REPRINT FROM AUGUST 19, 2013

BioCentury

THE BERNSTEIN REPORT ON BIOBUSINESS™

Product Discovery & Development

The next wave in ADCs

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After about 25 years of development, antibody-drug conjugates have gained acceptance as a validated approach to the targeted killing of cells, with two drugs approved in the past two years. While **Seattle Genetics Inc.** and **ImmunoGen Inc.** have owned the space, a variety of biotechs are developing next-generation ADCs that seek to improve the technology and broaden its scope beyond cancer to inflammation and autoimmune diseases.

These advances include producing a more homogeneous drug product and delivering more potent payloads that move beyond the delivery of blunt, cytotoxic agents. Other approaches include developing ADCs as a delivery system for targeted small molecules.

Further down the line companies are beginning to think outside the box, which now is defined as "antibody linked to a payload." A mAb that has cytotoxic amino acids built into its sequence exemplifies such new thinking.

> ***"The therapeutic window for these conventional ADCs is not optimized and can not be optimized because you've got this heterogeneous population."***
>
> **Lawson Macartney, Ambrx**

The combination of product approvals and new approaches has increased investor interest in the field. Indeed, Seattle Genetics' stock is up 145% since the beginning of 2012, while ImmunoGen is up 40%.

In 2012, four next-generation ADC companies raised more than $150 million in venture funding.

None of these companies have raised money so far in 2013, but last week **Bind Therapeutics Inc.** filed to raise up to $80.5 million in an IPO underwritten by Credit Suisse; Cowen; Stifel; and JMP Securities.

What these next-generation technologies need is clinical data that can validate the platforms and attract big industry players. Most of those are at least a year or two away, as there are no molecules in the clinic from next-generation ADC companies except for Bind's Accurins, which employ a nanoparticle with targeting ligands.

ADCs: Take One

Seattle Genetics and ImmunoGen developed similar technologies that have been widely out-licensed.

Both technologies use naturally occurring amino acids in the antibody as anchor points for attaching a cytotoxic drug. Seattle Genetics' technology uses cysteine, whereas ImmunoGen's Targeted Antibody Payload (TAP) platform uses lysine.

The technologies use different cytotoxic payloads, although both are microtubule-disrupting agents. Seattle Genetics employs auristatins like monomethyl auristatin E (MMAE), while ImmunoGen uses maytansine derivatives like DM1.

Both have engineered linkers that aim to keep the cytotoxic agents welded to the mAbs until the ADC reaches the cancer cell. Once internalized, the linker is cleaved, releasing the cytotoxic agent to kill the cell.

In August 2011, FDA granted accelerated approval to Seattle Genetics' Adcetris brentuximab vedotin to treat Hodgkin's lymphoma and anaplastic large cell lymphoma. In November 2012, the EC granted conditional approval to the ADC, which is composed of an anti-CD30 mAb and MMAE.

The **Millennium Pharmaceuticals Inc.** unit of **Takeda Pharmaceutical Co. Ltd.** has marketing rights outside the U.S. and Canada, where Seattle Genetics retains rights.

In February, FDA approved Kadcyla ado-trastuzumab emtansine (T-DM1) from ImmunoGen and partner **Genentech Inc.** to treat HER2-positive metastatic breast cancer in patients who have received prior treatment with Herceptin trastuzumab and a taxane-based chemotherapy.

The humanized mAb against epidermal growth factor receptor 2 (EGFR2; HER2) linked to DM1 is under review in Europe and Japan.

Even as first-generation ADCs are crossing the goal line, the next-generation ADC companies see room for improvement.

For example, because any given mAb contains numerous cysteine and lysine sequences, conjugating the payload to a naturally occurring amino acid results in a heterogeneous drug mixture. Some mAbs may have no cytotoxic drugs attached, while others in the same product may have six or more linked payloads.

In addition, naked antibodies can compete with ADCs to bind the target, affecting efficacy or pharmacokinetics.

The cytotoxic agent also can link to different sites on the mAb, affecting the stability or functionality of the therapeutic.

