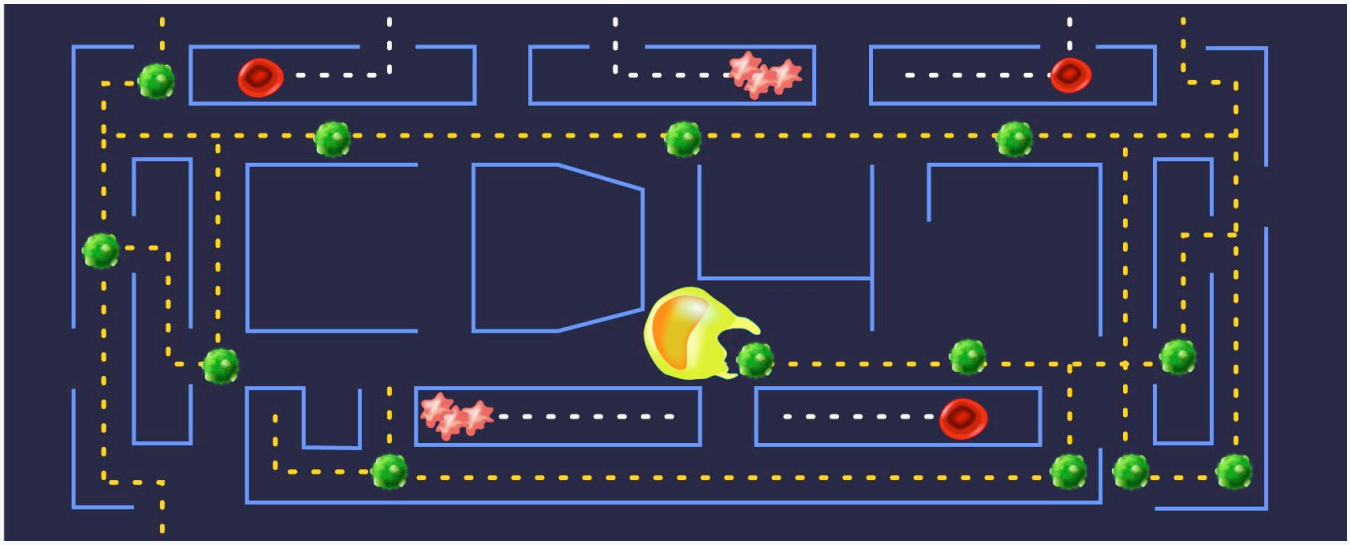
BIOCENTURY

REPRINT FROM AUGUST 12, 2019



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PRODUCT DEVELOPMENT

Eat this, don't eat that: CD47 companies' first hurdle

BY KAREN TKACH TUZMAN, ASSOCIATE EDITOR

The rush to drug CD47 shows no signs of abating, with five companies launching clinical programs for cancer this year and three moving forward on the back of encouraging Phase I data. The differentiator may be in which company develops the cleverest strategy to tell macrophages which cells to eat and which to leave alone.

Having briefly occupied the limelight as the next PD-1, CD47 is moving to the harsh light of day as companies work out how to capitalize on its broad potential in cancer, without falling foul of the safety issues that have already downgraded at least one candidate.

Interest in the target was kicked off by findings from Irv Weissman's lab at Stanford University, which demonstrated anti-tumor effects of blocking CD47, a "don't eat me" signal that prevents cells from being engulfed by macrophages. The idea is that preventing the interaction between CD47 on tumor cells and SIRPA, its binding partner on macrophages, would relieve a blockade on innate antitumor immunity, similar to the way PD-1 inhibitors release the brakes on antitumor T cells (see "Forty Seven and Counting").

Weissman co-founded Forty Seven Inc. in 2015. Since then, at least 11 other companies have announced CD47 programs, and at least 24 compounds are in development targeting CD47 or SIRPA, according to BioCentury's BCIQ database.

Weissman, a professor of pathology and developmental biology, is also director of the Institute of Stem Cell Biology and Regenerative Medicine at Stanford.

