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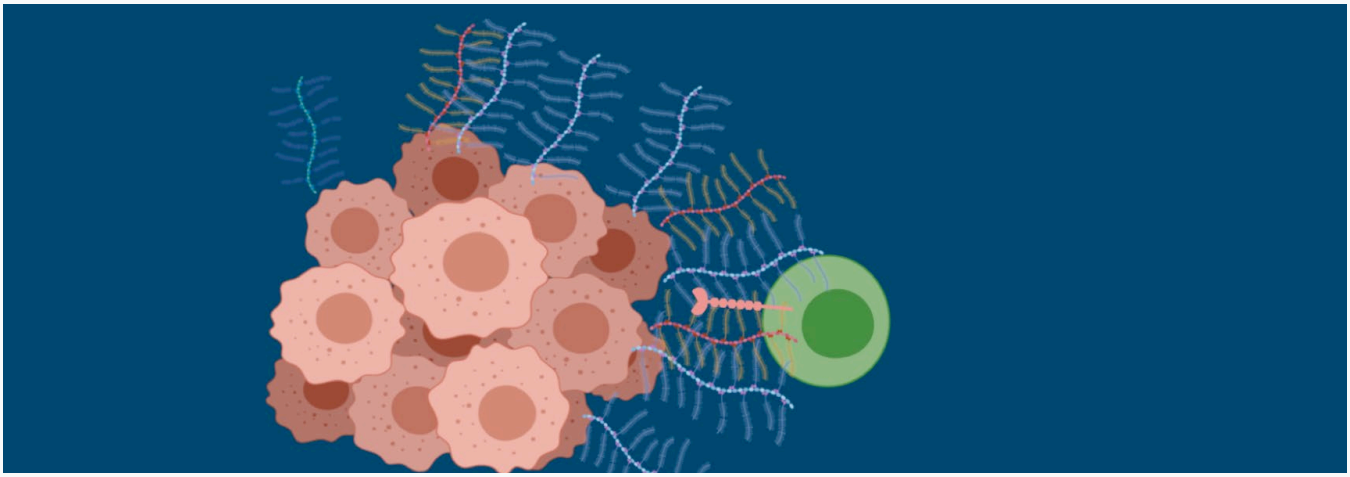


BIOCENTURY & BIORENDER

TARGETS & MECHANISMS

SIGLECs go from homing beacons to next-generation checkpoints

BY KAREN TKACH TUZMAN, ASSOCIATE EDITOR

Propelled by growing understanding of their biology, and an expression pattern that goes well beyond T cells, SIGLEC proteins are rising as the next set of checkpoint targets to challenge PD-1. The sugar-binding proteins could prove useful outside of cancer as well.

Two members of the family, CD22 (SIGLEC2) and CD33 (SIGLEC3), have already spawned commercial products as cell-surface markers that direct antibody-based therapies to kill B cell and myeloid cancers, respectively.

That strategy gained momentum last year when shares of Allakos Inc. (NASDAQ:ALLK) more than tripled in value on takeout rumors following positive Phase II GI data from its SIGLEC8-targeting mAb AK002. The therapy triggers antibody-dependent cytotoxicity (ADCC) against SIGLEC8-expressing eosinophils and mast cells that drive inflammatory diseases like eosinophilic gastritis (see "Allakos Sustains Gains on Strength of GI Readout").

Advances in chemical tools and disease models have paved the way for a new set of therapies that use SIGLECs (sialic acid-binding immunoglobulin-like lectins) as switches to boost or dampen the activity of immune cells, rather than deplete them (see Figure: "SIGLECs: More than GPS Coordinates").

The majority of SIGLECs suppress immune activation via mechanisms similar to the T cell checkpoint PD-1, but have the potential to act on a wider range of cell types, including NK cells and myeloid cells.

"We think that this is actually a dominant mechanism of immunosuppression because the SIGLECs are expressed in a very broad way," said Jim Broderick, CEO and founder of Palleon Pharmaceuticals Inc. "This is a broader spectrum checkpoint pathway than the first generation."