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For instance, a cytotoxic compound conjugated near the mAb's binding site can affect its ability to bind the antigen.

If the cytotoxic agent happens to destabilize the mAb, this destabilized material can aggregate with other mAbs in the drug product and reduce performance.

Conventional ADCs also may have a narrow therapeutic window, according to **Ambrx Inc.** CEO Lawson Macartney. "The therapeutic window for these conventional ADCs is not optimized and cannot be optimized because you've got this heterogeneous population," he said.

Getting specific

One goal of next-generation technologies thus is to create homogeneous ADC products.

For example, rather than relying on native amino acids as conjugation sites, Ambrx and **Sutro Biopharma Inc.** are engineering non-native amino acids into their mAbs for site-specific conjugation.

Ambrx does this by altering the antibody's genetic sequence, according to Chief Technology Officer Ho Sung Cho. He said the normal amber stop codon in the genetic sequence is altered to code for an artificial amino acid.

Drug designers can then specify where in the mAb the novel amino acid should be placed. "We choose to put the Ambrx amino acid into the antibody framework in a region where it is designed to minimally perturb all the other functions of the antibody," Cho told BioCentury.

The non-native amino acid also comes with a chemical handle that can be conjugated to the payload.

Cho said the result is a homogeneous ADC in which a known number of cytotoxic drugs are conjugated to each mAb at specific sites. He added that these products are very stable under physiological conditions, so there should be few issues around aggregation.

> ***"You can attach drugs to Fleximer that you couldn't dream of attaching to an antibody chemically."***
>
> **Nicholas Bacopoulos, Mersana**

This stability plus the fact that the conjugation sites don't interfere with the mAb's binding characteristics should translate into greater efficiency in getting the drug to the target cell, said Cho. This should mean less drug is necessary and broaden the therapeutic window.

Ambrx uses commercially validated systems based on *E. coli* and CHO cells, meaning its ADCs can be readily manufactured in existing systems used by CMOs and large biopharma companies.

By contrast, Sutro's platform generates ADCs in a cell-free environment.

According to CSO Trevor Hallam, an often overlooked but important aspect to ADC design is the specific site chosen to conjugate the payload to the mAb. He said Sutro can generate and empirically test almost any combination of conjugation sites because its cell-free technology allows the company to create all potential variations "in a few hours" *(see BioCentury, Jan. 10, 2011)*.

"A lot of companies have made assumptions as they approach ADCs that if the payload is on the surface and it doesn't interfere with the stability or binding of the antibody, then that's good enough," Hallam said. "But that's not actually the case. Sutro's data show it really can be quite variable in terms of how effective that killing can be depending purely on the site of the conjugation."

He said preclinical data show the killing efficiency of an ADC can vary by up to two orders of magnitude based solely on the site of conjugation.

Both Ambrx and Sutro acknowledge that adding non-natural amino acids to a mAb could raise questions about immunogenicity.

Ambrex's Cho expects these compounds shouldn't be much more immunogenic than a conventional ADC. Regardless of whether a non-native or native amino acid is used, he noted, there is still a foreign chemical moiety linked to the mAb that could be immunogenic.

"We don't introduce additional risk; we introduce a slightly different chemical risk," Cho told BioCentury. "But because we are able to do rational, structure-guided engineering, we can prevent the main cause of immunogenicity, which is aggregation."

Ambrx's ARX788, an ADC against HER2, is in preclinical development for breast cancer and gastric cancer. In June, Ambrx granted Chinese rights to **Zhejiang Medicine Co. Ltd.**

Ambrx also has ADC discovery deals with **Astellas Pharma Inc.**, **Bristol-Myers Squibb Co.**, **Fabrus LLC** and **Merck & Co. Inc.**

Additional internal and partnered ADC programs are undisclosed.

Sutro's internal ADC programs, which are undisclosed, are in preclinical development. The biotech also has an ADC discovery and development deal with **Celgene Corp.**

Like Ambrx and Sutro, **Redwood Bioscience Inc.** is developing site-specific, homogeneous ADCs. Redwood uses an aldehyde tag incorporated into the mAb *(see BioCentury, Aug. 1, 2011)*.