However, early clinical studies, in particular by Surface Oncology Inc., have revealed dose-limiting hematological toxicity to be a sticking point for the target.

Surface saw two neutropenia-related dose-limiting toxicities at a lower than expected dose in an all-comer Phase I trial of its anti-CD47 mAb SRF231, and has deprioritized its program. Celgene

"THE HEMATOLOGICAL TOXICITY IS NOT A CLASS EFFECT, IT'S A MOLECULE EFFECT."

SOPHIA RANDOLPH, ALX ONCOLOGY

Corp. discontinued a Phase I trial of its anti-CD47 mAb CC-90002 in myelodysplastic syndrome (MDS) and acute myelogenous leukemia (AML), but has not disclosed data.

Other companies have also reported toxicity due to excessive elimination of red blood cells (RBCs), platelets or neutrophils.

The problem is that because CD47 is ubiquitously expressed, healthy cells act as antigen sinks that soak up anti-CD47 mAbs, which means high doses are needed to target tumors. But those high doses also suppress the "don't eat me signal" on blood cells, prompting macrophages to engulf them and causing hematological toxicity.

According to Surface CEO Jeff Goater, animals haven't been a viable testing ground to tease out differences in toxicity.

"You have a set of antibodies all more or less looked similar from a preclinical perspective. You moved them into the clinic, and they all look very different," said Goater.

Forty Seven, Trillium Therapeutics Inc. and ALX Oncology Ltd. have each reported clinical data they see as proof-of-concept for their compounds (see Table: "CD47's Clinical Report Card"). The next two years should see more results from the latest wave of entrants to the clinic.

All ten companies with clinical-stage compounds have disclosed strategies to solve the toxicity, with a common theme being addition of a second component that creates a tumor-specific "eat me" signal. This leverages the fact that binding to CD47 alone is not enough to induce cancer killing; it needs to be coupled with active stimulation of phagocytosis from an anti-CD47 mAb's Fc domain or another source, either of which can be delivered separately, in a more tumor-specific fashion.

At least three companies with clinical-stage CD47 inhibitors are also developing candidates against SIRPA, whose narrower expression profile reduces tox risk (see Sidebar: "The Eater's Side").

Surface and Celgene have not completely abandoned their CD47 programs. The former is testing whether lower, more frequent dosing could reduce the neutropenia risk, and sees an opportunity for the compound in transplant conditioning; the latter is continuing a Phase I study of the compound for advanced solid tumors and hematological cancers. Celgene did not return requests for comment.

Weighing independence

What strategy a company chooses will determine whether it can pursue the product as a monotherapy, or whether its candidate will require a partner compound. Competitors run the spectrum, with some prioritizing single agent activity at the cost of more limited dosing or indication options, and others entirely focused on combos for greater flexibility on both counts (see Figure: "Restrictive Diet").

ALX's approach only works as a combination. Its ALX148 contains an inactive Fc domain, and requires co-delivery of antibodies against tumor antigens to provide macrophages with Fc receptor-driven "eat me" signals.

"The antibody provides that activating signal in a tumor-specific way, which leads the macrophage only to the cancer cell," said CMO Sophia Randolph.

Forty Seven's anti-CD47 mAb 5F9 uses an IgG4 Fc domain, which CEO Mark McCamish said triggers phagocytosis but not antibody-dependent cellular cytotoxicity (ADCC), making it less likely to cause off-tumor toxicity. While the company has seen some single agent activity, it is banking on combinations with tumor-targeting mAbs, chemotherapy or checkpoint inhibitors to maximize efficacy.

"The signal we got from monotherapy, from an FDA registration approach, was not adequate," said McCamish. "We had to come up with ways to augment the pro-phagocytic signals on the tumor itself."

Trillium is aiming to create a monotherapy. Its lead candidate TTI-621 uses an IgG1 Fc domain, which strongly induces killing of tumor cells but is more prone to killing platelets, in particular. The company is tuning dosing and delivery to mitigate toxicity.

