ADCs and solid tumours

the payload revolution that drives therapeutic opportunities in unmet need

The fight against cancer is ongoing. Despite progress in many indications, mortality rates for some of the most difficult to treat solid tumour types have not improved substantially since the early 1970s.

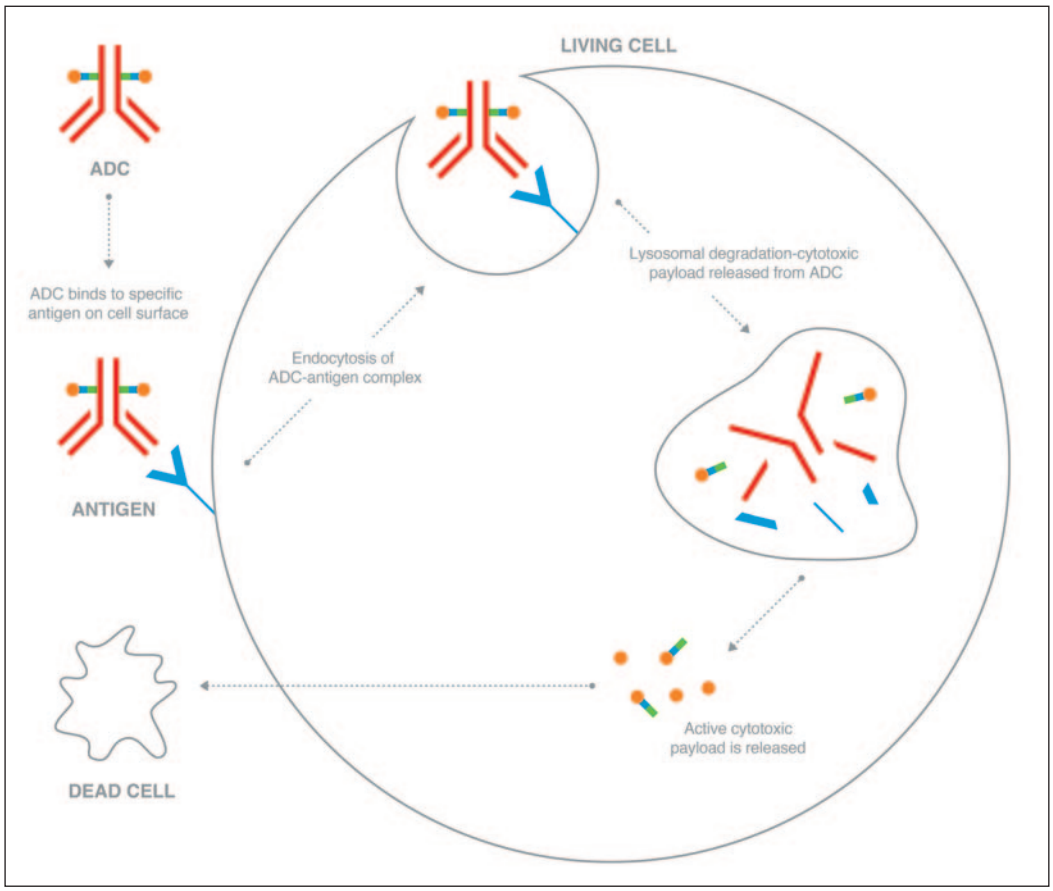
By Dr Jenny Thirlway

Ten-year survival statistics for oesophagus and lung cancer have shown an increase of less than 10% and pancreatic cancer has shown no improvement in survival rates at all[1]. Even for cancers where survival rates have improved, such as malignant melanoma, breast and uterine, there is still a need for more efficacious treatments which importantly, do not come with debilitating side effects.

Antibody Drug Conjugate (ADC) technology has been the focus of intense interest as a means to provide selective tumour killing with increased efficacy and fewer side-effects than standard of care chemotherapies. ADCs comprise a monoclonal antibody (or antibody fragment) that targets a tumour-associated antigen, conjugated via a chemical linker to a highly cytotoxic entity. Binding of the antibody to the cell surface triggers internalisation, and processing within endosomes or lysosomes releases the potent cell-killing molecule. Combining the targeting power of an antibody with a potent cytotoxic agent makes it possible to eradicate cancer cells more effectively and selectively, while reducing the side-effects which undermine patient quality of life. Currently, seven ADCs have been approved (four for haematological cancers and three for solid tumours) and the clinical and commercial potential of ADC technology is reflected in the burgeoning pipeline, with around 90 ADCs in clinical trials as of January 2020[2]. The global market for ADCs is anticipated to reach $7.5 billion by 2025.