Allozyne Inc. is developing a site-specific ADC platform that incorporates azide non-natural amino acids into the mAb to serve as a conjugation point for cytotoxic agents.

Another company in this space is **PolyTherics Ltd.** Its ThioBridge conjugation technology is site-specific and the company says its technology also provides a less heterogeneous ADC product with improved stability.

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The Bernstein Report on BioBusiness

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Keyhole specificity

A variation on the site-specific conjugation approach is **Meditope Biosciences Inc.**'s meditope-enabled mAb technology.

According to scientific co-founder John Williams, his lab at **City of Hope** discovered a unique site within the Fab arm of Erbitux cetuximab that acts as a very specific keyhole in which a specific small peptide is able to non-covalently bind — similar to a ligand-receptor interaction.

Meditope is taking this specific site from the Erbitux Fab arm and grafting it into the Fab arm of any other mAb, thus enabling the antibody to interact with the small peptide key.

The peptide then acts as an anchor point to which cytotoxic drugs can be attached.

Rather than welding the cytotoxin to the mAb, which requires specific conditions, the peptide payload can be hitched to the mAb under a variety of conditions.

"Once you have grafted that site into an antibody, then you have any myriad of combinations of that one peptide that binds there and whatever you want to put on that peptide. The peptide is like a trailer hitch," Williams said.

The interaction is non-covalent, which Williams said opens a variety of opportunities. For instance, he said the technology could be used for imaging specific cells with a specific antigen. The modified mAb would be administered without the peptide, and then the peptide conjugated to copper 64 would be administered.

Williams said the peptide would seek out the keyhole in the mAb to allow imaging of the cells of interest.

President and CEO Stephanie Hsieh said the company has biochemical data showing the modified keyhole site and peptide do not affect the natural binding of the mAb to the antigen.

She said the company, which was founded a year ago, still has some work to do validating the approach *in vivo*. The company's recent $3.6 million series A round should support those efforts.

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Next generation ADCs

At least 13 companies are developing new approaches to improve the next generation of antibody-drug conjugates, either through site-specific conjugation, mAb trailers that carry therapeutic payloads, or improvements to ADC payloads. *Sources: BioCentury reporting; company documents/websites*

Company	Technology	Status
	Site-specific conjugation	
Allozyne Inc.	Site-specific ADC technology that uses azide non-natural amino acids as conjugation point	Preclin
Ambrx Inc.	ADCs produced in cell-based system that inserts non-native amino acid for site-specific conjugation	Preclin
Igenica Inc.	ADCs with payload linked to disulfide bonds of mAb for site-specific conjugation	Preclin
Meditope Biosciences Inc.	Meditope-enabled mAb technology that grafts cetuximab-derived residues into Fab arm of mAb to create keyhole for non-covalent conjugation of peptide linked to a payload	NA (drug discovery not started yet)
PolyTherics Ltd.	ThioBridge conjugation technology for site-specific attachment of payload to mAb	NA (co provides drug discovery services and does no internal drug development)
Redwood Bioscience Inc.	ADC technology that uses an aldehyde tag incorporated into mAb for site-specific conjugation	Preclin
Sutro Biopharma Inc.	ADCs produced in cell-free system that inserts non-native amino acid for site-specific conjugation	Preclin
	mAb trailers	
Bind Therapeutics Inc.	Accurins technology consisting of a nanoparticle carrying a therapeutic payload that is linked to targeting ligands	Ph II
Immune Pharmaceuticals Ltd.	NanomAb technology conjugates a nanoparticle carrying a therapeutic payload to a mAb	Preclin
Mersana Therapeutics Inc.	Fleximer polymer technology conjugates a biodegradable hydrophilic polymer linked to a therapeutic payload to a mAb	Preclin
	Improved payloads	
ADC Therapeutics S.a.r.l.	Pyrrolobenzodiazepine (PDB) payloads that cross-link DNA	Preclin
Esperance Pharmaceuticals Inc.	Antibody conjugated to a membrane disrupting peptide (MDP) that interacts with cell membrane to cause cell lysis	Preclin
Synthon B.V.	Potent Payload ADC technology uses prodrug formulated duocarmycins payloads	Preclin

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> ***"The things we are now exploring are really quite wild."***
>
> **Trevor Hallam, Sutro**

Better *au naturale*

While mAb modification has been a popular approach for site-specific conjugation, **Igenica Inc.** thinks it can create a homogeneous ADC using the mAb's natural structure.