"We've made no secret of the fact that we think the IgG1-bearing CD47 blockers are the highest likelihood for monotherapy activity, so

we're continuing to pursue that with TTI-621, both intratumorally and intravenously," said CEO Bob Uger.

Forty Seven's compound is a mAb against CD47, while ALX's and Trillium's compounds are bivalent SIRPA fusion proteins.

ALX wants company

ALX thinks that the advantage of ALX148 is that it separates blockade of "don't eat me" signals from instigation of "eat me" signals. "When you have an asset that has those two functional ends on the same molecule, you lose your specificity," Randolph said.

The company has shown that swapping an active Fc domain into ALX148 induces anemia and thrombocytopenia in preclinical models. "The hematological toxicity is not a class effect, it's a molecule effect," said Randolph.

She says its strategy could open indications that would be challenging for a CD47-targeting agent alone. "In the solid tumor space, we can dose higher, and not be limited by hematological toxicity."

She also believes ALX148 will be better positioned for use in earlier rounds of therapy and in combinations with other agents known to have hematological toxicity.

Randolph added ALX has looser clinical trial enrollment criteria than competitors with regards to pre-treatment hemoglobin levels and prior transfusions.

CD47's clinical report card

At least three companies have reported clinical efficacy and toxicity data from antibodies or fusion proteins against CD47. Adverse event rates represent sums across all grades of toxicity. **Trillium Therapeutics Inc**. (TSX:TRIL; NASDAQ:TRIL) data is from IV administration of TTI-621. Two more companies have reported issues with their programs, but not detailed data. **Celgene Corp**. (NASDAQ:CELG) terminated a Phase I trial of CC-90002 in patients with acute myelogenous leukemia (AML) or myelodysplastic syndromes (MDS) when "preliminary monotherapy data in relapsed/refractory AML and high-risk MDS did not offer a sufficiently encouraging profile for further dose escalation/expansion." A separate Phase I trial in combination with Rituxan rituximab is ongoing. **Surface Oncology Inc.** (NASDAQ:SURF) said it would not open dose expansion cohorts for SRF231 after seeing two hematologic dose-limiting toxicities in the first 18 patients treated in a Phase I trial; the trial in solid and hematological cancer patients is ongoing to test other dosing schedules.

ORR = overall response rate; CR = complete response; PR = partial response; SD = stable disease; ALT = alanine aminotransferase, increase associated with liver damage; NR = not reported; (A) Forty Seven reported Complete Remission with Incomplete Hematologic Recovery (CRi), morphologic leukemia-free state (MLFS)/ marrow complete response and hematologic improvement (HI) in its calculation of overall response rates *Source: company presentations and announcements, ClinicalTrials.gov*

Company	Product	Status	Combo	Neutro-penia	Anemia	Thrombo-cytopenia	ALT increase	Indication	ORR (A)	CR	PR	SD
Forty Seven Inc. (NASDAQ:FTSV)	5F9	Ph I/II	Rituxan rituximab (n=115)	14%	29%	19%	NR	Diffuse large B-cell lymphoma (DLBCL) (n=59)	36%	15%	20%	12%
								Indolent lymphoma (n=38)	61%	24%	37%	24%
		Ph I/II	Monotherapy (n=10)	0%	20%	0%	0%	Acute myelogenous leukemia (AML) or myelodysplastic syndromes (MDS) (n=10)	10%	0%	0%	70%
			Vidaza azacitadine (n=36)	25%	37%	26%	12%	AML (n=14)	64%	36%	0%	36%
								MDS (n=9)	100%	55%	0%	0%
ALX Oncology Ltd.	ALX148	Ph I	Herceptin trastuzumab (n=30)	7%	7%	13%	NR	HER2+ gastric and and gastroesophageal junction cancer (n=18)	22%	0%	22%	28%
			Keytruda pembrolizumab (n=52)	4%	8%	8%	14%	Squamous cell carcinoma of the head and neck (SCCHN) (n=19)	16%	0%	16%	32%
								Non-small cell lung cancer (NSCLC) (n=18)	0%	0%	0%	44%
Trillium Therapeutics Inc. (TSX:TRIL; NASDAQ:TRIL)	TTI-621	Ph I	Monotherapy (n=179)	7%	11%	25%	NR	Mycosis Fungoides (n=24)	17%	0%	17%	NR
								Sézary Syndrome (n=5)	20%	20%	0%	NR
								Peripheral T-cell Lymphoma (n=11)	18%	0%	18%	NR
								DLBCL (n=8)	25%	13%	13%	NR
			Rituxan (n=24)	NR	NR	NR	NR	DLBCL (n=24)	25%	4%	21%	NR

