BUILDING ANTIBODY-DRUG CONJUGATES

Participants in a complex supply chain are gearing up to meet demand for an expected flood of **TARGETED THERAPIES**

ANN M. THAYER, C&EN HOUSTON

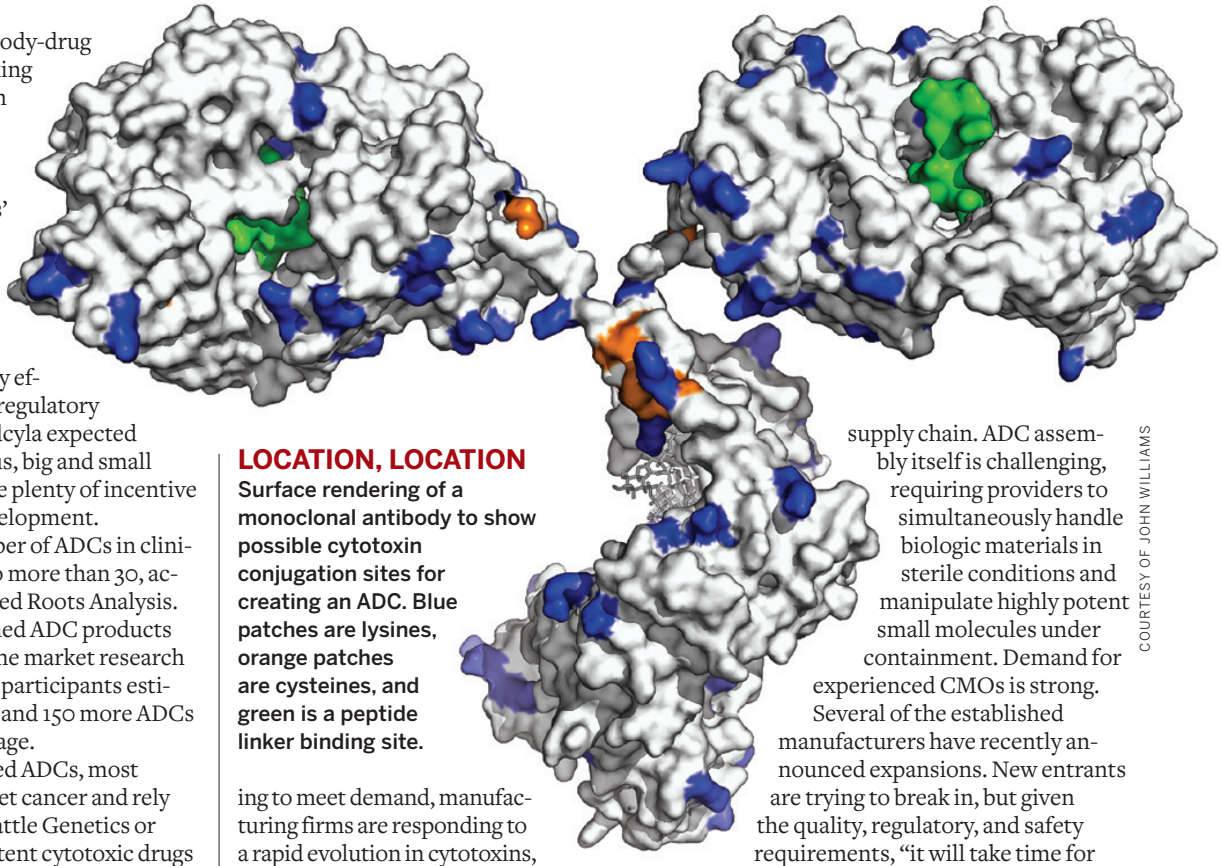
THEY'RE HERE. Antibody-drug conjugates, target-seeking molecular missiles with lethal payloads, have arrived.

Although just two ADCs, Seattle Genetics' Adcetris and Roche's Kadcyla, are on the market, they are proof that the products are technically feasible, therapeutically effective, and able to get regulatory approval. And with Kadcyla expected to hit blockbuster status, big and small pharma firms alike have plenty of incentive to invest in further development.

As a result, the number of ADCs in clinical trials has climbed to more than 30, according to London-based Roots Analysis. By 2018, sales of launched ADC products will exceed $5 billion, the market research firm predicts. Industry participants estimate that between 100 and 150 more ADCs are in the preclinical stage.