Rather than using a cysteine or lysine as the anchor point, Igenica's Stapled ADC technology conjugates the payload to the mAb's disulfide bonds.

While the number of cysteine or lysine amino acids can be highly variable, every mAb has four distinct disulfide bonds: one for each heavy and light chain pair and two between the heavy chains.

"It is the same site for every antibody. You don't have to optimize the site, and you don't have to do any mutagenesis of the antibody or use any proprietary or unvalidated expression system," said CEO Mary Haak-Frendscho. "So there's a speed and beauty there in terms of CMC aspects."

According to Haak-Frendscho, mAbs can have up to 50 lysine residues, meaning conjugation reactions cannot be driven to completion, making production of a homogeneous product difficult.

Haak-Frendscho noted the four disulfide links provide a drug-antibody ratio (DAR) of four, which fits well with lessons learned from Seattle Genetics, ImmunoGen and their partners that have been published in the scientific literature.

"They did all this work that showed a drug-antibody ratio of four appears to be optimal. That is something else we are trying to copy and learn from," she said.

Haak-Frendscho said the conjugation should not affect the binding characteristics of the mAb because the disulfide bonds are distal to the mAb's binding site and Fc portion. Additionally, conjugated payloads don't distort or alter the conformation of the mAb.

Igenica has seven internal ADC programs in discovery or preclinical testing. The most advanced is IGN786, an ADC against an undisclosed target that is in preclinical testing for chronic lymphocytic leukemia (CLL) and acute myelogenous leukemia (AML).

The biotech raised $33 million in a series C round in 2012, enough to get some programs into the clinic.

mAb trailer

While conventional and site-specific ADC approaches are focused on conjugating the drug to the antibody, other companies are attaching polymers or nanoparticles that are then conjugated to drugs.

Of three companies using this mAb trailer approach, Bind is the most advanced. Its Accurins consist of a nanoparticle that contains a therapeutic payload. But instead of using mAbs, the nanoparticle is attached to targeting ligands that bind cell-surface antigens, allowing for targeted delivery of the Accurin.

BIND-014 is in Phase II testing to treat non-small cell lung cancer (NSCLC) and has completed Phase I testing in metastatic castration-resistant prostate cancer (CRPC) and bladder cancer. The compound is a polymeric nanoparticle containing docetaxel that concentrates in the neovasculature surrounding tumors and targets prostate-specific membrane antigen (PSMA; FOLH1; GCPII).

Bind has Accurin development deals with **Amgen Inc.**, **AstraZeneca plc** and **Pfizer Inc.**

Mersana Therapeutics Inc. is using polymers with its Fleximer technology.

The technology conjugates a biodegradable hydrophilic polymer to the mAb using a non-releasing linker. This trailer can be loaded with the compound of choice using cleavable or non-cleavable linkers.

The conjugation is specific in that a known amount of compound is attached to every mAb, creating a homogeneous product. However, the trailer system enables the use of a wider amount and variety of payloads.

A Fleximer can hold 20-30 molecules.

"The fact that we put Fleximer between the payload and the antibody allows us a huge number of choices pharmacologically, because you can attach drugs to Fleximer that you couldn't dream of attaching to an antibody chemically," said President and CEO Nicholas Bacopoulos.

According to the company, this means small molecule targeted agents like kinase inhibitors could be delivered directly to the tumor cell, potentially increasing their efficacy while reducing off-target effects.

Fleximer is not limited to full size mAbs. The polymer can be conjugated to almost any targeting moiety, such as an antibody fragment or other biologics.

