BIOCENTURY
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TARGETS & MECHANISMS

KEEPING THE GAS OFF AXL

By Michael Leviten, Senior Writer

With its development of an AXL decoy able to trap and sequester the native receptor's ligand with femtomolar affinity, Aravive Biologics Inc. believes it has a biologic that can bypass the potency and selectivity issues of the small molecule AXL inhibitors already in the clinic for cancer. The company plans to submit an IND in 2017 for its lead candidate, Aravive-S6, in leukemia.

Last month, Aravive changed its name from Ruga Corp., and moved from Palo Alto to Houston to accept a $20 million grant from the Cancer Prevention & Research Institute of Texas (CPRIT) to help bring the compound to the clinic.

AXL is a receptor tyrosine kinase (RTK) on the surface of cells that binds the ligand GAS6 to trigger a survival response. Cancers co-opt the pathway to metastasize and become drug resistant, and overactivity is associated with poor survival.

There are no drugs on the market targeting the pathway, but at least 10 products are in preclinical or clinical development. The most advanced are small molecules, but four, including Aravive-S6, are biologics (see "Putting the Brakes on AXL").

CEO Ray Tabibiazar told BioCentury the company's compound acts by a different mechanism than its competitors, which bind the receptor directly. Instead, Aravive-S6 binds to the ligand GAS6, outcompeting its interaction with the receptor.

"There is a large group of products that are going after the receptor and the vast majority of them are small molecules. But small molecules lack specificity and sensitivity," he said.

In a study in *The Journal of Clinical Investigation* published last month, a Stanford University group led by Amato Giaccia, a professor of radiation oncology and Aravive's CSO, showed the AXL decoy bound GAS6 with a K_d of 93 fM, killed leukemic cells *in vitro*, and decreased tumor growth and metastasis in mouse models of breast and ovarian cancer.

Giaccia told BioCentury the decoy's ultra-high affinity is the key to effective inhibition of AXL signaling due to the unusually strong interaction between GAS6 and native AXL, which bind with a K_d of 30 pM. The decoy's binding is also several orders of magnitude stronger than the affinities of the small molecule and antibody inhibitors in development.

He thinks the AXL decoy will also be safer than the small molecule inhibitors as it has fewer off-target effects. In addition, it's differentiated from the antibody-based compounds in development by its decoy mechanism. Two of those — HuMax-AXL-ADC from Genmab A/S and CAB-AXL-ADC from BioAtla LLC — are antibody-drug conjugates (ADCs) that bind the receptor in order to deliver a cytotoxic payload, and it is unclear whether or to what extent they inhibit AXL signaling. The third, ORB0003 from Oribase Pharma, is a humanized antibody against AXL.

BIOCENTURY PRODUCT PROFILE

BIOCENTURY PRODUCT PROFILE	
INNOVATION STAGE	
Product	AXL decoy receptor
Concept	A protein based on the extracellular portion of AXL that binds its ligand GAS6, sequestering it from the native receptor and preventing AXL-mediated signaling
Disease	Acute myelogenous leukemia (AML); metastatic solid tumors
Competition	Small molecule AXL inhibitors; AXL-targeted antibody-drug conjugates (ADCs) or antibodies
Differentiation	Increased selectivity avoids off-target toxicity; increased potency
Administration	IV or subcutaneous
Risks	Activity in non-cancerous cells expressing AXL
Development status	Preclinical
Patents	Patent applications filed
Company; lead investigator	Aravive Biologics Inc.; Amato Giaccia

MUTANT STRENGTH

Aravive-S6 is an optimized version of the company's first AXL decoy, MYD1, described in a 2014 *Nature Chemical Biology* paper, which was created by isolating and systematically mutating the extracellular domain of AXL, yielding a compound with a K_d of 5.8 pM.

In the *JCI* paper, the team returned to the original yeast screen for additional affinity-altering mutations, and identified an alanine-to-valine change in amino acid 72 that was present in

