. First, the target cells must express ligands that stimulate activating receptors on the NK cell surface. Second, they should lack [MHCI](#) molecules that bind inhibitory receptors on the NK cell surface. The latter is a protective mechanism that blocks

NK cells from destroying healthy self cells, because normal human cells express **MHCI** (see “Natural Death,” page 4).

When an NK cell finds a cell that meets the criteria, it induces cytotoxicity either by cell lysis or antibody-dependent cellular cytotoxicity (ADCC).

But as with T cell-based immunotherapies, tumors evolve to evade detection by NK cells, most commonly by shedding their NK cell-activating ligands.

For T cells, most strategies have been directed at re-engaging the adaptive immune system cells against tumors,

in particular via immune checkpoint inhibitors that relieve the suppression of tumor-targeting T cells.

For NK cells, one of the principal strategies is to deliver activated NK cells. In a 2005 study published in *Blood*, a group led by Jeffrey Miller at the [University of Minnesota](#) found the antitumor potency of NK cells in adoptive transfer could be improved by using allogeneic NK cells instead of autologous cells. The difference boiled down to a mismatch between the MHC molecules on the tumor and the NK cells, which brought the NK cells out of their inactive state. Miller

NATURAL KILLER CELL THERAPEUTICS

Select natural killer (NK) cell therapies and NK cell-directed antibodies in clinical and preclinical development for cancer. There are two primary modalities involving NK cells in development: adoptive cell therapies using allogeneic NK cells and antibodies that target NK cell receptors or ligands to increase antitumor activity of endogenous cells. *Source: BCIQ: BioCentury Online Intelligence; company websites*

MODALITY	COMPANY	PRODUCT	DESCRIPTION	INDICATION	STATUS
Cell Therapy	Fortress Biotech Inc. (NASDAQ:FBIO).	CNDO-109	Donor NK cells primed with cell lysate from the CTV-1 cancer cell line	Acute myelogenous leukemia (AML)	Phase I/II
	Glycostem Therapeutics	oNKord	Allogeneic NK cell therapy derived from umbilical cord blood	AML	Phase I
	NantKwest Inc. (NASDAQ:NK)	Activated Natural Killer Off-The-Shelf Cell (aNK Cell)	Donor NK cells lacking killer inhibitory receptors (KIRs) to prevent tumor evasion	AML; lung cancer; lymphoma; melanoma; Merkel cell carcinoma; kidney cancer	Phase I
		High-affinity Natural Killer cells (haNK cells)	Donor NK cells modified to express high-affinity Fcy receptor III (FCGR3; CD16), which promotes antibody-dependent cell cytotoxicity (ADCC)	Cancer	Preclinical
		Target-activated Natural Killer cells (taNK cells)	Activated NK cells engineered with one or more chimeric antigen receptors to target cancers	Cancer	Preclinical
	Celgene Corp. (NASDAQ:CELG)	Natural killer (NK) cells	NK cell therapy derived from placenta and umbilical cord blood	Cancer	Preclinical
	Fate Therapeutics Inc. (NASDAQ:FATE)	Adaptive NK cells	A long-lived and high-potency NK cell subpopulation expressing a maturation marker and cytomegalovirus-induced memory-like activating receptor killer cell lectin like receptor 2 (KLRC2; NKG2C)	AML	Preclinical
		iNK cells	Induced pluripotent stem (iPS) cell-derived NK cells	Cancer	Preclinical
	NantKwest Inc.; Sorrento Therapeutics Inc. (NASDAQ:SRNE)	Chimeric Antigen Receptor Tumor-attacking NK cells (CAR.TNK)	NantKwest's NK cell line modified a CAR targeting programmed cell death 1 ligand 1 (PD-L1; B7-H1; CD274) to increase tumor-homing	Cancer	Preclinical
		CAR.TNK	NantKwest's NK cell line modified a CAR targeting receptor tyrosine kinase-like orphan receptor 1 (ROR1) to increase tumor-homing	Cancer	Preclinical
Ziopharm Oncology Inc. (NASDAQ:ZIOP)	NK cells	Adoptive NK cell therapy	Cancer	Preclinical	