In a Phase I study, ALX combined its product with the anti-HER2 mAb trastuzumab or Merck & Co.'s anti-PD1 mAb Keytruda pembrolizumab.

According to Randolph, the ALX148/trastuzumab combo acts by triggering Fc-dependent phagocytosis, whereas the ALX148/Keytruda combo recruits macrophages and T cells into tumor, and possibly induces M1 macrophage polarization in an Fc cell-independent manner. In a Phase I study presented at the 2019 American Society of Clinical Oncology (ASCO) meeting, the compound induced single digit rates of anemia and neutropenia and an 8-13% rate of thrombocytopenia when dosed at 10 mg/kg per week in combination with standard regimens of trastuzumab in patients with HER2-positive gastric and gastroesophageal junction cancer, or Keytruda in patients with squamous cell carcinoma of the head and neck (SCCHN) or non-small cell lung cancer (NSCLC).

Forty Seven ways to detox

Forty Seven is minimizing 5F9-induced anemia using a priming dose of 1 mg/kg to clear the CD47 from RBC surfaces before delivering higher doses capable of targeting the tumor.

"Within about two hours of the initial dose, the binding of our molecule to CD47 on red blood cells causes a cleavage — what we call pruning — of CD47 on all red blood cells, old and new," said McCamish. FortySeven presented the priming strategy at last year's American Society of Hematology (ASH) meeting, and is continuing to investigate the mechanism.

The company has patented the dosing strategy in the U.S., Europe and Japan. "The priming dose is something we patented early on, and that's the crux of this. We do believe it will be on the label, which will be a key part of the patent protection, because it is a safety issue," McCamish said.

Forty Seven is testing combinations with the anti-CD20 mAb rituximab, which provides an extra 'eat me' signal via its IgG1 Fc receptor, and the chemotherapy azacitidine, which the company believes promotes upregulation by the tumor of endogenous eat-me signals like calreticulin.

At June's Congress of the European Hematology Association (EHA) meeting, the company reported data from a Phase I/II study of its rituximab/5F9 combo in non-Hodgkin lymphoma (NHL), and a Phase Ib study of its monotherapy and azacitidine combo in AML and MDS patients.

CBO Craig Gibbs told BioCentury the compound's safety profile is now "well-established," with experience in 290 patients of doses up to 45 mg/kg, and some patients treated for more than two years. The company is in discussions with FDA about accelerated approval for 5F9 plus azacitidine for MDS and 5F9 plus rituximab for diffuse large B cell lymphoma (DLBCL), a type of NHL, based on single arm studies with historical controls.

The compound is also in Phase I or I/II testing for other hematological cancers and solid tumors, and FortySeven is exploring preclinical combinations with newer agents like CD24 inhibitors (see "Combo Opportunities for Weissman's Latest 'Don't Eat Me' Signal").

The eater's side

Blocking the macrophage side of the CD47-SIRPA interaction could offer a safer way to promote tumor phagocytosis.

While CD47 is expressed ubiquitously, SIRPA is primarily expressed on macrophages. As a result, SIRPA inhibitors could have more limited effects on off-target cells, and avoid the need to overcome the "antigen sink" phenomenon that has pushed up dosing of anti-CD47 therapies.

Two anti-SIRPA mAbs began Phase I testing this year.

OSE Immunotherapeutics S.A. and Boehringer Ingelheim GmbH are testing the SIRPA inhibitor BI 765063 in solid tumors as a monotherapy and in combination with Boehringer's anti-PD1 mAb BI 754091.

