Antibody–drug conjugates for cancer: poised to deliver?

Highlighted by Genentech's recent US regulatory submission for trastuzumab–DM1, antibody–drug conjugation technology could be heading for the mainstream in anticancer drug development.

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In July this year, Genentech submitted a biologic license application to the US Food and Drug Administration (FDA) for the antibody–drug conjugate (ADC) trastuzumab–DM1 (T–DM1). It is hoped that by coupling trastuzumab (Herceptin; Genentech/Roche) — a humanized monoclonal antibody (mAb) specific for the human epidermal growth factor receptor 2 (HER2; also known as ERBB2) — to the cytotoxic agent DM1 (emtansine; ImmunoGen), T–DM1 could provide more potent anticancer effects than trastuzumab alone, which is a blockbuster therapy for HER2-positive breast cancer.

Genentech's application is based on the results of a Phase II trial known as TDM4374g, a single-arm trial that assessed the efficacy of T–DM1 in 110 women with HER2-positive advanced breast cancer whose disease had worsened after receiving at least two prior HER2-targeted treatments: trastuzumab and lapatinib (Tykerb; GlaxoSmithKline), as well as an anthracycline, a taxane and capecitabine (Xeloda; Roche). On average, the women had received seven drugs to treat their metastatic disease prior to receiving T–DM1.

"One of the reasons that we think that [TDM4374g] is important is because this is the first trial that looked at patients who have received the standard chemotherapies and both the HER2-directed agents. ▸

Table 1 | **Selected antibody–drug conjugates in Phase II or III trials***

Drug (developer)	Antibody–drug conjugate	Indication (phase)
Glembatumumab vedotin (Celldex Therapeutics)	A fully human mAb specific for GPNMB conjugated to monomethyl auristatin E‡	Metastatic breast cancer and melanoma (II)
Trastuzumab emtansine (Roche/Genentech/ Chugai)	A humanized mAb specific for HER2 conjugated to the maytansine derivative DM1§	HER2-positive metastatic breast cancer (II/III)
Lorvotuzumab mertansine (ImmunoGen)	A humanized mAb specific for CD56 conjugated to the maytansine derivative DM1	Small cell lung cancer, Merkel cell carcinoma, ovarian cancer and multiple myeloma (II)
SAR-3419 (Sanofi–Aventis)	A humanized mAb specific for CD19 conjugated to the maytansine derivative DM4§	Non-Hodgkin's lymphoma (II)
Brentuximab vedotin (Seattle Genetics/ Millennium Pharmaceuticals)	A chimeric mAb specific for CD30 conjugated to monomethyl auristatin E	Anaplastic large cell lymphoma (II), relapsed or refractory Hodgkin's lymphoma (II) and Hodgkin's lymphoma following autologous stem cell transplant (III)
Inotuzumab ozogamicin (Pfizer)	A humanized mAb specific for CD22 conjugated to calicheamicin	Diffuse large B-cell lymphoma, indolent non-Hodgkin's lymphoma (II)

GPNMB, glycoprotein non-metastatic melanoma protein B; HER2, human epidermal growth factor receptor 2 (also known as ERBB2); mAb, monoclonal antibody. *This list only includes antibody–drug conjugates that link an antibody to a cytotoxic agent; it does not include antibodies conjugated to radioisotopes or immunotoxins. ‡Licensed from Seattle Genetics. §Licensed from ImmunoGen.

Currently, there is no standard treatment for these patients," says Ian Krop, an oncologist at the Dana–Farber Cancer Institute in Boston, USA, and lead investigator of the TDM4374g trial.

Of the 110 women in the TDM4374g trial, 32.7% achieved an objective response (a complete or partial tumour shrinkage of at least 30%), which analysts suggest is a strong response for such a sick patient population.

"What is interesting about these data is that they indicate that, despite the patients having cancers which have become resistant to trastuzumab, a large proportion of the cancers retain HER2 expression," says Krop. So, in this population, trastuzumab can still be used to target HER2. "The advantage of using T–DM1 is that you considerably improve the therapeutic index because the antibody delivers the chemotherapy directly to the cancer cell. Also, because DM1 is tethered to the antibody, it minimizes the exposure of DM1 to the normal tissues, thereby reducing toxicity," he adds.