It also opens ADCs to indications beyond cancer.

"If you add a payload that has a therapeutic benefit in rheumatoid arthritis, one has the possibility to make an ADC for that indication," CSO Timothy Lowinger said. "You can't do that with just a cytotoxic payload."

As an example, Lowinger said a potent steroid could be conjugated to a mAb against an inflammatory target. "By delivering it specifically to the site of that inflammation with an antibody, one can overcome the systemic side effects of steroids and only have the beneficial anti-inflammatory effects," he said.

In 2012, Mersana partnered with **Endo Health Solutions Inc.** to develop ADCs against a single cancer target. The company also has an alliance with **Adimab LLC** offering prospective partners integrated access to Adimab's antibody discovery technology and Mersana's Fleximer technology.

Immune Pharmaceuticals Ltd. is using a nanoparticle instead of a polymer.

The company's NanomAb technology conjugates a nanoparticle to the mAb. CEO Daniel Teper said the nanoparticle can contain up to 20,000 molecules, which means chemotherapy combinations can be delivered in a targeted fashion directly to tumor cells.

He said one preclinical NanomAb program delivers a combination of paclitaxel and gemcitabine within the nanoparticle.

NanomAbs could be developed to target non-cancer indications, but Teper said the company currently is focused on cancer.

Differentiated warheads

ADC Therapeutics S.a.r.l. and **Synthon B.V.** are seeking to optimize the payload aspect of the ADC modality that could be complementary to most other ADC platforms.

Synthon gained its Potent Payload ADC technology through the 2011 acquisition of Syntarga B.V.

According to CSO Marco Timmers, Synthon's duocarmycin payloads have two advantages over conventional cytotoxic ADC

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> ***"A drug-antibody ratio of four appears to be optimal."***
>
> **Mary Haak-Frendscho, Igenica**

warheads. First, the duocarmycin drug is conjugated to the mAb as a prodrug, not in the active form. Once the ADC is internalized, a specific undisclosed enzyme typically only present in cancer cells cleaves the prodrug into the active form, which should limit systemic toxicity.

A second advantage is that duocarmycins have a very short half-life.

"You can imagine when the tumor cell is dying, the drug may leak out again. So the second mechanism comes into play because even if the drug gets back into circulation, it has a very short half-life," Timmers said.

Synthon's ADC programs use cysteine as a conjugation point like Seattle Genetics, but Timmers argued that using a prodrug should translate into a broader therapeutic window and better safety compared with conventional ADCs.

Synthon's lead program is an ADC against HER2; it is in preclinical development.

While Synthon is pursuing a safer payload, ADC Therapeutics is seeking more potency, which would give ADCs utility against targets that are only expressed at low levels.

According to Director and Collaboration Manager Chris Martin, targets like HER2 are low-hanging fruit for conventional ADCs because the targets are so highly expressed on cancer cells and rapidly internalized. As a result, ADCs can deliver a large cytotoxic dose to the cell for effective killing.

Other targets are not as attractive for conventional ADCs because the target antigen may have a low copy count or have a very slow rate of internalization.

Martin noted patients receiving ADCs are also typically heavily pretreated, "so their resistance machinery is highly upregulated."

In these cases, "you can picture a scenario where you deliver relatively low numbers of antibodies to the tumor cell. They are getting internalized quite slowly, and then when they do get in they are being pumped out quickly or any damage that is done is being repaired," he said. "The challenge then is to get enough of a really potent toxin in to kill the cell."

This is where ADC Therapeutics hopes its pyrrolobenzodiazepine (PDB) warheads can make a difference.

Martin said PDB payloads are two to four times more potent than other cytotoxic agents used in ADCs. Additionally, the PDBs have a different mechanism of action in that they cross-link DNA inside the cancer cell.

As a result, Martin said, "they are not affected at all by the cancer cell resistance machinery. This means that head-to-head with other toxins, ADCs armed with PDBs tend to be very active."

The cross-linking is permanent, he added, so that the PDB-armed ADCs can kill slowly dividing tumor cells like cancer stem cells.