First generation ADCs suffered from a lack of potency, as existing chemotherapeutic agents with known toxicity profiles such as methotrexate, vinblastine and doxorubicin were conjugated in an effort to increase their specificity. The cBR96-doxorubicin conjugate showed promise in pre-clinical studies and progressed to a Phase II clinical trial but was insufficiently efficacious. The effectiveness of an ADC depends on efficiency of internalisation and the failure of this conjugate was largely attributed to under-potency of the doxorubicin payload. It is estimated that 4-12 million doxorubicin molecules are required to kill a cell. Antigen expression levels are generally less than one million copies per cell making it difficult to achieve a critical concentration of toxin[3]. Lessons from the story of cBR96-doxorubicin established the use of more potent cytotoxic payloads to counteract issues with payload delivery.

The first ADC to be approved, Mylotarg® for treatment of acute myelogenous leukaemia (AML), is conjugated to calicheamicin. Mylotarg® was originally approved by the FDA in 2000 and subsequently withdrawn in 2010 following an unsuccessful confirmatory Phase III trial[4]. Calicheamicin is a potent anti-tumour antibiotic that associates with the minor groove of DNA causing double



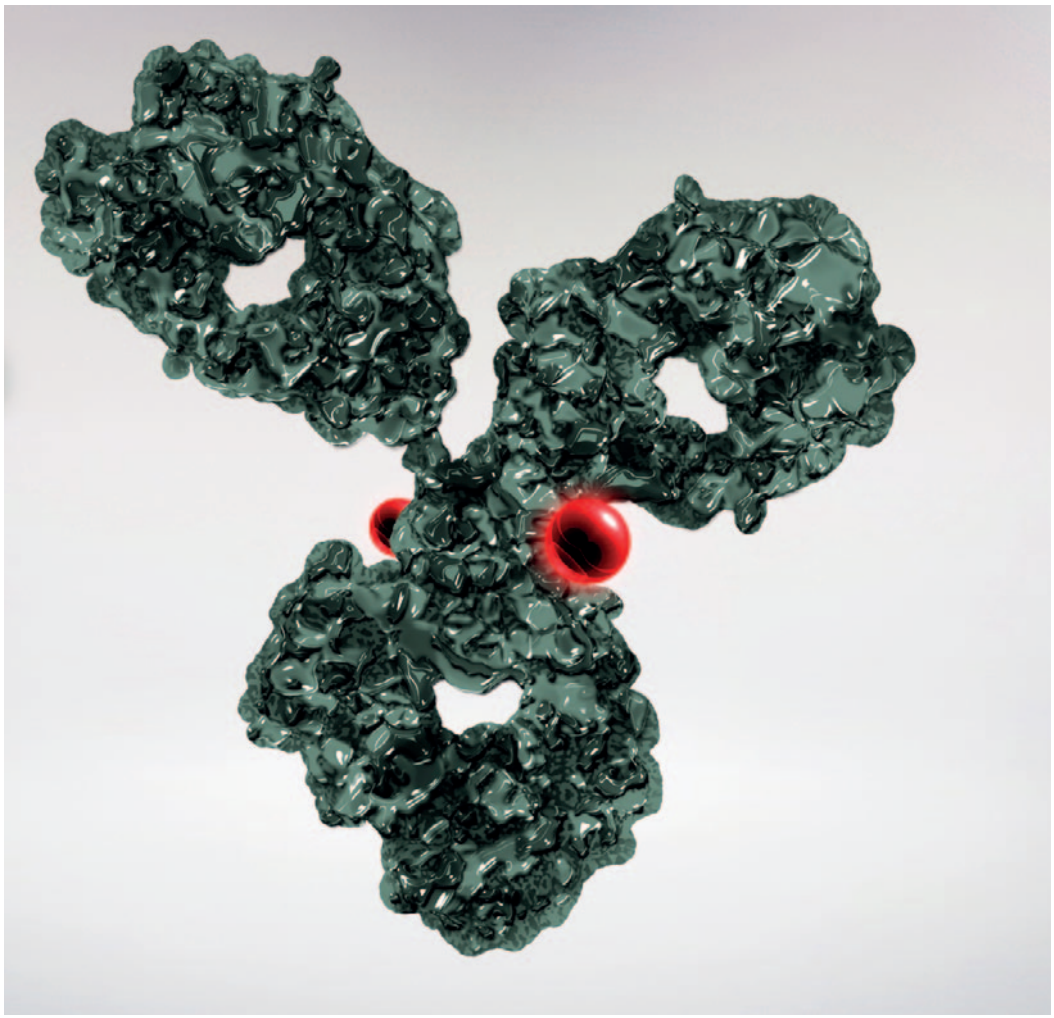
Mechanism of action of ADCs

strand DNA breaks. The failure of Mylotarg® was partly attributed to an unstable linker that released payload prematurely. It was reapproved by the FDA in September 2017 and by the EMA in April 2018 on an altered dosing regimen. Another calicheamicin-based ADC, Besponsa®, was also approved in 2017 for the treatment of relapsed or refractory acute lymphoblastic leukaemia, but three other calicheamicin ADCs in development have been discontinued due to having a very narrow Therapeutic Index (TI).

