ADCs as a targeted delivery system must be passed through many hurdles, including blood circulation, antigen binding and internalization. Antibody drug conjugates (ADCs) normally compose of a humanized antibody and small molecular drug via a chemical linker. After decades of preclinical and clinical studies, a series of ADCs have been widely used for treating specific tumor types in the clinic such as brentuximab vedotin (Adcetris®) for relapsed Hodgkin's lymphoma and systemic anaplastic large cell lymphoma, gemtuzumab ozogamicin (Mylotarg®) for acute myeloid leukemia, ado-trastuzumab emtansine (Kadcyla®) for HER2-positive metastatic breast cancer, inotuzumab ozogamicin (Besponsa®) and most recently polatuzumab vedotin-piiq (Polivy®) for B cell malignancies. More than eighty ADCs have been investigated in different clinical stages from approximately six hundred clinical trials to date.
INTRODUCTION

Conventional chemotherapy drugs target both proliferating cancer cells and normal cells and the lack of tumor specificity leads to severe off-target toxicity and limited efficacy. To overcome the limitations of chemotherapeutic agents, appreciable advances have been made in targeted cancer therapies. Several monoclonal antibodies have been approved for the treatment of various cancer types and have demonstrated promising clinical benefits [1]. However, they possess modest antitumor efficacy as a single agent, therefore alternative therapies have been developed.

Antibody–drug conjugate (ADC) technology has been the focus of intense interest as it is viewed as a sophisticated delivery system that combines the benefit of the highly specific tumor targeting of antibodies with the strong potency of a small molecule cytotoxic payload. This in turn reduces the likelihood of systemic exposure and off-target toxicity. ADCs display targeted therapy in a broad range of tumors as they can target a variety of protein markers that are overexpressed by cancer cells. ADCs have achieved considerable success in recent years with seven clinically approved to date: brentuximab vedotin (Adcetris™) for treatment of Hodgkin lymphoma systemic anaplastic large cell lymphoma; inotuzumab ozogamicin (Besponsa™) for treatment of B-cell precursor acute lymphoblastic leukemia; gemtuzumab ozogamicin (Mylotarg™) for treatment of acute myeloid leukemia; polatuzumab vedotin (Polivy™) for treatment of diffuse large B-cell lymphoma; trastuzumab emtansine (Kadcyla™) and trastuzumab deruxtecan (Enhertu™) for treatment of HER2+ metastatic breast cancer and enfortumab vedotin (Padcev™) for treatment of locally advanced or metastatic urothelial cancer. There are currently 91 ADC drug candidates in clinical trials and more than 200 ADCs in preclinical development [2]. The approved ADCs demonstrated clear benefits for patients over standard intensive chemotherapy, proving that the therapeutic index of highly potent cytotoxins can be elevated to a therapeutically beneficial level by coupling to an antibody.

ADCs are composed of three components: a cytotoxic drug, a tumor-targeting monoclonal antibody and a linker that connects the two. All components play a critical role in ADC design and the selection of a suitable cancer target, the antibody, linker chemistry, cytotoxic payload potency, site of drug attachment and the optimal number of drug molecules attached per antibody is crucial to balance efficacy against adverse toxic events.

The antibody component of an ADC is responsible for high tumor specificity and needs to be effectively internalized to deliver the payload inside the cancer cell. Many efforts have been made to optimize antibody affinity, selectivity and pharmacokinetics to improve tumor delivery. Antibodies with strong binding to a target show high accumulation in tumor but exhibit poor tumor penetration so a careful balancing act is required to achieve optimal therapeutic efficacy [3]. As many cancer targets are also expressed on normal cells, affinity-attenuated binders can be developed to improve specific targeting of cancer cells and decrease toxicities on normal cells [4]. Bispecific ADCs are in development as a viable route to increase tumor selectivity while retaining highly potent antitumor efficacy. Bispecific ADCs directed against c-MET and EGFR displayed efficacious killing of EGFR and c-MET overexpressing cancer cells with reduced toxicity in normal cells expressing EGFR [5].