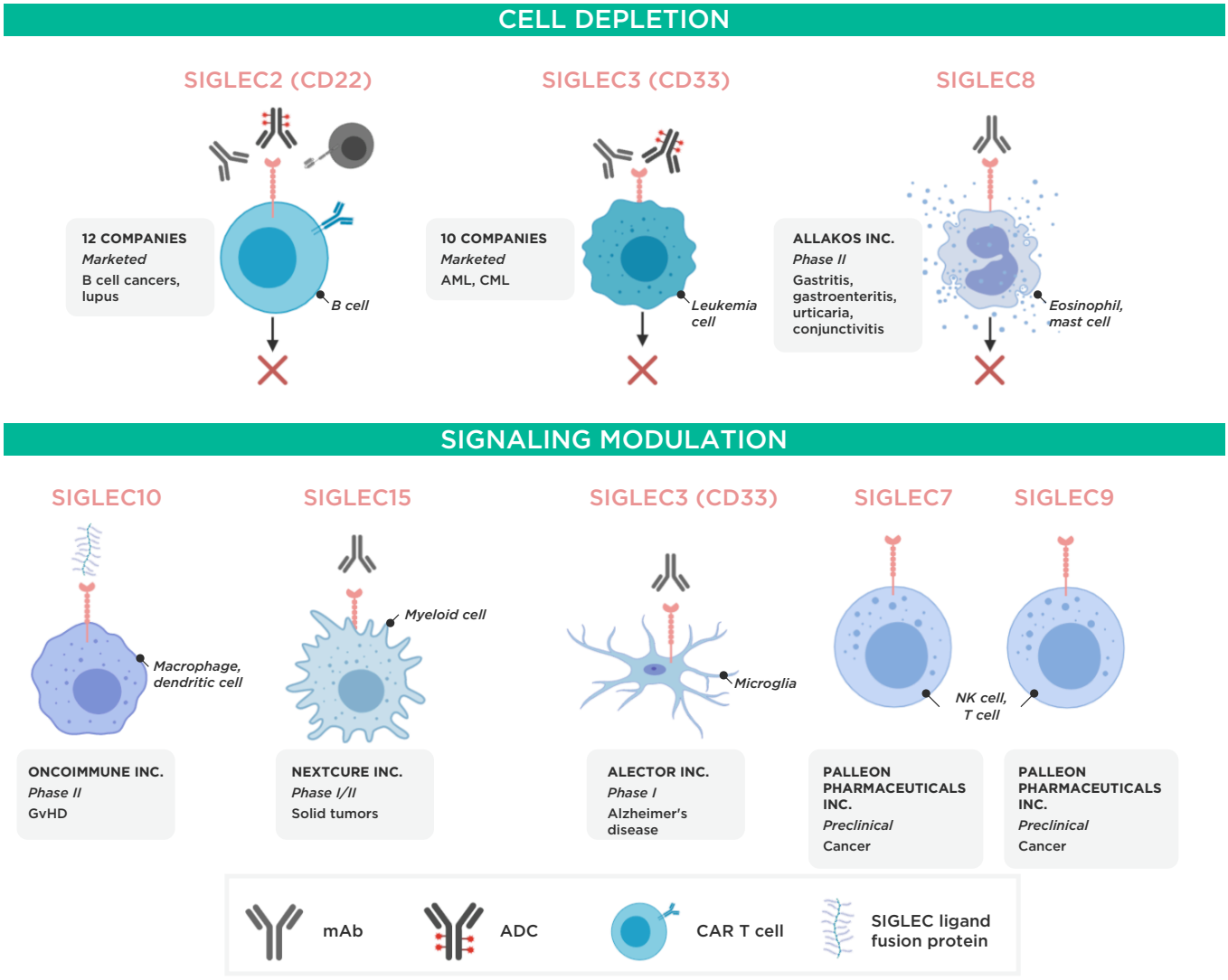
New territory: signaling modulation

Palleon's lead program is a preclinical tumor-targeted enzyme that shaves every SIGLEC's ligands from cancer cell surfaces. The company believes the product could have single-agent efficacy. Palleon is also inhibiting individual SIGLECs via blocking

SIGLECs: more than GPS coordinates

Sialic acid-binding immunoglobulin-like lectins (SIGLECs), a family of cell-surface proteins expressed on immune cells, have largely been used as markers to target pathogenic cell types for depletion (**top**) via mAbs, antibody-drug conjugates (ADCs) and CAR T cells. Now, a new set of therapies is emerging that use mAbs or fusion proteins to modulate signaling via a wide range of SIGLEC proteins (**bottom**). For each target, the most advanced development stage is listed.

Targets: CD22 (SIGLEC2); CD33 (SIGLEC3); SIGLEC7 - Sialic acid binding Ig like lectin 7; SIGLEC8 - Sialic acid binding Ig like lectin 8; SIGLEC9 - Sialic acid binding Ig like lectin 9; SIGLEC10 - Sialic acid binding Ig like lectin 10; SIGLEC15 - Sialic acid binding Ig like lectin 15;



antibodies, a strategy being pursued by at least three other companies in a range of indications.