OSE Immunotherapeutics CSO Nicolas Poirier told BioCentury BI 765063 overcomes resistance to checkpoint inhibition in preclinical models by modulating macrophage activity and allowing more T cells to enter the tumor microenvironment.

He said OSE has patented selective blockade of SIRPA but not SIRPG, which contributes to antitumor T cell responses.

Celgene Corp. is one of at least three companies developing both CD47 and SIRPA inhibitors.

The company is testing CC-95251 alone and in combination with anti-CD20 mAb rituximab or anti-EGFR mAb cetuximab in hematological malignancies and solid tumors, respectively.

Forty Seven Inc. President and CEO Mark McCamish believes the biotech's preclinical anti-SIRPA mAb FSI-189 will induce less anemia than its lead CD47 inhibitor 5F9, and sees opportunities for FSI-189 in transplant conditioning and infectious disease.

He thinks FSI-189 won't show single-agent activity in cancer, but could be used in combination regimens.

Arch Oncology Inc. presented preclinical data on a range of anti-SIRPA mAbs at this year's American Association for Cancer Research (AACR) meeting.

President and CEO Julie Cherrington said the company has not selected a lead compound, but is prioritizing agents that bind the most prevalent SIRPA variants, induce phagocytosis both as a single agent and in combination with the company's CD47 inhibitor AO-176, and do not suppress T cell activity, regardless of their ability to bind SIRPG.

— Karen Tkach Tuzman

A Restrictive diet for CD47

Companies with clinical **CD47** inhibitors are using an array of construct design and combination strategies to stimulate potent **macrophage** phagocytosis of **tumor cells** by simultaneously blocking the cancer's "don't eat me" signals and increasing "eat me" signals, all while avoiding engulfment of healthy hematological cells like red blood cells (**RBC**s).

Each competitor is using its biologic against CD47 to block the "don't eat me" interaction between CD47 on tumor cells and **SIRPA** on macrophages. The companies vary in how they deliver tumor-selective "eat me" signals via **FCGR**, a receptor on macrophages that binds the biologic compounds' long stalks, known as Fc domains. Fc domains derived from **IgG1** (**green**) antibodies trigger the strongest killing response, which can lead to more effective tumor killing but also greater toxicity; those derived from **IgG4** (**orange**) or **IgG2** (**pink**) have weaker killing effects.

1) ALX Oncology Ltd. is developing **ALX148**, a fusion protein against CD47 with an inactive Fc domain **(gray)**. To deliver "eat me" signals through FCGR, the compound must be combined with a tumor-specific biologic with an active Fc domain, like the anti-**HER2** IgG1 mAb herceptin.

2) Forty Seven Inc. (NASDAQ:FTSV), **Trillium Therapeutics Inc.** (NASDAQ:TRIL; TSX:TRIL) and **Innovent Biologics Inc.** (HKEX:1801) are developing **5F9**, **TTI-622** and **IBI188**, respectively. These compounds moderately activate FCGR via their IgG4 Fc domains; Forty Seven and Trillium are combining their compounds with additional pro-phagocytic signals like the anti-**CD20** IgG1 mAb rituximab, which targets B cell cancers. Innovent has not disclosed its strategy.