Payloads currently undergoing evaluation in clinical trials generally fall into three categories: tubulin inhibitors, DNA-damaging agents and transcription inhibitors. Heterogeneous and variable target expression on cancer cells has driven payload selection toward highly potent drugs in order to enhance the therapeutic potential of ADCs. Optimally, payloads should be extremely potent for intracellular targets as limited tumor penetration of antibodies, low-to-moderate target expression and inefficient internalization may result in very low toxin concentrations in
the cell. Other strategies include the use of cytotoxic payloads with lower potency and alternative mechanisms of activity, particularly for targets expressed on normal cells. Auristatins and maytansinoids are highly potent drugs with a sub-nanomolar IC50 that each bind to tubulin and inhibit tubulin polymerization. This results in G2/M phase cell cycle arrest and cell death [6]. Maytansine- and auristatin-based ADCs dominate the clinical landscape with brentuximab vedotin, polatuzumab vedotin, trastuzumab emtansine and enfortumab vedotin already approved and approximately 50% of ADCs in clinical trials employing tubulin inhibitors. Novel tubulin inhibitors, such as tubulysins, have also been developed; they cause rapid disintegration of the cytoskeleton and mitotic machinery of dividing cells, leading to apoptosis. They are also less effective substrates of drug resistance transporters [7]. Although tubulin inhibitors showed robust activity through a substantial number of clinical trials, a modest response rate was observed in the colon and gastric tumors [8].

The other major toxin class used in ADC development is DNA damaging agents. These include the calicheamicin payload employed in both inotuzumab ozogamicin and gemtuzumab ozogamicin, duocarmycins, topoisomerase inhibitors, pyrrolobenzodiazepine (PBD) dimers and indolino benzodiazepines (IGNs). PBD dimers are among the most potent cytotoxic compounds, exhibiting low-to-sub-picomolar potency in vitro assays. PBDs bind covalently to the minor groove of DNA and they cross-link DNA without distorting the DNA helix [9]. The binding activity of these agents interferes with DNA processes, including transcription and replication, leading to the death of both proliferating and nonproliferating cells. The increase in potency of PBDs allows for targeting of tumor-specific antigens that are expressed at a lower density. There are currently six PBD dimer-based ADCs in clinical trials targeting Axl (ADCT-601), CD19 (ADCT-402), CD25 Camidanlumab Tesirine, PSMA (ADCT-401), HER-2 (DHES0815A) and ASCT2 antigen (MEDI7247).

Duocarmycins and the IGNs are DNA monoalkylators that alkylate adenine or guanine bases in the DNA minor groove, respectively [10,11]. They maintain sub-nanomolar in vitro potency but display a better safety profile than DNA cross-linking agents such as PBDs. SYD985, an ADC conjugated to a new duocarmycin prodrug, has recently obtained fast-track designation from the US FDA for treating patients with HER2+ metastatic breast cancer [12]. The IGNs have a higher maximum tolerated dose in in vivo models, suggesting that ADCs containing this type of payload may have a higher therapeutic index in the clinic. The IMGN779 ADC, based on IGNs, is presently in Phase I clinical trials for the treatment of acute myeloid leukemia.

Many cancer targets are expressed on normal tissues; to overcome this limitation, cytotoxic drugs with lower potencies, such as topoisomerase inhibitors, have been successfully applied to ADC technology [13]. Trastuzumab deruxtecan for treatment of breast and gastric cancer was recently approved by the FDA and sacituzumab govitecan- targeting Trop2 was granted fast-track designation for patients with triple-negative breast cancer, small-cell lung cancer or non-small-cell lung cancer [14].

Another promising compound in nonclinical stage development includes α-amanitin, which disrupts DNA transcription and causes cell death by binding to RNA polymerase II [15]. Iksuda Therapeutics (Newcastle, UK) is currently working on the development of a payload with a novel protein alkylating mechanism of action.

ADCs as a targeted delivery system must be passed through many hurdles, including blood circulation, antigen binding and internalization. Therefore, conjugate stability is critical for drug delivery to the site of action, particularly with the development of very highly potent payloads. Extensive research is being conducted to develop novel conjugation technologies with improved stability such as
ring-opened maleimides and the introduction of non-natural amino acids containing azide handles for drug attachment. Iksuda’s vinyl pyridine-based PermaLink R technology is highly selective for cysteine residues and does not undergo the retro-Michael reaction, providing stability to ADCs. PermaLink technology was employed by Iksuda in the development of IKS01, an ADC-targeting folate receptor that is highly effective in causing tumor regression in FRA-expressing xenograft models at doses that are well tolerated. IKS01 was found to be significantly more active than a benchmark ADC and caused complete regressions in low/moderate FRA-expressing models [16].

Clinical trials and research conducted on existing ADCs have paved the way to identify new strategies for ADC improvement and increased the understanding of the intricacies of ADC design. As a result, the number of ADCs showing promising outcomes in clinical trials is rapidly growing, offering exciting opportunities for cancer patients.

REFERENCES