One of these, NextCure Inc. (NASDAQ:NXTC), drew attention in November with the first clinical data from a SIGLEC inhibitor, its stock rising and falling within a week as more data were announced.

NextCure's shares went up $65.79 (248%) on data from its anti-SIGLEC15 mAb NC318 in non-small cell lung cancer (NSCLC) patients, where results were comparable to early clinical data from Keytruda pembrolizumab and Opdivo nivolumab, the anti-PD1 mAbs from Merck & Co. Inc. (NYSE:MRK) and Bristol-Myers Squibb Co. (NYSE:BMY), respectively.

Then to now

The cell surface expression patterns and endocytic nature of CD22 and CD33 made these initial SIGLEC targets natural fits for antibody-drug conjugate (ADC) therapies against hematological cancers.

Those includes the first FDA-approved ADC, Mylotarg gemtuzumab from Pfizer Inc. (NYSE:PFE), which targets CD33 and is marketed for acute myelogenous leukemia (AML). Pfizer and partner UCB S.A. (Euronext:UCB) also market the anti-CD22 ADC Besponsa inotuzumab for acute lymphoblastic leukemia (ALL). Dozens of other companies have CD22- and CD33-targeting mAbs, ADCs or cell therapies in development.

"THIS IS A BROADER SPECTRUM CHECKPOINT PATHWAY THAN THE FIRST GENERATION."

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NextCure lost more than half those gains a few days later, its share price dropping to $39.02, when it presented data from additional patients that brought the observed response rate (ORR) down to 15% from 27%, and its disease control rate to 46% from 71%. But even then, the single therapy data were in the range that Keytruda showed in the early days, where Phase I data gave it an ORR of 9-33% and a disease control rate of 40-70%. NextCure's share price is now $56.45 (see "Updated Data Bring NextCure Back to Earth").

Other companies, including OncoImmune Inc., are working in the opposite direction, co-opting SIGLEC ligands to develop immune-tolerizing therapies for diseases driven by excess inflammation. OncoImmune's founders first showed SIGLEC10 binding to the glycoprotein CD24 induces immune tolerance in a 2009 *Science* study. The company is in the process of launching a Phase III pivotal trial for its CD24Fc fusion protein in graft-versus-host disease (GvHD).

Key questions include the trade-offs of targeting all SIGLECs versus individual ones, and how the membrane localization and endocytosis of SIGLEC proteins will affect therapeutic outcomes.

"The family of SIGLEC receptors are clearly thought to be critical players in regulation of immune cell responses, but the details are far from worked out," James Paulson, a professor of chemistry and molecular medicine at Scripps Research and SIGLEC pioneer, told BioCentury.

"Long before SIGLECs were known to be sialic acid-binding receptors, they were known to be specifically expressed on myeloid cells and B cells," said Paulson.

More recently, SIGLECs have been recognized for their role in preventing immune responses against mammalian tissues, which, unlike microbial pathogens, express SIGLEC ligands.

"Since sialic acids don't really exist in the microbial world, we developed this pattern system to sense the self, which turns off immunity to minimize collateral damage," said Broderick.

In the last five years, the success of checkpoint inhibitors and advances in glycobiology tools have catalyzed an explosion of research showing cancer cells exploit this mechanism of self/non-self discrimination to evade immune recognition.