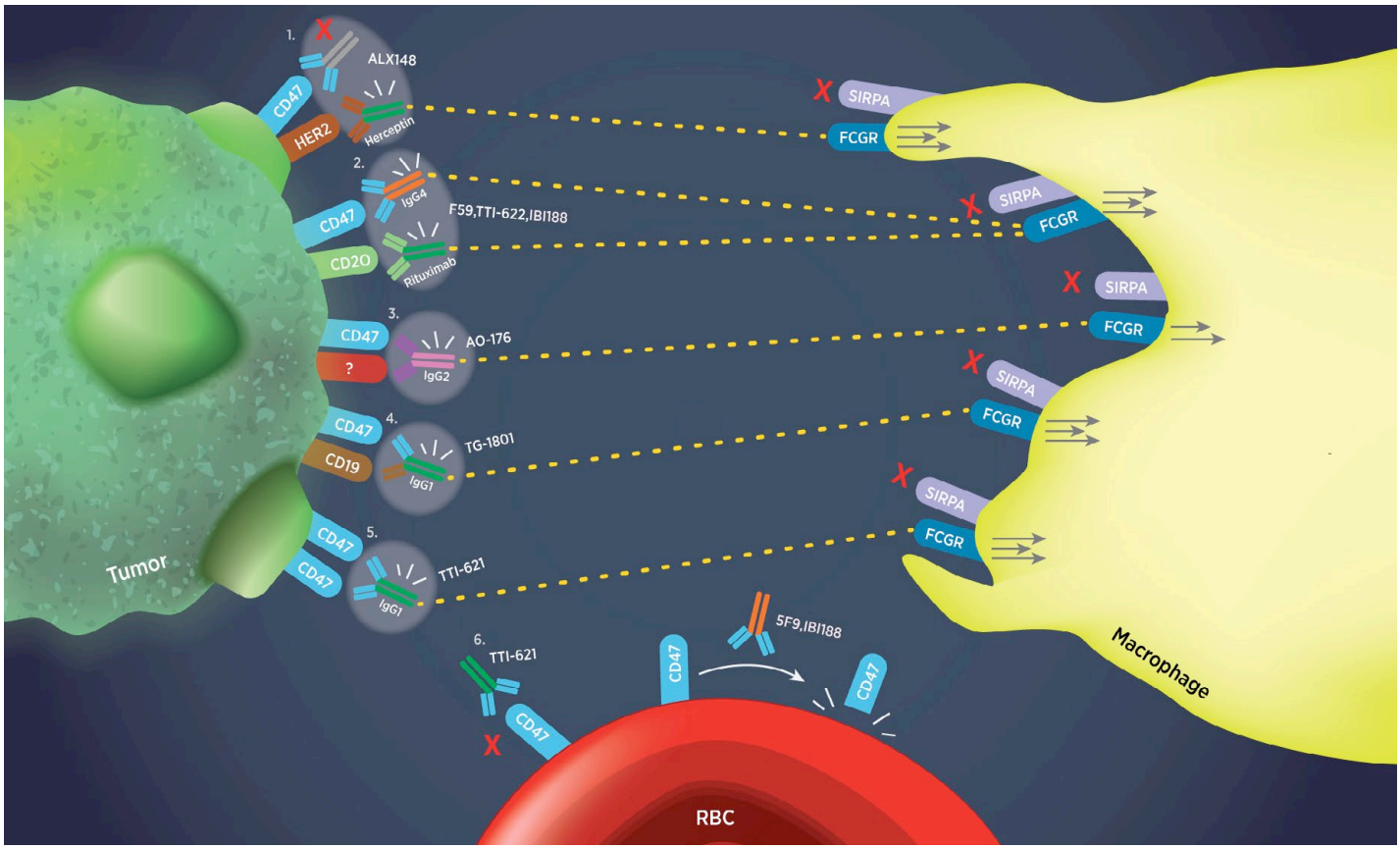
3) Arch Oncology Inc. is developing **AO-176**, a mAb that selectively targets CD47 in the presence of undisclosed tumor-specific proteins. The compound activates FCGR moderately via its IgG2 Fc domain, and induces direct tumor killing via a programmed cell death pathway.

4) TG Therapeutics Inc. (NASDAQ:TGTX) is developing **TG-1801**, an IgG1 bispecific antibody that weakly binds CD47 and strongly binds the B cell cancer antigen **CD19**, making CD47 blockade dependent on the presence of CD19.

5) Trillium's lead compound **TTI-621** is a fusion protein that requires CD47 clustering to bind the target and activates FCGR strongly via its IgG1 domain.