Despite their higher potency, Martin said PDBs have a broader therapeutic window than other cytotoxic agents that are used as payloads, based on dosing in an ongoing Phase II trial of a PDB as a standalone agent to treat leukemia. Information from that study has not been disclosed.

ADC Therapeutics has in-licensed two mAbs to develop as ADCs: VM101 for hematological cancers from **BioAtla LLC**, and a mAb against PSMA to treat prostate cancer from **BZL Biologics LLC**. Both are in preclinical testing.

In June, the company partnered with **Genmab A/S** to develop an ADC for Genmab's HuMax-TAC antibody, which targets IL-2 receptor alpha chain (CD25). Last year, ADC Therapeutics partnered with **Cancer Research Technology Ltd.** to develop ADCs.

A third company developing improved payloads is **Esperance Pharmaceuticals Inc.** The biotech's technology involves conjugating a moiety — such as an antibody — to a membrane disrupting peptide (MDP) that interacts with the cell membrane to cause cell lysis.

Beyond better warheads, some companies are envisioning a future of ADCs without the "C."

Sutro's Hallam said the company is exploring ways to do away with the conjugation altogether.

"The things we are now exploring are really quite wild. What we can actually do is make the non-natural amino acid the cytotoxin itself," he said.

This type of antibody would carry the cytotoxic agent in the antibody structure itself, giving it a type of super effector function. He said this would be difficult to do in cell-based systems as the toxic sequence would likely kill the cell during production.

While it is still early days, he said Sutro's cell-free system may be able to do it.

"That's the direction we will take the field and that could revolutionize it," he said.

COMPANIES AND INSTITUTIONS MENTIONED

ADC Therapeutics S.a.r.l., Lausanne, Switzerland
Adimab LLC, Lebanon, N.H.
Allozyne Inc., Seattle, Wash.
Ambrx Inc., La Jolla, Calif.
Amgen Inc. (NASDAQ:AMGN), Thousand Oaks. Calif.
Astellas Pharma Inc. (Tokyo:4503), Tokyo, Japan
AstraZeneca plc (LSE:AZN; NYSE:AZN), London, U.K.
Bind Therapeutics Inc., Cambridge, Mass.
BioAtla LLC, San Diego, Calif.
Bristol-Myers Squibb Co. (NYSE:BMY), New York, N.Y.
BZL Biologics LLC, New York, N.Y.
Cancer Research Technology Ltd., London, U.K.
Celgene Corp. (NASDAQ:CELG), Summit, N.J.
City of Hope, Duarte, Calif.
Endo Health Solutions Inc. (NASDAQ:ENDP), Chadds Ford, Pa.
Esperance Pharmaceuticals Inc., Baton Rouge, La.
Fabrus LLC, La Jolla, Calif.
Genentech Inc., South San Francisco, Calif.
Genmab A/S (CSE:GEN; OTCBB:GMXAY), Copenhagen, Denmark
Igenica Inc., Burlingame, Calif.
Immune Pharmaceuticals Ltd., Herliya-Pituach, Israel
ImmunoGen Inc. (NASDAQ:IMGN), Waltham, Mass.
Meditope Biosciences Inc., Pasadena, Calif.
Merck & Co. Inc. (NYSE:MRK), Whitehouse Station, N.J.
Mersana Therapeutics Inc., Cambridge, Mass.
Millennium Pharmaceuticals Inc., Cambridge, Mass.
Pfizer Inc. (NYSE:PFE), New York, N.Y.
PolyTherics Ltd., London, U.K.
Redwood Bioscience Inc., Burlingame, Calif.
Seattle Genetics Inc. (NASDAQ:SGEN), Bothell, Wash.
Sutro Biopharma Inc., South San Francisco, Calif.
Synthon B.V., Nijmegen, the Netherlands
Takeda Pharmaceutical Co. Ltd. (Tokyo:4502), Osaka, Japan
Zhejiang Medicine Co. Ltd. (Shanghai:600216), Zhejiang, China