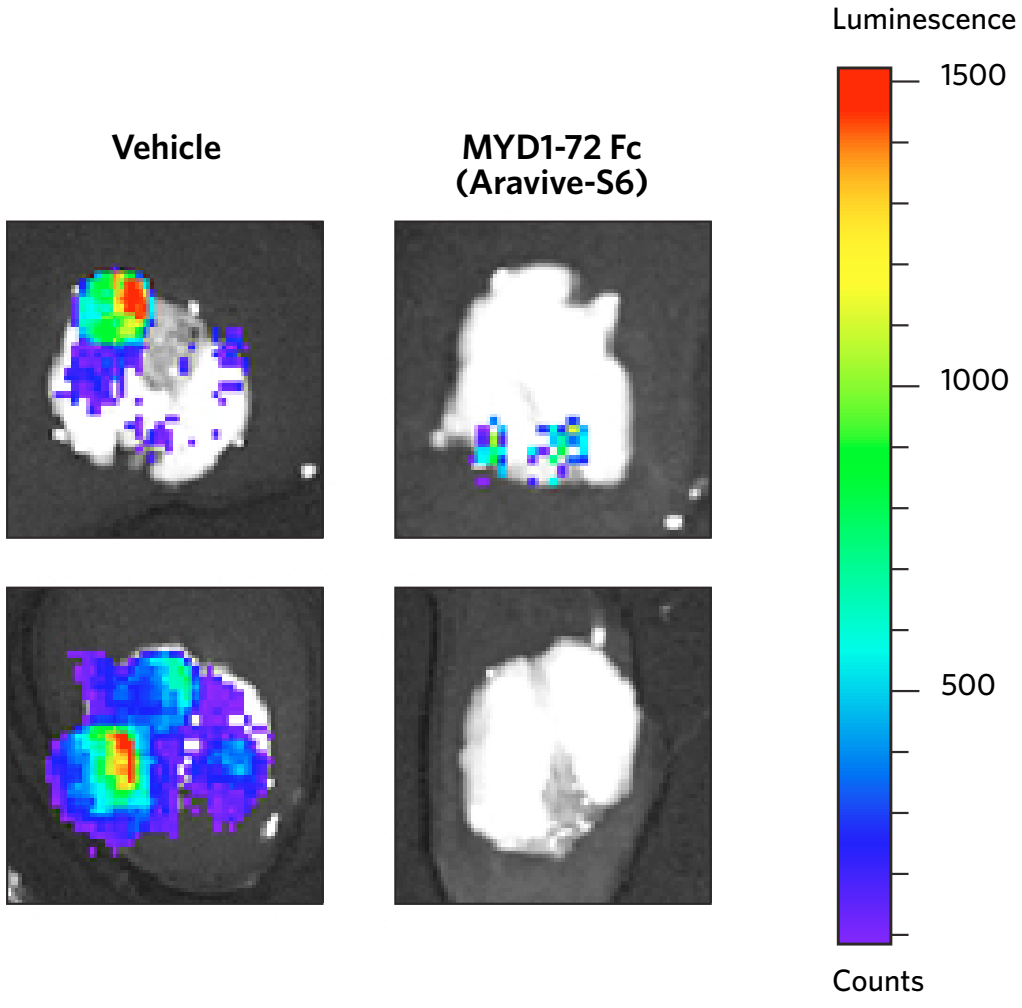
METASTASES OUT OF GAS6

In a paper in *The Journal of Clinical Investigation*, researchers from **Stanford University** and **Aravive Biologics Inc.** described an AXL decoy, MYD1-72 Fc — dubbed Aravive-S6 — that sequestered the AXL ligand GAS6 and suppressed growth and metastasis of tumors in mouse models of cancer.

The team used luciferase imaging to monitor lung metastasis burden in mice injected with a luciferase-expressing breast cancer cell line. Lungs from two mice that received daily subcutaneous injections of a vehicle **(left),** and two that received Aravive-S6 **(right)** were isolated and imaged after two weeks of treatment. The absence of luciferase-based color in the Aravive-S6-injected tissue reflects the smaller number and size of lung metastases than in vehicle-treated tissue.

Aravive has the compound in preclinical testing for acute myelogenous leukemia (AML) and solid tumors.

AXL (UFO) - AXL receptor tyrosine kinase; GAS6 - growth arrest-specific 6. *Source: Stanford University*



PUTTING THE BRAKES ON AXL

Select AXL receptor tyrosine kinase (AXL; UFO)-targeted therapeutics in clinical and preclinical development. At least three AXL inhibitors are in clinical development and seven others are in preclinical development, including Aravive-S6 from **Aravive Biologics Inc.** Aravive-S6 is an Fc fusion protein containing a mutant extracellular AXL domain that binds its ligand, growth arrest-specific 6 (GAS6), with high affinity. By binding GAS6, Aravive-S6 inhibits ligand-mediated activation of AXL. *Source: BCIQ: BioCentury Online Intelligence*