6) Trillium has shown TTI-621 is not effective at binding RBCs because CD47 is difficult to cluster on RBC membranes. Forty Seven and Innovent are administering their anti-CD47 mAbs at low priming doses before giving higher therapeutic doses; the priming dose cleaves CD47 from the surfaces of RBCs, reducing anemia induction by the subsequent therapeutic dose.

FCGR - Fc gamma receptor (FCGR); HER2 (EGFR2; ErbB2; neu) - Epidermal growth factor receptor 2 ; IgG1 - Immunoglobulin G1; IgG2 - Immunoglobulin G2; IgG4 - Immunoglobulin G4; SIRPA (CD172a; SHPS-1) - Signal regulatory protein α



Trillium takes two

To minimize the risk profile of TTI-621, Trillium is taking a "cautious" dosing strategy for its IV studies, and focusing on indications where the compound can be delivered intratumorally, said Uger. He said intratumoral delivery "gets us around the systemic exposure issue, and gets a lot of drug locally to where we want it to be."

Intratumoral TTI-621 is in Phase I/II testing for solid tumors and mycosis fungoides, a type of cutaneous T cell lymphoma (CTCL), both as a monotherapy and in combination with checkpoint inhibitors or IFNα. Trillium presented data on its intratumoral monotherapy for CTCL at a January T-Cell Lymphoma Forum, and Uger said the company is in discussions with FDA about a registrational path for early stage disease.

The IV form of the compound is in Phase I/II testing to treat four forms of lymphoma as a monotherapy, or in combination with checkpoint inhibitors or rituximab. According to Uger, Trillium stuck to a low dose of 0.2 mg/kg in response to grade four thrombocytopenia events, but recently raised the dose to 0.5 mg/kg following discussions with FDA after it became clear that platelet counts rebounded within a week and the compound did not cause bleeding.

"We've seen single agent activity with our drug across a number of heme indications," said Uger. "We're encouraged by that, because frankly, we believe we've been underdosing."

He said the company has developed an assay to measure receptor occupancy in tumor microenvironment, rather than the more commonly tested bloodstream, which he said will be key "to better define how far up in dose we need to go."

Trillium has developed a second compound, TTI-622, with an Fc domain from IgG4 instead of IgG1; the agent is in Phase I testing alone and in combination with rituximab, checkpoint inhibition or a proteasome inhibitor regimen. "We are under no illusions that TI-622 is going to have significant monotherapy activity. For us, it's a combination play," Uger said.

He argues TTI-621 and TTI-622 will have a lower risk of anemia than competitors because the Trillium molecules don't bind CD47 on RBC surfaces. The company has biochemical data suggesting the compounds require bivalent interactions with clustered CD47 molecules for high avidity binding, and CD47 molecules on RBCs are less capable of clustering because they are embedded in stiff cytoskeletal networks.

Next in line

Companies entering the clinic have made it clear that tackling the target's toxicity will be their priority.

Arch Oncology Inc. CEO Julie Cherrington told BioCentury its anti-CD47 IgG2 mAb AO176 preferentially targets tumor cells because it selectively binds CD47 in the presence of undisclosed proteins that are specifically expressed on cancer cells.

The company presented data at this year's meeting of the American Association for Cancer Research (AACR) showing the compound preferentially bound cancer cells over T cells, epithelial cells or RBCs, and exhibited a clustered binding pattern on tumor cell surfaces not seen for Forty Seven's 5F9, which bound tumor cell surfaces uniformly. The data also show AO176 is capable of directly killing tumor cells, which the company believes occurs via a programmed cell death pathway.

In March, Arch announced $50 million in series B funding to advance AO176 in the clinic; the compound is in Phase I testing to treat solid tumors. Formerly known as Tioma Therapeutics Inc., Arch raised $86 million in its 2016 series A round.

> "ALL MORE OR LESS LOOKED SIMILAR FROM A PRECLINICAL PERSPECTIVE. YOU MOVED THEM INTO THE CLINIC, AND THEY ALL LOOK VERY DIFFERENT."
>
> JEFF GOATER, SURFACE ONCOLOGY

TG Therapeutics Inc. has TG-1801, a bispecific IgG1 antibody targeting CD47 and CD19 licensed from Novimmune S.A. last year, in Phase I testing for B cell lymphoma. Because the compound binds CD47 weakly and CD19 strongly, it preferentially binds CD47 on cells expressing the B cell marker CD19.