The idea of using an antibody to target a cytotoxic agent to cancer cells has existed since mAbs were first being developed for clinical use in the 1980s. But, the clinical success of antibodies conjugated to cytotoxic drugs has been limited compared with that of naked antibodies. So far, only one drug has been approved by the FDA: gemtuzumab ozogamicin (Mylotarg; Pfizer), a humanized mAb specific for CD33, conjugated to the cytotoxic drug calicheamicin, was approved in 2000 for the treatment of patients with acute myeloid leukaemia.

However, on 21 June this year, Pfizer voluntarily withdrew Mylotarg from the US market. "The post-approval study required by the FDA that combined chemotherapy and Mylotarg did not demonstrate improved survival. Furthermore, the rate of fatal toxicity was higher in patients treated with chemotherapy and Mylotarg compared with patients treated with chemotherapy alone," says Alain Beck, Head of the Physicochemistry Department at the Centre d'Immunologie Pierre Fabre, Saint-Julien-en-Genevois, France.

With the withdrawal of Mylotarg, Genentech's T–DM1 is now the latest-stage ADC among a total of six ADCs (which link an antibody to a cytotoxic agent; this does not include antibodies conjugated to radioisotopes or immunotoxins) currently in Phase II or III development (TABLE 1). Given the huge investment in antibody development technologies, particularly in recent years as large pharmaceutical companies have been expanding their expertise in biologicals, a key question is why more ADCs have not been approved by now.

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Peter Senter, Vice President of Chemistry at Seattle Genetics, thinks that one reason is that the ADC field progressed to the clinic before the technology was ready. "The original clinical trials tested antibodies that are directed against antigens that hadn't been well characterized. So, for example, they were present on many normal tissues. Also, some of the antibodies were of mouse origin [and caused increased rates of immunogenicity] and the drugs had low potency," he explains.

However, over the past decade, companies including Seattle Genetics and ImmunoGen have extensively researched the ideal characteristics of an ADC. "It took a while to figure out that you need to have an antibody against a well-characterized antigen and a highly potent drug, mixed with incredibly stable linker systems and conjugation technologies that preserve the characteristics of the antibody," says Senter.

HER2 (the target of T–DM1), for example, has the three characteristics of an ideal antigen for an ADC, says Krop. "It is expressed in millions of copies on a HER2-positive cancer cell, whereas other tissues express low levels of HER2. It is internalized fairly quickly, which is also important, and it doesn't get downregulated. So, once you bind T–DM1, HER2 does not disappear from the cell; it keeps making more."

Characterizing the target antigen is also something that Tibor Keler, Senior Vice President and Chief Scientific Officer at Celldex Therapeutics, highlights: "One of the benefits of HER2 is the amount that is known about the target. Similarly, we have been putting a lot of effort into understanding

our target, glycoprotein non-metastatic melanoma protein B, and its expression to help design our clinical studies and to select the patient population that will have the greatest opportunity to benefit."

The second important component of an ADC is a highly potent cytotoxic drug. T–DM1 contains an agent derived from maytansine that is 100-fold to 10,000-fold more potent than standard chemotherapeutic agents. "Maytansine was tested as a monotherapy in the 1970s, but it was too toxic and had no therapeutic window. However, our insight was that we needed to have agents that were potent enough to have effective payloads for antibody delivery. So, we made derivatives of maytansine known as DM1 and DM4 that can be linked to antibodies and have the appropriate potency," says John Lambert, Chief Medical Officer at ImmunoGen.

Similarly, Seattle Genetics have also developed their own highly potent drugs, known as auristatins. These are synthetic drugs that have been engineered to have functionalities for attaching to a linker to conjugate the antibody to the drug, as well as broad activity against a wide variety of tumour types. "Another advantage is that by developing a robust synthetic pathway to make the auristatins, we can reproducibly make them in large amounts, whereas it is often difficult to obtain large quantities of natural products," says Senter.