MODALITY	COMPANY	PRODUCT	DESCRIPTION	INDICATION	STATUS
Antibodies	Affimed N.V. (NASDAQ:AFMD)	AFM13	Bispecific antibody targeting Fcγ receptor IIIa (FCGR3A; FcγRIIIa; CD16A) on NK cells and CD30 on cancer cells	Hodgkin's lymphoma; lymphoma;	Phase II
		AFM22	Bispecific antibody targeting FCGR3A on NK cells and a mutant form of epidermal growth factor receptor (EGFR) on cancer cells	Head and neck cancer; solid tumors	Preclinical
		AFM24	Bispecific antibody targeting FCGR3A on NK cells and EGFR on cancer cells	Colon cancer; head and neck cancer; lung cancer; solid tumors	Preclinical
	Innate Pharma S.A. (Euronext:IPH); Bristol-Myers Squibb Co. (NYSE:BMJ)	Lirilumab	mAb targeting killer cell immunoglobulin-like receptor two domains long cytoplasmic tail 1 (KIR2DL1), KIR2DL2 and KIR2DL3 to prevent NK cell inactivation	AML; solid tumors; hematologic malignancies	Phase II
	Innate Pharma S.A.; AstraZeneca plc (LSE:AZN; NYSE:AZN)	Monalizumab	mAb targeting NK cell inhibitory receptor killer cell lectin-like receptor subfamily C member 1 (KLRC1; CD159a; NKG2A)	Head and neck cancer; ovarian cancer; chronic lymphocytic leukemia (CLL); solid tumors	Phase I/II
	Innate Pharma S.A.	IPH4102	Humanized cytotoxic antibody targeting killer cell immunoglobulin-like receptor three domains long cytoplasmic tail 2 (KIR3DL2; CD158K)	Cutaneous T cell lymphoma (CTCL)	Phase I
IPH4301		Therapeutic antibody targeting NK cell-activating ligands MHC class I polypeptide-related sequence A (MICA) and MICB	Cancer	Preclinical	

is a professor of medicine in the division of hematology, oncology and transplantation at the university.

More recently, companies and academics have extended the finding by improving the protocols for allogeneic adoptive transfer or using antibodies to reactivate and redirect a patient's NK cells to his or her tumor.

IMPROVING TRANSFER

Researchers have found three major flaws in the early adoptive transfer methods: tumors can still inactivate the NK cells; cell survival is too short to elicit a meaningful antitumor response; and the cells might not be able to find the tumors efficiently.

Although the early methods increased NK cell activation *in vivo* by treatment with cytokines such as IL-2, they produced inflammatory risks and proved dangerous. The method has been replaced by priming NK cells *ex vivo* — a strategy used widely among companies in the space.

For example, Fortress Biotech primes its NK cells *ex vivo* with a cancer cell line lysate.

Second, the short lifespan in the circulation has limited the efficacy of some NK cell therapies, according to Scott Wolchko, president and CEO of [Fate Therapeutics Inc.](#)

Fate has a collaboration with Miller, who discovered a subset of NK cells — dubbed adaptive NK cells — that persists longer than most NK cells *in vivo* and has a high level of cytotoxicity against solid tumor cells.

Last year, Fate and the [University of Minnesota](#) formed a partnership to develop the adaptive NK cells and off-the-shelf NK cell therapy products derived from engineered induced pluripotent stem (iPS) cells. The adaptive NK cells could be tested in patients as early as next year, and the off-the-shelf engineered NK cells could be tested in the clinic within two years.

[NantKwest Inc.](#) is using repeated dosing to overcome low persistence of its aNK cell therapy, which is in Phase I testing for lung cancer, melanoma, lymphoma and acute myelogenous leukemia (AML).

Kaufman told BioCentury that repeat administrations are particularly important for NantKwest's product because the cells are developed from an NK tumor cell line, which needs to be irradiated and inactivated before being given to a patient. That "dramatically decreases their activity because cell survival is shorter after that process," he said.

NATURAL DEATH

NK cell activity is dictated by activating and inhibitory receptors on the cell surface that work together to distinguish tumor cells from healthy cells. NK cell attack is triggered when stimulatory ligands engage activating receptors, and inhibitory receptors are unbound by MHC I molecules.

(1) Although **healthy cells** express **stimulatory ligands** that would otherwise activate an **NK cell**, **MHC I** molecules on the surface of healthy cells put the brakes on NK cell-mediated lysis by binding **inhibitory receptors** on the immune cells.