Like the two approved ADCs, most clinical candidates target cancer and rely on technology from Seattle Genetics or ImmunoGen to link potent cytotoxic drugs to disease-targeting antibodies. When they are successful as a therapy, the resulting conjugates remain stable in circulation and deliver their toxic payload only when they enter tumor cells, sparing healthy cells and reducing side effects.

Although the idea behind ADCs is straightforward, drug developers have had to overcome technical challenges to make them a reality. Now their manufacturing partners are being tested, as the growing number of development-stage ADCs taxes their production capacity. And beyond trying to meet demand, manufacturing firms are responding to a rapid evolution in cytotoxins, linking chemistries, and antibody design for the next generation of ADCs.



COURTESY OF JOHN WILLIAMS

LOCATION, LOCATION
Surface rendering of a monoclonal antibody to show possible cytotoxin conjugation sites for creating an ADC. Blue patches are lysines, orange patches are cysteines, and green is a peptide linker binding site.

"The technology around all three components of the ADC is changing rapidly," says Brian O'Neill, North American sales manager at Carbogen Amcis, a Swiss contract manufacturing organization, or CMO. The goals are typically improving specificity, lowering dosage, lowering costs, and targeting the right patient population.

ADCs are still a niche area of manufacturing, and few CMOs offer all of the drugs' pieces, which makes for a complex supply chain. ADC assembly itself is challenging, requiring providers to simultaneously handle biologic materials in sterile conditions and manipulate highly potent small molecules under containment. Demand for experienced CMOs is strong. Several of the established manufacturers have recently announced expansions. New entrants are trying to break in, but given the quality, regulatory, and safety requirements, "it will take time for additional CMOs to become established in ADC manufacturing," says Scott Miller, head of special projects at Carbogen Amcis.

"There clearly is a need for manufacturing capacity for ADCs, particularly at the clinical and commercial scale," Miller adds. Carbogen Amcis is increasing its clinical-scale conjugation capacity in Bubendorf, Switzerland, where it also makes potent drugs, and upgrading product-finishing capabilities in Riom, France.

Because of their potency and frequent use in niche disease areas, production runs for ADCs are small. Hundreds of grams are enough for early clinical studies, and kilogram amounts suffice for commercial

& MORE ONLINE Learn more about modifying antibodies for improved site-specific conjugation at http://cenm.ag/adcon.

SITE SPECIFIC

Developers Aim To Create Well-Defined Drug Conjugates

Homogeneity may be bad for creativity, but it's good for antibody-drug conjugates. A well-defined loading—in the number and position of drug molecules conjugated to an antibody—can make an ADC easier to purify and characterize, and possibly more stable and functional as a therapy.

Some companies are using mutagenesis or nonnatural amino acids to create linkage sites on an antibody to attach drug molecules, but another tack is to take advantage of an antibody's natural structure.

This approach is at the core of the technologies used in the two approved ADCs and many of those in clinical development. A method from ImmunoGen attaches cytotoxins to lysines in the antibody. Because there are dozens of conjugatable lysines, the result is a mix of positional isomers with an average drug-to-antibody ratio (DAR) of about 3.5.

"One of the advantages of linking through the lysine residues is that they are not necessarily integral to the structure of the antibody, and therefore it is unlikely to have a major impact on pharmacokinetics," says Charles Morris, ImmunoGen's chief development officer.

Alternatively, Seattle Genetics targets four interchain disulfide bridges in the antibody's hinge region. Breaking these bridges creates eight cysteine thiols as linkage points. Although the locations are more specific than lysine, the ADC can have DARs from zero to eight, with the average controlled around four.

Two ADC technology developers, PolyTherics and Igenica, are targeting this same cysteine-rich region but with new conjugation routes. PolyTherics's ThioBridge reagent is designed to maintain an antibody's shape and stability by reconnecting the cysteines through a three-carbon bridge to which a toxic payload is already conjugated. The firm is partnering with payload developers to access a range of drug-linker combinations to use with its bis-thiol alkylating reagent.