Around 40% of clinically-active ADCs employ tubulin inhibitors from the auristatin and maytansine classes[5]. Auristatins are derived from dolastatin 10, isolated from the marine sea hare *Dolabella auricularia*. They block tubulin assembly and cause G2/M phase cell cycle arrest. One of the first ADCs to be approved, Adectris®, for treatment of Hodgkin lymphoma and systemic anaplastic large cell lymphoma, is conjugated to Monomethyl auristatin E (MMAE), as is Polivy™ for the treatment of relapsed refractory diffuse large B cell lymphoma. Maytansine was isolated from the bark of the African shrub *Maytenus ovatus* and is a potent inhibitor of microtubulin assembly. Maytansines bind to the 'plus' end of growing microtubules blocking polymerisation of tubulin dimers. Two out of three approvals of ADCs in solid tumours employ tubulin inhibitors as the payload. Kadcyla® was approved by the FDA in February 2013 for the treatment of Her2 positive metastatic breast cancer and is comprised of the anti-Her2 antibody trastuzumab conjugated to the maytansine DM1. Padcev™ was granted accelerated approval by the FDA in December 2019 for treatment of locally-advanced or metastatic urothelial cancer and is conjugated to the auristatin MMAE.

Tubulysins have been developed as next generation tubulin inhibitors. Tubulysins bind to the vinca binding site of tubulin and exhibit potent cytotoxic activity in the picomolar (pM) range. Unlike the auristatins and maytansines, tubulysins have been shown to bypass drug efflux pumps and retain potency in MDR1 expressing cell lines[6]. Three out of four of the tubulysin-based conjugates (two Small Molecule Drug Conjugates and one ADC) to have entered the clinical development phase have been discontinued due to either safety or efficacy reasons.

Although first-generation tubulin inhibitors



A structural model of an Antibody Drug Conjugate (*photograph courtesy of Iksuda Therapeutics*)

have been approved in several indications, they are not as effective in indications with lower expression of target antigen or cells that are less sensitive to tubulin inhibition. It has become clear that there is a need for payloads with increased potency and alternative mechanism of action to the tubulin inhibitor class. There has therefore been a push towards the design of payloads to address targets where tubulin ADCs are proving to be insufficiently active, such as colon cancer.

Third-generation ADCs have looked to payloads with novel modes of action and activity against non-proliferating cells so targets can be widened to include tumour initiating cells (TICs). Higher potency allows for targeting of antigens with relatively low tumour expression levels.

Pyrrolobenzodiazepines (PBD) are based on naturally-occurring, anti-tumour antibiotics that bind to the DNA minor groove in a sequence-specific manner. PBD dimers can form interstrand and intrastrand DNA cross-links by covalently binding to the nucleophilic C2-amino group of a guanine base and are 600 times more potent than PBD monomers *in vitro*. As with tubulysins, PBDs avoid MDR1 mediated drug resistance[7]. The first PBD dimer-based ADCs entered the clinic in 2013. Vadastuximab talirine targets CD33 which is over-expressed in AML. Pre-clinically vadastuximab talirine was shown to be more potent than Mylotarg® against a panel of AML cell lines and primary AML cells *in vitro* and *in vivo*. Unlike Mylotarg®, anti-leukaemic activity was observed in AML models with the multidrug-resistant phenotype[8]. However, in June of 2017 Seattle Genetics announced the discontinuation of the Phase III CASCADE clinical trial of vadastuximab talirine (SGN-CD33A) in frontline older AML patients following consultation with the Independent Data Monitoring Committee (IDMC) and after reviewing unblinded data. The data indicated a higher rate of deaths, including fatal infections in the vadastuximab talirine-containing arm versus the

control arm of the trial. In March 2018 Seattle Genetics announced it was no longer developing vadastuximab tailrine as a result of recent portfolio and resource prioritisation decisions.