"Our drug would be essentially useless for any other diseases other than B cell malignancies, which is the focus of our company, but in those diseases, it's the ideal manifestation of a CD47 inhibitor," CEO Michael Weiss told BioCentury. He anticipates TG-1801 will have single-agent activity, but sees opportunities to combine the compound with ublituximab, the company's glycoengineered anti-CD20 mAb in Phase III testing for chronic lymphocytic leukemia (CLL) and multiple sclerosis (MS).

The CD47 space has also seen a flurry of activity in China.

Innovent Biologics Inc. has initiated U.S. and Chinese Phase I studies of its anti-CD47 IgG4 mAb IBI188 in advanced malignant cancers. The company has described IBI188 as having "stronger receptor blocking ability than similar drugs."

In an email to BioCentury, CEO Michael Yu said the company is managing toxicity via a priming dose strategy. Though the dosing plan is similar to Forty Seven's approach, Yu said Innovent has a developed a new delivery strategy "to make sure that we respect their IP and have our own freedom of operations."

In June, I-Mab Biopharma began U.S. Phase I testing of its anti-CD47 mAb TJC4 for multiple cancers. The company identified the mAb via a screening strategy that selected for clones that bound CD47 with high

affinity, but seldom or minimally bind RBCs. I-Mab also plans to initiate Chinese Phase I trials for AML and NHL.

ImmuneOnco Biopharma Co. Ltd. has IMM001, an Fc fusion protein targeting CD47, in Phase I testing for leukemia in China. The company disclosed it resubmitted its IND to China's National Medical Products Administration (NMPA) after the agency asked for additional preclinical data showing the compound had "good safety" against human RBCs and platelets.

I-Mab and ImmuneOnco did not comment in time for publication.bc

COMPANIES AND INSTITUTIONS MENTIONED

ALX Oncology Ltd., Dublin, Ireland
American Association for Cancer Research (AACR), Philadelphia, Pa.
American Society of Clinical Oncology (ASCO), Alexandria, Va.
American Society of Hematology (ASH), Washington, D.C.
Arch Oncology Inc., Brisbane, Calif.
Boehringer Ingelheim GmbH, Ingelheim, Germany
Celgene Corp. (NASDAQ:CELG), Summit, N.J.
European Hematology Association (EHA), the Hague, the Netherlands
Forty Seven Inc. (NASDAQ:FTSV), Menlo Park, Calif.
I-Mab Biopharma, Shanghai, China
ImmuneOnco Biopharma Co. Ltd., Shanghai, China
Innovent Biologics Inc. (HKEX:1801), Suzhou, China
Merck & Co. Inc. (NYSE:MRK), Kenilworth, N.J.
National Medical Products Administration, Beijing, China
Novimmune S.A., Plan-les-Ouates, Switzerland
OSE Immunotherapeutics S.A. (Euronext:OSE), Nantes, France
Stanford University, Stanford, Calif.
Surface Oncology Inc. (NASDAQ:SURF), Cambridge, Mass.
TG Therapeutics Inc. (NASDAQ:TGTX), New York, N.Y.
Trillium Therapeutics Inc. (NASDAQ:TRIL; TSX:TRIL), Toronto, Ontario
U.S. Food and Drug Administration (FDA), Silver Spring, Md.

TARGETS

IFNα - Interferon α
IgG1 - Immunoglobulin G1
IgG2 - Immunoglobulin G2
IgG4 - Immunoglobulin G4
HER2 (EGFR2; ErbB2; neu) - Epidermal growth factor receptor 2
PD-1 (PDCD1; CD279) - Programmed cell death 1
SIRPA (CD172a; SHPS-1) - Signal regulatory protein α
SIRPG (CD172g) - Signal regulatory protein γ

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