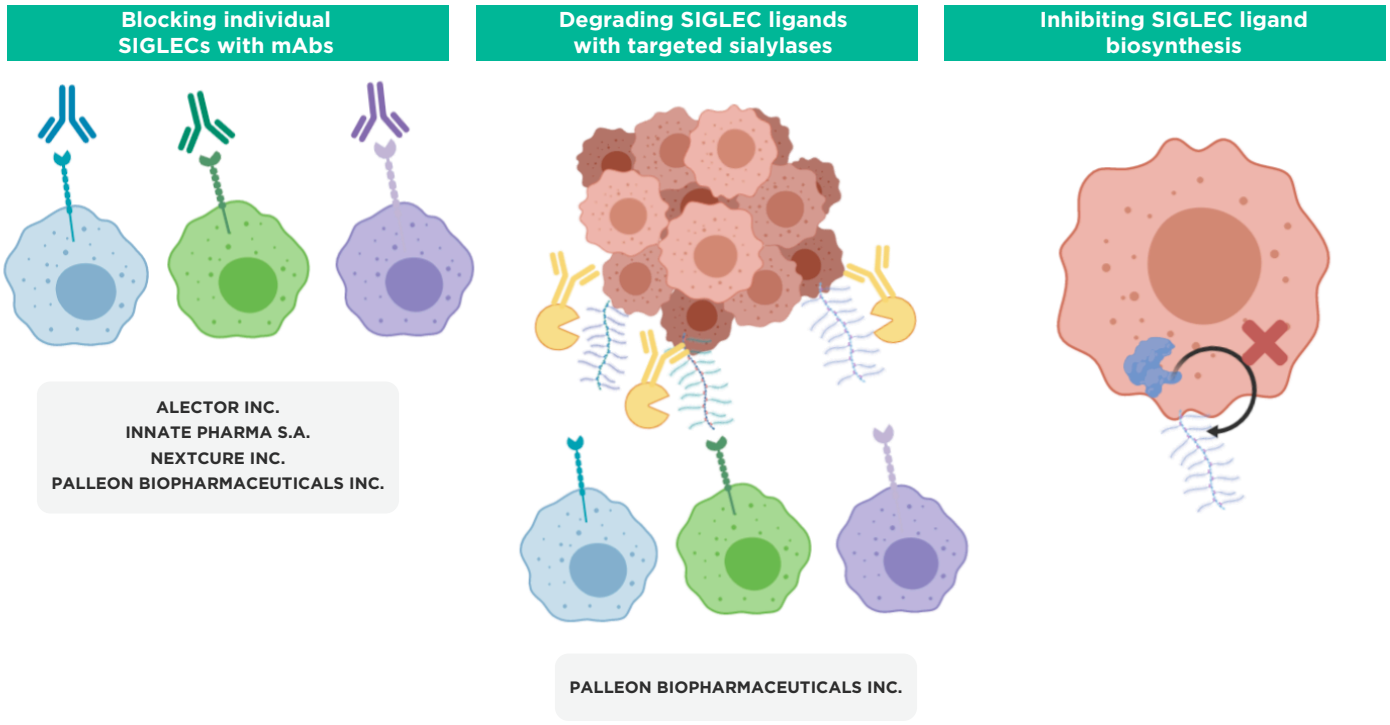
Like PD-1, most of the 15 SIGLEC proteins expressed by human cells have an immunoreceptor tyrosine-based inhibition motif (ITIM) in their intracellular domain, which opposes immune-activating kinase signaling by recruiting the phosphatase SHP-1.

Paulson said that SIGLECs are "basically the same kind of receptor" as PD-1. "They're both members of the immunoglobulin superfamily, they have Ig domains that recognize ligands on other cells — it's just that the ligands in this case happen to be sialic acids," said Paulson.

A key breakthrough in understanding SIGLEC function came from the lab of Palleon founder Carolyn Bertozzi, a professor of chemistry and

Sugar block

At least three strategies have emerged to inhibit sialic acid-binding immunoglobulin-like lectin (SIGLEC) signaling triggered by sialylated sugar ligands: using mAbs to block individual SIGLECs; shaving ligands for all SIGLECs from cancer cell surfaces by fusing tumor-targeted mAbs to sialylase enzymes (yellow); and blocking synthesis of SIGLEC ligands by inhibiting sialyltransferase enzymes. The latter approach has been described in academic studies but is not the focus of any disclosed company programs.



chemical and systems biology at Stanford University. In a 2014 study, her team described a chemical tool to coat living cells with specific glycans, and used it to showed SIGLEC7 ligands protected tumors from NK cell immunity.

"She was able to do these 'IF/THEN' experiments: if I coat the cell with these glycans, what happens from an immune perspective?" Broderick said. He said the field was also bolstered by the growing availability of transgenic mice and human-derived *in vitro* systems that could capture the biology of human SIGLECs not conserved in mice.

Role in tumors, and beyond

According to Broderick, the results explain the longstanding correlation between tumor expression of sialylated glycoproteins and poor prognosis, and the observation that tumor sialylation is elevated in syngeneic mouse cancer models with functional immune systems, but not in xenograft models, which are engineered to be immune-compromised.

A slew of studies have added to the evidence supporting a role for SIGLECs in immuno-oncology, including a 2018 paper from the lab of Heinz Läubli at the University of Basel, showing SIGLEC9 upregulation impairs tumor-infiltrating lymphocyte (TIL) function, and a March study from the lab of NextCure founder and Yale immunobiology professor Lieping Chen that showed SIGLEC15 on tumor-associated macrophages (TAMs) suppressed T cell responses to cancer.

"By different means, different groups have identified SIGLECs as potential players in cancer immunotherapy," said Läubli.

Innate Pharma S.A. (Euronext:IPH; NASDAQ:IPHA) has joined the space. The company presented data on its preclinical SIGLEC9 mAb at

last year's American Association for Cancer Research (AACR) meeting (see "NK Cell Check-in").