MODALITY	COMPANY	PRODUCT	DESCRIPTION	INDICATION	PHASE OF DEVELOPMENT
Small molecules	**Astellas Pharma Inc.** (Tokyo:4503); **Kotobuki Pharmaceutical Co.**	Gilteritinib (ASP2215)	AXL and FMS-like tyrosine kinase 3 (FLT3; CD135) inhibitor	Acute myelogenous leukemia (AML); non-small cell lung cancer (NSCLC)	Phase III; Phase I
	Mirati Therapeutics Inc. (NASDAQ:MRTX)	Glesatinib (MGCD265)	Dual inhibitor of AXL and c-Met receptor tyrosine kinase (c-MET; MET; HGFR; c-Met proto-oncogene)	Solid tumors; NSCLC	Phase II
	BerGenBio A/S; Rigel Pharmaceuticals Inc. (NASDAQ:RIGL)	BGB324	AXL inhibitor	NSCLC; AML	Phase I
	Qurient Co. Ltd. (KOSDAQ:115180)	Q-4	AXL inhibitor	Cancer	Preclinical
	Tolero Pharmaceuticals Inc.	TP-0903	AXL inhibitor	Leukemia; pancreatic cancer	Preclinical
	Ignyta Inc. (NASDAQ:RXDX)	RXDX-106	Selective, pseudo-irreversible inhibitor of AXL and c-Met	Cancer	Preclinical
Biologics	**Aravive Biologics Inc.**	Aravive-S6	Fc-fusion protein that blocks activation of the GAS6-AXL signaling pathway by preventing binding of GAS6 to AXL	AML; solid tumors	Preclinical
	BioAtla LLC	CAB-AXL-ADC	Antibody-drug conjugate (ADC) containing a conditionally active biologic (CAB) and targeting AXL	Solid tumors	Preclinical
	Genmab A/S (CSE:GEN)	HuMax-AXL-ADC	ADC targeting AXL	Lung cancer; solid tumors	Preclinical
	Oribase Pharma	ORB0003	Humanized antibody against AXL	Pancreatic cancer	Preclinical

a large percentage of yeast clones. The group incorporated the mutation into MYD1 and then fused the new decoy to a human Fc domain to increase the protein's *in vivo* half-life, producing Aravive-S6.

Giaccia said the group would never have been able to create the compound had it cut corners and mutagenized only the GAS6-binding region of AXL rather than the entire extracellular domain. That's because the mutation at amino acid 72 fell outside the GAS6 binding domain and there would have been no way to predict the change would alter affinity so profoundly.

Structural analyses of Aravive-S6 in complex with GAS6 revealed the alanine-to-valine mutation increased affinity of the MYD1 parent compound in two ways: by introducing an additional electrostatic interaction within the loop containing the valine, and increasing van der Waals contacts due to the larger volume of the valine residue compared with alanine.

UP-ING THE COMPETITION

In mice, the Aravive-S6 decoy bound circulating GAS6, eliminating the ligand from the blood for several hours.

The team then performed side-by-side comparisons of Aravive-S6, foretinib (GSK1363089; 1363089) and BGB324.

Foretinib is a c-Met and VEGFR-2 inhibitor with activity against AXL that Exelixis Inc. and partner GlaxoSmithKline plc have in

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> Ray Tabibiazar, Aravive Biologics

Phase I and Phase II trials for a variety of cancers. BGB324 is a small molecule AXL inhibitor that BerGenBio A/S and Rigel Pharmaceuticals Inc. have in Phase I/II testing for non-small cell lung cancer (NSCLC) and in Phase I for acute myelogenous leukemia (AML).

In a mouse xenograft breast cancer model, all three compounds decreased primary tumor size; Aravive-S6 produced effects comparable to foretinib and was superior to BGB324.

Aravive-S6 and foretinib decreased the number of lung metastases compared with vehicle by 71% and 55%, respectively.

Despite the similar efficacy, foretinib was more toxic than Aravive-S6. The team had to stop treatment early in roughly half of the mice in the foretinib group due to excessive weight loss not seen in the decoy-treated animals. In histological analyses of retinal and other tissues, Giaccia's team found no signs of toxicity in Aravive-S6 treated mice.

BerGenBio CEO Richard Godfrey told BioCentury the weak efficacy of BGB324 in the study may have been due to Giaccia's team selecting a dose on the low end of what BerGenBio typically uses in mouse studies.

Moreover, he expects BGB324 will also be safer than foretinib. "Our molecule, BGB324, is the only highly selective small molecule AXL inhibitor that's in development. It's quite exquisitely selective for AXL, as opposed to any other kinase or indeed other members of the so-called TAM [TYRO3-AXL-MERTK] family." Giaccia's team did not cite any toxicity associated with BGB32 at the dose used in the *JCI* paper.

BRAKING MECHANISM

Mechanistic studies showed Aravive-S6 killed cancer cells by inducing a DNA damage response that led to apoptosis.