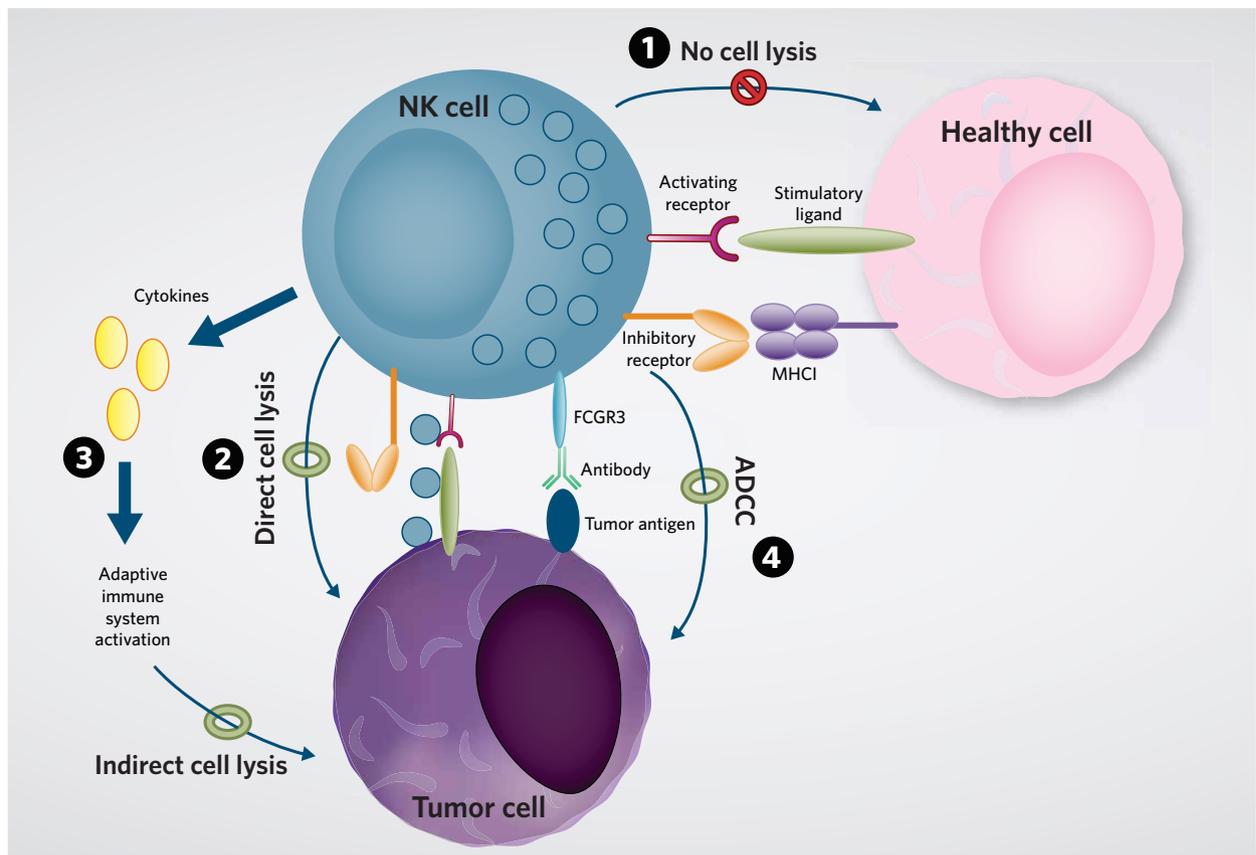
(2) By contrast, most tumor cells express stimulatory ligands but not MHC I molecules, prompting NK cells to release cytotoxic granules (**blue circles**) that

directly lyse tumor cells.

(3) Activated NK cells also indirectly induce tumor cell lysis by releasing **cytokines** that activate the **adaptive immune system**, generating a specific antitumor response.

(4) NK cells use antibody-dependent cellular cytotoxicity (ADCC) as a third mechanism to kill cancer cells. NK cells express the antibody-binding receptor FCGR3. ADCC is initiated when FCGR3 binds an antibody that recognizes a **tumor antigen**.

FCGR3 (CD16) - *Fcγ receptor III*; MHC I - major histocompatibility complex class I



NantKwest is also investigating other NK cell-based platforms that could be more potent, including one designed to increase NK cell homing to tumors. In 2014, the company partnered with [Sorrento Therapeutics Inc.](#) to jointly develop Chimeric Antigen Receptor Tumor-attacking NK cells (CAR.TNKs), which incorporate CARs into NantKwest's NK cell lines to help direct the cells to their targets.

In addition, NantKwest is modifying NK cells with a high-affinity variant of the activating receptor [FCGR3](#) to boost the cells' natural ability to induce ADCC.

According to Adi Hoess, CEO of cancer immunotherapy company [Affimed N.V.](#), adding a tumor-targeting component could be a difference-maker.

"Just giving the body a surplus of NK cells, but not using an antigen-specific approach, might not be as effective as a more tumor-directed strategy," Hoess said. "Even if you load patients with NK cells that are primed to kill, there is no guarantee that these cells will reach the tumor. In their natural state, they lack a steering wheel."

CHECKPOINT NK

Companies such as Innate Pharma and Affimed are focusing on reactivating and redirecting a patient's NK cells to kill the tumor by developing antibodies that either stimulate activating NK receptors or block inhibitory NK receptors.

"Right now in immuno-oncology, the most promising therapies are checkpoint-blocking antibodies. It will be interesting to see if blocking NK cell checkpoints will have similar potential," said Wagtmann.