Efficient and predictable, the conjugation process improves solubility and reduces aggregation of the ADC while also resulting in a narrow DAR distribution, according to PolyTherics. "We achieve very high yields, up to 90%, with a DAR of four," says Antony Godwin, the firm's vice president of chemistry. ADCs with ThioBridge don't suffer from deconjugation or cross-conjugation reactions, he says.

Similarly, Igenica's SNAP technology uses bifunctional linkers to maintain the interchain bonding. It works in conventional manufacturing processes and with most linkers and payloads.

supply. Nevertheless, the small-scale capacity additions now under way "may be a little bit shortsighted," suggests Charlie Johnson, chief executive officer of ADC Biotechnology, a process technology firm based in Wales.

Kadcyla has been a wake-up call for suppliers. The ADC is widely expected to succeed the established forerunner antibody drug Herceptin as a treatment for advanced breast cancer, which means that demand will quickly call for hundreds of kilograms per year. Although the demand for most products will remain small, other big opportunities still could emerge for lung, breast, and colorectal cancer treatments, Johnson says.

IN FACT, ROCHE decided late last year to spend $200 million to build its own production facility in Basel, Switzerland, to support Kadcyla and another eight ADCs in its clinical pipeline. Custom chemicals firm Lonza, which also manufactures Kadcyla, is investing $15 million to add a second commercial-scale ADC facility in Visp, Switzerland, later this year.

Among CMOs, Lonza claims one of the most complete offerings, covering antibody production, linker and toxin synthesis, and conjugation of the components. Expansion into the fill and finish of the final product is a "strategic interest," says Syed T. Husain, head of business development for chemical manufacturing.

Working with a single supplier that can handle all the stages of ADC manufacturing avoids having to transfer technology and hand off highly toxic compounds. "In splitting up the supply chain between the toxin, conjugation, and fill-finish, customers become concerned because these are such high-value materials, and shipping them around the world adds risk," Husain argues.

Like Lonza, France's Novasep can supply antibodies, potent compounds, and conjugations, but not every customer wants all these services from a single source, acknowledges Jean-François Marcoux, the firm's technical business development manager. "The demands for the biological and small-molecule aspects are very different and are sometimes treated separately by our customers," he says.

"It is good, though, for a CMO to have experience in all areas because you will not develop your payload the same way if you know what it will entail in the conjugation and vice versa," Marcoux adds. To meet growing customer demand, the company invested $4 million in 2012 to expand capacity for synthesizing ADC payloads.

Executives with companies that don't offer antibody production note that many customers come to them with antibodies in hand. "Antibody manufacturing, for the most part, has not been a bottleneck," says Cynthia Wooge, a market manager at SAFC, the custom manufacturing arm of Sigma-Aldrich. "Many people have developed the antibody first because they want a specific targeting effect, and then they look at how to make it into a more effective therapeutic by arming it."

For example, by turning the antibody used in Kadcyla into an ADC, Roche has helped extend its life, because patents on Herceptin, Roche's third-largest product, will expire in Europe this year and in the U.S. within five years. In other instances, constructing an ADC can salvage an

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"We have performed proof of concept in rodents and have demonstrated that our linkers are at least as efficacious if not better than the conventional linkages," Igenica CEO Mary Haak-Frendscho says. "We suspect that as the industry gets more experience the drive will be toward homogeneous ADCs, not just to create the drug quality but the uniformity and the reproducibility of the drug product" that regulators want to see.

To date, however, none of the new approaches has been fully developed or has passed regulatory muster. Meanwhile, ImmunoGen's Morris notes that his firm has proven that its technology works and is acceptable to regulators: It is used in the Roche cancer drug Kadcyla. The company is advancing its ADC technology through new linkers, toxins, and antibodies, but "there is still a lot to be done with the current generation of ADC technology," Morris says.

antibody that was ineffective alone as a therapeutic.

SAFC supports bioprocessing and cell-line development but doesn't make antibodies. Its strength is in supplying toxins and linkers. The business recently began expanding its conjugation capabilities in St. Louis and its highly potent drug facility in Verona, Wis. "Now is the right time to invest in commercial-scale conjugation capability," Wooge says.

India's Piramal Healthcare is another chemistry-based CMO that is trying to make the supply chain more seamless. "A number of customers, particularly the small entrants, find it difficult to manage complex supply chains that are fragmented between various suppliers," says Aidan Walker, Piramal's president for formulation services. At the same time, "customers want to ensure that they are working with the best provider of each part of the value chain."