Rovalpituzumab tesirine (Rova-T) targets cancer stem cell-associated target delta-like protein 3 (DLL3) which is over-expressed in small cell lung cancer (SCLC), a solid tumour with few treatment options. In Phase I/II studies of relapsed SCLC patients who had previously failed one or more standard therapies, Rova-T demonstrated overall response rates of 44% in the patients identified with high expression of DLL3. Based on these results, in 2016 Abbvie announced that it would acquire Stemcentrx and its lead late-stage asset rovalpituzumab tesirine in a deal worth upwards of $9 billion. By 2018, things were looking less promising when disappointing results were announced for the Phase II TRINITY trial. An independent review committee assessed efficacy as an overall response rate of 16% (95% CI, 11, 22) with the most common (≥5%) grade ≥3 severe toxicities being thrombocytopenia (11%), photosensitivity reaction (7%) and pleural effusion (5%)[9]. The development of Rova-T was discontinued in August 2019 after the IDMC recommended terminating the Phase III MERU study due to lack of survival benefit.

Since 2013, 20 PBD dimer-based ADCs have entered clinical development with eight being discontinued. The apparent narrow TI of PBD dimer-based drugs has led to a shift away from cross-linkers towards DNA monoalkylators such as the indolinobenzodiazepine pseudodimers (IGNs) and duocarmycins.

Within the IGN class, it was discovered that the cross-linking analogues had an unfavourable toxicity profile in mice with the animals displaying prolonged body weight loss, as well as delayed lethality. Development of a DNA alkylating IGN payload that does not have cross linking capability provided a distinct mode of action compared with that of the PBDs. *In vivo* testing revealed that the alkylating IGN ADC did not display any signs of prolonged or delayed toxicity[10].

To date, there are four pre-clinical IGN-based

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Continued on page 29

ADCs and three have entered clinical trials. IMGN779 was the first IGN-based ADC to enter clinical trials in 2016. In 2018 Immunogen reported that IMGN779 displayed tolerability with repeat dosing across a wide range of doses in patients with relapsed AML. One Dose Limiting Toxicity was reported and Adverse Events were consistent with underlying disease. No cumulative toxicity was observed following multiple doses (up to 40). IMGN779 demonstrated anti-leukaemia activity in 41% (12 of 29) patients with evaluable bone marrows (≥ 0.39mg/kg). However, the company discontinued the development of IMGN779 in June 2019 following portfolio prioritisation and restructuring initiatives.

Immunogen has another IGN-based ADC in clinical trials in collaboration with Jazz Pharmaceuticals. IMGN632 is being developed for patients with relapsed AML, Blastic Plasmacytoid Dendritic Cell Neoplasm and other CD123 positive haematologic malignancies. In 2019 the FDA granted Orphan-Drug Designation for IMGN632 as a treatment for AML. Data presented at ASH in 2019 showed that IMGN632 displays a tolerable safety profile and activity at doses up to 0.3mg/kg and a dose and schedule of 0.045mg/kg given on day one every three weeks has been selected for Phase II development. In the assessable AML population (n = 66), 37 (55%) had a reduction in bone marrow blasts and 13 (20%) achieved an objective response across all dose levels. Three of seven evaluable BPDCN patients (43%) achieved a response after a single dose of IMGN632.

TAK164 is an IGN-based ADC targeting Guanyl Cyclase C in solid tumour indications. Clinical trial NCT03449030, A Study of TAK-164 in Participants with Advanced Gastrointestinal (GI) Cancer Expressing Guanylyl Cyclase C (GCC), was initiated in 2018 based on promising pre-clinical data in cell line-derived and patient-derived xenograft models.

The success of the IGNs is dependent on the payload class being able to overcome the TI issues of the PBD class of payload. The ADC community will be monitoring the progress of the IGN-based ADCs with interest.

The duocarmycins were initially isolated from *Streptomyces zelensis* in the late 1970s. Duocarmycins have pM potency and bind to the minor groove of DNA in a sequence selective manner forming a covalent linkage to the N3 of adenine. Currently there are five duocarmycin-based ADCs in the development pipeline, predominantly concentrating on solid tumour indications.

The furthest progressed duocarmycin-based ADC is trastuzumab duocarmazine which is being assessed in a Phase III trial in patients with Her-2-positive locally-advanced or metastatic breast cancer[11]. Trastuzumab duocarmazine is essentially a 'biobetter' version of Kadcyla®. Kadcyla® is approved for the treatment of Her2 positive metastatic breast cancer defined by immunohistochemistry testing as being 3+. Based on these criteria 20-25% of metastatic breast cancer patients would be eligible for treatment with Kadcyla®. Trastuzumab duocarmazine aims to extend the target population to patients with lower Her2 expression levels[12].