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It is important to use a highly potent drug because research in the 1980s and 1990s indicated that the amount of mAb that is found at a tumour is only about 0.01% of injected dose per gram of tumour tissue (at best) in humans, says Lambert. "Recent studies have shown that one cannot do much better in terms of the amount of mAb delivered than with an intact immunoglobulin G1 antibody, even with all the advances in engineering," he adds. Hence, the cytotoxic agent bound to the antibody needs to be highly potent so that the ADCs that do reach the target cell have the maximum killing potential. Other characteristics of the drug component of the ADC are that it must be non-immunogenic and non-toxic (dormant or inactive) when circulating in the blood.

Importantly, says Senter, there is also an optimal amount of drug that should be added to each antibody to avoid changing the characteristics of the antibody. "The prevailing view was that the more drug molecules that you could add to an antibody the better. But we found that as you attach more and more drug molecules to each antibody, the more the ADC becomes like a small-molecule drug — it clears rapidly, it does not distribute properly and the antibody does not bind well to its target tumour antigen. We found that between two and four drug molecules were required per antibody to ensure the characteristics of the antibody were not changed."

Last, but not least, the ADCs that are being developed now benefit from advanced linker technology. "The greatest amount of advancement and development in this field has been in the linker that links the toxin molecule to the antibody," says Keler. "The linker has to be stable enough to ensure that the ADC remains intact until it reaches its target cell, so it has to be resistant to enzymes in the serum, for example. Then, once the ADC is internalized, the linker and drug have to be released," he adds.

ImmunoGen has developed a family of linkers that have varying chemistries that they can use for different types of antigen target. "We use linker chemistries that attach to lysine amino acids on the surface of antibodies. Lysines are surface-accessible, hydrophilic, charged residues, and you can modify them without disturbing the structure of the antibody. Essentially, we create ADCs that are indistinguishable from a naked antibody in terms of the *in vivo* properties of the antibody (such as the pharmacokinetics)," says Lambert.

T–DM1 uses an ImmunoGen linker that is non-cleavable in all biological systems. Other linkers from ImmunoGen utilize disulphide bonds that are cleavable inside cancer cells and, once the cytotoxic drug is released, will allow for the possibility of bystander killing of neighbouring cancer cells. "This may be important when there is antigen heterogeneity (in which different tumour cells express varying amounts of antigen). Bystander killing may also be useful for the treatment of solid tumours to help overcome the uneven penetration of antibodies throughout the solid tumour," says Lambert. ImmunoGen have also recently developed a new class of linkers that use hydrophilic elements, such as charged residues or short polyethylene glycol sequences. With these linkers, once the cytotoxic drug has been released into the cell, the hydrophilic elements help the drug to stay inside the cell. "This is particularly important because some cancers overexpress multidrug-resistance efflux pumps. With this newer class of linker we will be able to develop ADCs that target those cancers," says Lambert.

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Seattle Genetics has also designed its own linker. "When we first started working with the auristatins, we used conventional linkers such as acid-labile hydrozones and disulphide bonds, but we found that most of the drug was coming off the antibody before it had reached the tumour. So, we have developed conditionally labile peptide bonds that are stable in the circulation. The peptides are not cleaved by proteases in the systemic circulation, so when the conjugate gets to the tumour, the peptides are cleaved by intracellular tumour-associated proteases."

Although only a few ADCs are in the late stages of clinical development, both Seattle Genetics and ImmunoGen have multiple collaborators. Seattle Genetics is working with Genentech (who recently extended their ADC development deal with them), Millennium Pharmaceuticals, GlaxoSmithKline, Celldex Therapeutics, Progenics Pharmaceuticals, Bayer Schering Pharma, Daiichi Sankyo, MedImmune and Astellas Pharma. ImmunoGen is working with Genentech, Sanofi–Aventis, Amgen, Biogen Idec, Biotest and Bayer Schering Pharma.

With so many companies seeking access to ADC technology, the field looks poised to take-off. Lambert concludes: "We have technology that works and there are many antibodies that have been developed to bind to a wide variety of antigens that may need an additional factor to turn them into effective and potent drugs."