Piramal sees partnerships as a way to achieve both goals. "There may be opportunities to both shorten the development time and improve the service if we are able to talk to and work alongside the antibody supplier." Through an alliance with Fujifilm Diosynth Biotechnologies, Piramal can offer customers biologics manufacturing along with its own conjugation services.

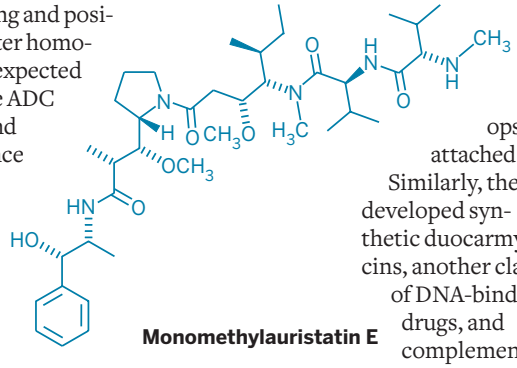
In July, Piramal began a $2.5 million project to add a second commercial-scale ADC conjugation suite at its Grangemouth, Scotland, site. "The market seems reasonably comfortable with the supply of toxin and linker," Walker says. "The constraint, and where we frequently get asked questions, is around the fill-finish capacity, and we are more actively pursuing that as a potential area of growth."

"Now is the right time to invest in commercial-scale conjugation capability,"

New Jersey-based Catalent Pharma Solutions is also taking a partnership approach to filling out its offering. Already an antibody manufacturer and provider of analytical services for ADCs, it expanded further into ADC technology in April 2013 by taking an equity interest in Redwood Bioscience. It has exclusive rights to market Redwood's SMARTag technology for engineering antibodies for conjugation. The partners hope to develop the technology and attract additional partners.

Redwood's approach involves introducing amino acids into the antibody as linkage points. From there, using existing linkers or new ones developed in-house, it can chemoenzymatically attach commonly used cytotoxins and other payloads at defined locations. The company says it has successfully tested more than 50 such combinations against multiple targets.

THE AIM OF THIS SO-CALLED site-specific conjugation is to create homogeneous ADC structures in terms of their drug loading and position. Greater homogeneity is expected to improve ADC stability and performance while greatly simplifying the demanding analytics needed to characterize their complex structures.



Monomethylauristatin E

Some CMOs have reported that they spend more than 50% of their time on an ADC project developing analytical methods, validating production, and testing. Always critical in drug manufacturing, analytics are doubly important with ADCs because both chemical analysis and biological testing are involved, SAFC's Wooge explains. For example, beyond the usual impurity and stability testing, manufacturers must assess whether the conjugation has gone as planned and if both the antibody and drug remain active.

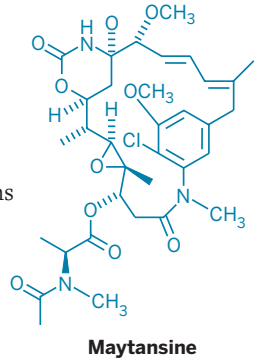
And regulators have indicated that they'll expect the same degree of characterization and impurity control for the separate biologic and toxin as for the final combination. "More and more, the payload or toxin is considered as an active ingredient by the regulatory agencies, even if it later will be conjugated to an antibody," Novasep's Marcoux says.

At the same time, more potent classes of toxins, having different mechanisms of action, are emerging as alternatives to the auristatins from Seattle Genetics and the maytansines from ImmunoGen, all of which act by inhibiting tubulins, which are proteins involved in cell growth.

In October, ImmunoGen presented preclinical data on new indolinobenzodiazepine DNA-alkylating agents, or IGNs. The firm created the IGNs to expand the development of ADCs against cancers that express few binding sites or are resistant or not sensitive to other drugs.

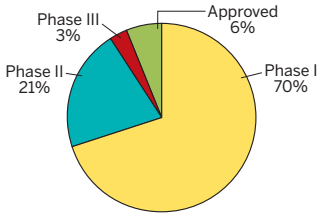
DNA-binding pyrrolobenzodiazepines (PBDs) from Spirogen have garnered enough attention that AstraZeneca paid $440 million last year to buy the London-based firm. AstraZeneca also has a stake in Switzerland's ADC Therapeutics, which develops ADCs using PBD dimers attached via biodegradable linkers.