Innate Pharma is developing mAbs that block [KIRs](#) and other inhibitory ligands. NK cells express three KIR receptor types on their cell surface that engage [MHCI](#) molecules on normal cells and suppress activation. Innate's anti-KIR mAb [lirilumab](#) was licensed to [Bristol-Myers Squibb Co.](#) in 2011 and is in Phase II testing for multiple cancers.

In addition, Innate Pharma and other companies are developing bispecific antibodies that activate NK cells and direct them to the tumor. The company partnered with [Sanofi](#) earlier this year to develop two bispecifics targeting the [NCR1](#) activation receptor and two undisclosed tumor antigens selected by [Sanofi](#).

Affimed has three bispecific antibodies targeting [FCGR3A](#) on tumor cells: [AFM13](#), which binds the tumor target [CD30](#), is in Phase II for Hodgkin's lymphoma; and [AFM22](#) and [AFM24](#), which target mutant and wild-type [EGFR](#), respectively, are in preclinical testing.

New studies are uncovering additional NK cell receptors that could be targets for antibodies. In May, a team at the [Walter and Eliza Hall Institute of Medical Research](#) published in *Nature* a study showing that knocking out the NK receptor [SOCS](#) decreased tumor growth in mice.

"If you're looking for the next big thing to come down the road in cancer immunology, NK cells are certainly it."

Daniel Kaufman, UCSD

KILLER COMBINATIONS

Stakeholders who spoke with BioCentury agreed that NK cells will likely find their place in immuno-oncology as components of combination therapies with other products, in particular those acting via the adaptive immune system.

Innate Pharma's Wagtmann told BioCentury the cells could be the piece of the immuno-oncology puzzle the field has been missing.

"The T cell field is driving interest also in innate immune system modulators, and there is definitely cross-fertilization between the two subsets. I think combining both arms of the immune system may be the answer to some of our questions about how to drive more potent cancer immunotherapy responses," he said.

Henry Ji, co-founder, president and CEO of Sorrento, added that combining the highly specialized and specific CAR T cells with the general, first-line-of-defense NK cells could optimize treatment. "I don't see NK cells as an alternative to T cells, but as synergistic," he said.

Affimed is already looking at combination possibilities. "When we combine our NK bispecifics with checkpoint inhibitors, we are seeing synergies based on true cooperativity between the innate immune system and adaptive immune system. There is a cross-talk between NK cells and T cells, enhancing the uptake of NK and T cells by the tumor," said Hoess.

But he noted there remains much more biology to learn, and said NK cells are still "lagging behind the T cell field by about 10 years."

In particular, Hoess thinks more information is needed about the different NK cell subsets and how tumors protect themselves from NK cells.

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Nicolai Wagtmann, Innate Pharma

"We also still need to learn, by treating different sets of tumors, where NK cells can work the best. With checkpoint inhibitors, we're learning that tumors with immune cell infiltration are the most highly responsive to immunotherapy. We need to study totally immune cell-naïve tumors that don't respond to checkpoint inhibitors to see what barriers prevent immune cells from moving in there, and how we can overcome them," said Hoess. ▮

COMPANIES AND INSTITUTIONS MENTIONED

Affimed N.V. (NASDAQ:AFMD), Heidelberg, Germany

Bristol-Myers Squibb Co. (NYSE:BMJ), New York, N.Y.
Fate Therapeutics Inc. (NASDAQ:FATE), San Diego, Calif.
Fortress Biotech Inc. (NASDAQ:FBIO), New York, N.Y.
Innate Pharma S.A. (Euronext:IPH), Marseille, France
Juno Therapeutics Inc. (NASDAQ:JUNO), Seattle, Wash.
NantKwest Inc. (NASDAQ:NK), Cardiff-by-the-Sea, Calif.
Sanofi (Euronext:SAN; NYSE:SNY), Paris, France
Sorrento Therapeutics Inc. (NASDAQ:SRNE), San Diego, Calif.
University of California San Diego, La Jolla, Calif.
University of Minnesota, Minneapolis, Minn.
U.S. Food and Drug Administration, Silver Spring, Md.
Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia

TARGETS AND COMPOUNDS

EGFR - Epidermal growth factor receptor
FCGR3 (CD16) - [Fcy receptor III](#)
FCGR3A (FcyRIIIa; CD16a) - [Fcy receptor IIIa](#)
IL-2 - Interleukin-2
KIRs - Killer cell immunoglobulin-like receptors
MHC - Major histocompatibility complex
MHCI - Major histocompatibility complex class I
NCR1 (NKP46; CD335) - Natural cytotoxicity triggering receptor 1
SOCS (CISH; CIS) - Suppressor of cytokine signaling

REFERENCES

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