Giaccia said that result was "very surprising," because AXL inhibition had been thought to starve cancer cells of survival signals rather than induce DNA damage. "I think most people that would have said it's regulating survival factors such as AKT and MAP kinase."

However, in retrospect he said the result "makes sense, especially because we were seeing such significant interactions with cytotoxic chemotherapy. But the concept that a growth factor receptor can regulate the DNA damage response was pretty cool."

In an ovarian cancer cell line *in vitro*, Aravive-S6 and doxorubicin additively induced expression of DNA damage regulators.

The group tested the combination in two mouse models of human ovarian cancer. In the less aggressive OVCAR8 model, the combination therapy decreased the number of lung metastases to only two to three per mouse from nearly 750 with vehicle. Three out of 10 mice treated with Aravive-S6 and doxorubicin had no detectable signs of disease (see "Metastases Out of GAS6").

In the more aggressive skov3.ip model, Aravive-S6 and doxorubicin decreased tumor burden by 51% and 91% respectively, while their combination eliminated 99% of tumors.

Aravive's Tabibiazar said the data support the company's plan to test Aravive-S6 plus doxorubicin in the clinic.

PEDAL TO THE METAL

Tabibiazar told BioCentury the company's name change signals a transition from its initial stage of research-based exploration of the protein-folding space to a more focused pursuit of AXL inhibition for cancer. "I'm not calling it a single-product company but it's a product-focused company. Before it was a big R and a little D company and now it's a little R and a big D," said Tabibiazar.

While a move from Palo Alto to Houston may seem unusual in the biotech world, Tabibiazar told BioCentury the change

was largely driven by the CPRIT award, which he said was the largest single grant ever made by the organization.

"Twenty million dollars of non-dilutive financing, plus a scientific milieu in which our company can do well, caused us to make the move there," said Tabibiazar.

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>
> Ray Tabibiazar, Aravive Biologics

He noted the patient reservoir provided by the Texas Medical Center and research strength of the University of Texas MD Anderson Cancer Center, Baylor University and Rice University make Houston a cancer research destination. Still, he acknowledged: "Houston is not Boston or San Francisco, so I think there is going to be a little bit more work to do to recruit talent that knows about drug development."

Giaccia said the Aravive-S6 program is far enough along that he does not anticipate major roadblocks in its path to the clinic. He said Aravive has already initiated GMP manufacturing and plans to complete GLP toxicology studies in time for a 2017 IND submission.

"Right now everything is going very smoothly. We've got high-producing clones, no aggregates and good PK," said Giaccia.

COMPANIES AND INSTITUTIONS MENTIONED

Aravive Biologics Inc., Houston, Texas
Baylor University, Waco, Texas
BerGenBio A/S, Bergen, Norway
BioAtla LLC, San Diego, Calif.
Cancer Prevention & Research Institute of Texas (CPRIT), Austin, Texas
Exelixis Inc. (NASDAQ:EXEL), South San Francisco, Calif.
Genmab A/S (CSE:GEN), Copenhagen, Denmark
GlaxoSmithKline plc (LSE:GSK; NYSE:GSK), London, U.K.
Oribase Pharma, Montpelier, France
Rice University, Houston, Texas
Rigel Pharmaceuticals Inc. (NASDAQ:RIGL), South San Francisco, Calif.
Stanford University, Stanford, Calif.
Texas Medical Center, Houston, Texas
University of Texas MD Anderson Cancer Center, Houston, Texas

TARGETS

AXL (UFO) - AXL receptor tyrosine kinase
c-Met (MET; HGFR; c-Met proto-oncogene) - c-Met receptor tyrosine kinase
GAS6 - Growth arrest-specific 6
MAPK (ERK) - MAP kinase
MERTK - c-Mer proto-oncogene tyrosine kinase
AKT (AKT1; PKB; PKBA) - Protein kinase B
TYRO3 (Sky) - TYRO3 protein tyrosine kinase
VEGFR-2 (KDR/Flk-1) - Vascular endothelial growth factor receptor 2

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Leviten, M. "Next stop: Houston" *BioCentury Innovations* **(2016)**

BIOCENTURY INC.

NEWSROOM
pressreleases@biocentury.com

SAN CARLOS, CA
+1 650-595-5333; Fax: +1 650-595-5589

CHICAGO
+1 312-755-0798; Fax: +1 650-595-5589

WASHINGTON, DC
+1 202-462-9582; Fax: +1 202-667-2922

UNITED KINGDOM
+44 (0)1865-512184; Fax: +1 650-595-5589