Similarly, the Dutch firm Synthon has developed synthetic duocarmycins, another class of DNA-binding drugs, and complementary linker chemistry. Other new cytotoxins of note, according to Roots Analysis, include Heidelberg Pharma's α-amanitin, which comes from

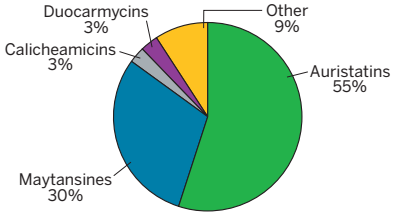


Maytansine

INCREASED FLOW Of the more than 30 antibody-drug conjugates in development, most are in early stages ...



... and most use one of two cytotoxin drug classes.



ADCs in development[a] = 33

a As of mid-2013.
SOURCE: Roots Analysis

the green death cap mushroom, and the plant-derived deBouganin from Canada's Viventia Bio.

Orders of magnitude more potent than the marketed cytotoxic agents, these new compounds are harder to deal with in the manufacturing plant, ADC Bio's Johnson explains. "If we are going to continue to go down this route, then we are going to have to get smarter about how we conjugate them." Over the next five to 10 years, he contends, "the manufacturability of ADCs is really going to become key."

For example, Johnson says his firm is seeing a lot of hard-to-conjugate drugs, hydrophobic ones in particular. With those, if the ratio of drug molecule to antibody gets too high, the ADC molecules can aggregate and can't be purified.

To avoid these problems, ADC Bio has developed a "lock and release" technology through which it immobilizes the antibody on a solid support, conjugates it, and then releases the ADC in a soluble form. The process can use any drug-linker chemistry and be run on columns in flow mode, the firm claims.

In May, Johnson says, ADC Bio opened a tech service lab. Like others in the field, it is starting to work on next-generation linker and toxin technology. For example, in addition to improving the solubility of the drug-linker and the conjugate, many ADC developers are looking for ways to prereact the linker and toxin and attach them to the antibody in a one-step process.

Linker and toxin pairings significantly influence an ADC's stability, pharmacokinetics, and efficacy. All the parts must function correctly if the ADC is to bind selectively to the target before the toxin is internalized and released in a tumor cell. If they don't, premature release of the drug can poison the patient while naked antibodies compete with the ADC at target-binding sites.

The ADC field has been dominated by Seattle Genetics' technology, which links cytotoxins to cysteines in the antibody structure, and ImmunoGen's, which attaches drugs to surface-accessible lysines. Although these approaches have been validated by approved products and multiple licenses from big pharma firms, more can be learned about improving potency, linker stability, and targeting (C&EN, June 18, 2012, page 12).

"People are trying to push the envelope around new linkers and toxins," Piramal's Walker says. And the company is starting to see customer requests for development and manufacturing of these new materials for toxicology and early clinical testing. "We've also been approached by many small companies that have a linker, a toxin, or an antibody but never all three, and little way of proving whether it could be conjugated."

IN RESPONSE, Piramal has added a proof-of-concept service. Taking whatever the customer brings, the company adds the other needed components and then quickly creates and tests an ADC to see if the customer's idea is worth pursuing. The service, Walker believes, "will give some new players a chance to expand the potential of the ADC business."

Site-specific conjugation technologies designed to make more homogeneous ADCs are making their way into manufacturers' plants. Piramal, for example, will soon start its first development and production run for a customer that is using a new site-specific approach, Walker says. "It's the first one that we have seen but is certainly an area of discussion that is becoming more prevalent."

Almost all the ADCs in clinical development use established conjugation chemistries and cytotoxins. Manufacturers have become adept at working with them, but they may soon be challenged by new technologies.

New cytotoxin classes, linker chemistries, and antibody designs will only multiply the possible combinations that CMOs will face. Looking ahead, suppliers also point to growing interest in ADCs outside oncology in areas such as inflammatory and autoimmune diseases. And requests are coming in for combining an array of payloads with antibodies, nanoparticles, and other carriers. Challenges like these should keep ADC manufacturers busy for some time to come. ■

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