NextCure CSO Sol Langermann said the company's mAb against SIGLEC15, which doesn't have ITIM domains, has a different mechanism of action than other SIGLEC-targeting mAbs. Instead of blocking intracellular signaling downstream of SIGLEC15, the mAb prevents SIGLEC15 from binding an unidentified receptor on T cells that induces immunosuppressive signals. The company is investigating the identity of the receptor.

> "SHE WAS ABLE TO DO THESE 'IF/THEN' EXPERIMENTS: IF I COAT THE CELL WITH THESE GLYCANS, WHAT HAPPENS FROM AN IMMUNE PERSPECTIVE?"
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> JIM BRODERICK, PALLEON

Another emerging area for SIGLEC inhibitors is Alzheimer's disease (AD), where genetic and functional studies suggest CD33 signaling in microglial cells prevents clearance of amyloid plaques. Alector Inc. (NASDAQ:ALEC) is leading with an anti-CD33 mAb AL003 in Phase I testing; Paulson said at least three undisclosed companies are developing CD33 inhibitors for the disease.

Paulson's lab aims to promote CD33 signaling to treat allergic diseases. In a January study, the company showed liposomes containing CD33's ligand plus an allergen decreased allergic responses in a mouse model and human tissue samples.

The SIGLEC10/CD24 axis is also gaining traction as a therapeutic target, beyond OncoImmune's GvHD program, with a study from the lab of Forty Seven Inc. (NASDAQ:FTSV) founder Irv Weissman suggesting it could be another "don't eat me" signal (see: "Weissman's Latest 'Don't Eat Me' Signal").

OncoImmune CEO and founder Yang Liu said the company plans to launch a clinical trial testing CD24Fc in metabolic disorders, and is exploring inhibiting the pathway in cancer.

Innate Pharma and Alector did not return requests for comment in time for publication.

Control over sugar

The dominant strategy among companies developing SIGLEC inhibitors is to block the receptors using mAbs, but there are at least two alternative approaches that instead target their sialylated sugar ligands (see Figure: "Sugar Block").

Palleon is developing both receptor- and ligand-targeting platforms; the former uses targeted mAbs, and the latter deploys a fusion protein that connects a tumor-targeting antibody to an enzyme that cleaves all sialylated glycans, blocking signaling via all SIGLECs simultaneously (see "Sweeter Checkpoints").

Broderick told BioCentury the company is prioritizing the enzyme-based approach because it has the ability to prevent immunosuppression in a broader range of cell types, and it overcomes the challenges posed by heterogeneity and redundancy in SIGLEC ligands.

He likened the company's approach to targeted degradation therapies, which send proteins considered "undruggable" by standard approaches to enzymes that can break them down.

"Here we're just bringing an enzyme to the target," Broderick said. "The SIGLEC ligand is 'undruggable' by conventional means because of the vast structural heterogeneity, and the fact that SIGLECs bind multiple ligands."

A third approach involves preventing the generation of SIGLEC ligands by inhibiting sialyltransferases. A 2018 paper from a Dutch group described a sialic acid mimetic that blocked sialyltransferase activity in tumor models, but no companies have disclosed programs using the approach.

Läubli said the consequences of reducing all sialic acid residues at once are not yet clear, particularly since the approach could impact the function of adhesion molecules that bind sialylated glycoproteins. That might have the added benefit of reducing tumor metastasis, but could also change the way lymphocytes traffic to tumors, he said.

Langermann thinks targeting a single SIGLEC introduces fewer safety risks than targeting the whole class. "What we like about our SIGLEC15 program is it's very specific: it's targeted to a very specific protein that we know plays a role in immunosuppression."

Broderick said Palleon's enzyme approach has shown single-agent activity in multiple preclinical cancer models, and a wide safety window in non-human primates.

"Desialylation of a tumor exposes neoantigens and damage patterns that were masked," he said. "It's a removal of a mask, but if you have nothing to hide, there's nothing to be worried about." bc

TARGETS

CD22 (SIGLEC2)
CD33 (SIGLEC3)
PD-1 (PDCD1; CD279) - Programmed cell death 1
SHP-1 (SHPTP1; PTPN6)- Src homology protein tyrosine phosphatase 1
SIGLEC7 - Sialic acid binding Ig like lectin 7 (SIGLEC7)
SIGLEC8 - Sialic acid binding Ig like lectin 8 (SIGLEC8)
SIGLEC9 - Sialic acid binding Ig like lectin 9
SIGLEC10 - Sialic acid binding Ig like lectin 10
SIGLEC15 - Sialic acid binding Ig like lectin 15

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