Kadcyla® surpassed $1 billion in annual sales last year, becoming the first ADC to hit blockbuster status, so there are obvious incentives to develop biobetter versions. The most recently-approved ADC, Enhertu®, is another trastuzumab-based ADC (trastuzumab deruxtecan). In this case the cytotoxic moiety is the topoisomerase inhibitor I inhibitor, deruxtecan.

The topoisomerase I inhibitors employed in ADCs are camptothecin analogues. Camptothecin was originally isolated from the Chinese ornamental tree *Camptothecaacuminata* in the 1980s. Deruxtecan (also known as DX-8951 derivative, DXd) is a more water-soluble analogue of camptothecin and SN38 is an active metabolite of irinotecan which is a semi-synthetic analogue of camptothecin. Camptothecin analogues form a stable complex with human DNA topoisomerase I and DNA, which induces DNA damage and initiates the cell apoptosis pathway[7]. There are currently 12 topoisomerase inhibitor-based ADCs in the development pipeline. These are lower potency cytotoxics and are therefore more suitable than the DNA cross linkers/alkylating compounds for antigen targets that have greater expression in normal tissues.

Daiichi-Sankyo's Enhertu® was granted accelerated approval by the FDA for the treatment of Her2 positive metastatic breast cancer in December 2019 and is notable for being the first ADC to be approved for solid tumour indications that does not employ the tubulin inhibitor payload class. Approval was based on results from the DESTINY-Breast01 (NCT03248492) trial. The main efficacy outcome measures were confirmed objective response rate (ORR) assessed by independent central review. ORR was 60.3% (95% CI: 52.9, 67.4), with a 4.3% complete response rate and a 56% partial response rate. Median response duration was 14.8 months (95% CI: 13.8, 16.9). Earlier in 2019, Daiichi-Sankyo announced a global development and commercialisation collaboration with AstraZeneca. AstraZeneca is set to pay

Daiichi Sankyo up to $6.9 billion in total consideration, including a $1.35 billion upfront payment and up to an additional $5.55 billion contingent upon achievement of future regulatory and sales milestones. The companies will share equally development and commercialisation costs as well as profits worldwide from Enhertu® with Daiichi Sankyo maintaining exclusive rights in Japan.

One of the next ADCs on track for approval is Sacituzumab govitecan which is currently in Phase III trials for several solid tumour types. Sacituzumab govitecan is comprised of an anti-Trop2 antibody conjugated to the irinotecan derivative SN38. Immunomedics' Biologics Licence Application (BLA) submission for the treatment of metastatic triple-negative breast cancer was originally rejected by the FDA due to unresolved manufacturing issues. In December 2019 Immunomedics announced the FDA acceptance of its BLA resubmission for sacituzumab govitecan to treat metastatic triple-negative breast cancer. A Phase I/II clinical trial (NCT 01631552) showed efficacy with a 33% response rate, including complete responses in three patients (2.8%) in a heavily-pretreated population of patients with metastatic triple-negative breast cancer. The clinical benefit rate (including stable disease for at least six months) was 45.4%. Diarrhoea and myelosuppression were the primary adverse events, and discontinuation rates were low[13].

Amanitin-based ADCs represent a new class of ADC using a novel mode of action. Alpha and beta-amanitins were identified more than 40 years ago in the green death cap mushroom *Amanita Phalloides*. Amanitins bind with highest affinity to eukaryotic RNA Polymerase II resulting in a dramatic more than 1,000-fold decrease in transcription and protein synthesis. Amanitins are much more hydrophilic than other cytotoxic drugs and a consequence of this is that they do not passively penetrate the majority of human cells with the exception of hepatocytes. Kidney toxicity is also an issue for amanitins due to this being the excretion route. Heidelberg Pharma is developing amanitin-based ADCs which have potency in the pM range across a number of cell lines[14]. There are currently eight amanitin-based ADCs in pre-clinical development, predominantly in solid tumour indications.

What does the future hold for the development of payloads for solid tumour indications? ADCs have traditionally been more successful in haematological malignancies but the advent of higher potency and novel mechanism of action toxins is starting to bear fruit. Along with other companies in the field, Iksuda is working to develop payloads with high potency and novel mechanism of action with the aim of building the next generation of ADCs to treat the broadest patient population possible. DDW

Dr Jenny Thirlway *leads Iksuda's ADC programmes including the pre-clinical demonstration of Iksuda's proprietary PermaLink® conjugation chemistry, exemplification of novel toxin platforms and the evaluation of partnered antibodies/alternative scaffolds focused on the development of a proof of concept stage ADC portfolio.*

Continued from page 28

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