Antibody-drug conjugates in cancer therapy—filling in the potholes that lie ahead

MA Firer*

Abstract

Antibody-Drug Conjugates, composed of a cytotoxic drug conjugated to a tumour cell targeting antibody are being extensively studied and tested for the treatment of a variety of cancers. Antibody-Drug Conjugate systems hold the promise of more selective drug therapy compared to traditional chemotherapy, with fewer side effects. These important advantages have stimulated exciting developments in various aspects of Antibody-Drug Conjugate technology. Although several Antibody-Drug Conjugates have now entered the clinic, a number of biological and technical pitfalls exist that will need to be overcome if Antibody-Drug Conjugates are to fulfil their full therapeutic potential. This paper will discuss the more important of these challenges.

Editorial

The use of monoclonal antibody-drug conjugates has come a long way since the first attempts in the 1980s to use murine antibody-based antibody-drug conjugates as therapeutic agents. The concept of antibody-drug conjugates brings together the targeting advantages of antibodies and the cytotoxic potential of chemotherapy, heralding the promise of targeted accumulation of drug in the tumour cell or tissue. Antibody-drug conjugates are typically comprised of three components: a monoclonal antibody that acts as the cancer cell targeting moiety, a potent cytotoxic agent and a linker molecule to connect the two together. Disappointingly, the first antibody-drug conjugate constructs, which usually contained doxorubicin or methotrexate as the drug, not only showed little clinical efficacy, but also poor pharmacological parameters and toxicity profiles. Over the ensuing years, improvements in monoclonal antibody engineering and production were combined with advances in cytotoxic drug synthesis and selection, linker chemistry and conjugation methods (reviewed in) that together resulted in many antibody-drug conjugate candidates entering the preclinical development pipeline. Despite this progress, their passage into successful clinical application has been difficult and slow; only three ADCs have received Food and Drug Administration (FDA) approval for cancer therapy but only two are currently in use. Notwithstanding this sluggish growth, there are now about 30 new antibody-drug conjugates undergoing clinical assessment and at least another 12 are in preclinical development. Figure 1 and Table 1 show the number of different antibody-drug conjugates either approved or currently at different stages of development. Many of these are being tested in multiple applications.

While many pharmaceutical companies appear to have embraced antibody-drug conjugates as a source of potentially successful cancer therapeutics, whether as an extension of immunotherapy or simply as a format for targeted drug delivery, a number of important pitfalls still exist in this field. These will need to be addressed if antibody-drug conjugates are to fulfil their therapeutic potential. What are the most important of these challenges and where do we currently stand in overcoming them?

Target cell surface-specific molecules

One of the basic concepts of ADCs is their ability to deliver the drug payload to the correct cell, which assumes the ability to identify unique markers on the target cell surface. A variety of biochemical, immunologic, genetic and proteomic techniques have been used over the years to generate a plethora of cell surface-binding antibodies. Only rarely, however, have these antibodies targeted true tumour-specific antigens, such as clonotypic membrane immunoglobulins in some B-cell leukaemias. The vast majority of the cell surface targets can only be classed as tumour-associated cell surface antigens (TAAs) or overexpressed antigens (OEAs), for example the HER-2 receptor. Despite their lack of true tumour cell specificity, treatment with antibodies to TAAs and OEAs undoubtedly has made a clinical impact and they save lives. Nonetheless, their use is intrinsically associated with side effects that limit their efficacy.

This situation could change in the near future. The antibodies and ADCs currently in the clinic and many of those in clinical development were discovered using more traditional technology streams that began with immunization, production of hybridomas and selection of antibody candidates. This approach carries with its inherent limitations regarding the scope of antibodies identified. For example, ‘one-fit-for-all’ high-throughput screening methods tend to favour antibodies targeting dominant epitopes,

* Corresponding author
Email: firer@ariel.ac.il
Department of Chemical Engineering, Faculty of Engineering and Ariel Center for Applied Research, Ariel University, Ariel, 40700, Israel

Competing interests: none declared. Conflict of interests: none declared.
All authors contributed to conception and design, manuscript preparation, read and approved the final manuscript.
All authors abide by the Association for Medical Ethics (AME) ethical rules of disclosure.

Licensee OA Publishing London 2013. Creative Commons Attribution License (CC-BY)

Antibody internalization

Anti-cancer drug development has to date concentrated almost exclusively on compounds that act on intracellular targets. While this approach limits the scope of drugs that are being developed for cancer chemotherapy, the upshot for ADCs is antibody binding to a cell surface antigen must also efficiently activate receptor-mediated endocytosis in order to facilitate entry of the drug payload into the cell. Parenthetically, a number of antibodies that had failed clinically in their naked form as immunotherapeutics are now being revisited as potential components of ADCs. There may be several reasons for their failure, including the lack of relevance of their targeted antigens to cell survival, or that the antibodies themselves do not induce sufficient immune-related responses such as antibody-dependent cellular cytotoxicity (ADCC) or antibody-induced complement activation. Fortunately, these criteria are not required for a successful ADC. However, in most cases, attempts to revive these products will probably fail, because antibody internalization was also not a criteria used in the initial selection of those antibodies. This is not always the case, and some therapeutic monoclonals are indeed also good candidates for ADCs. For example, the successful immunotherapeutic Trastuzumab (Herceptin®) does induce its own uptake and was recently FDA approved as an ADC for HER-2+ metastatic breast cancer (Trastuzumab emtansine) (T-DM1, Kadcyla®).

Selection of internalizing antibodies can be somewhat tricky. CD19 for instance is a well-studied pan-B-lymphocyte differentiation marker. It is highly expressed on chronic lymphocytic leukemic (B-CLL) cells and is the target of several experimental ADCs, including SAR2319 which is currently in phase II clinical trials. CD19 is important for B-cell activation. It can also form dimers with CD21, another B-cell surface protein, following the latter’s binding to C3b during C3 activation, thus acting as a bridge between innate and acquired immune responses. However, whereas some anti-CD19 antibodies are rapidly internalized, the uptake of others is inhibited by CD21 expression. These studies underline the importance of early stage selection of antibody clones with the appropriate functionality and internalization. Appreciation of these factors has now led to testing for internalization as standard procedure and its use as an absolute criteria antibody candidate selection.

Another important criterion for antibody selection is antigen modulation induced by antibody binding. Studies by Ackerman and colleagues and further reviewed by Ritchie and colleagues demonstrate that maintaining antibody efficacy over time is dependent in part on selecting targets that are not expressed above a critical level and are not rapidly internalized following antibody binding as these features can have a deleterious effect on antigen modulation.

Antibody-drug conjugation and product heterogeneity

Another area of ADC development that has undergone important improvement is the chemistry of conjugation of the antibody protein to the drug moiety. With the early ADCs, the
methods used significantly reduced product efficacy, because the conjugation impaired the activity of either the drug, the antibody or both. Also of importance, the functional groups used to bind the two components were sensitive to enzymatic degradation in extracellular fluids. This extratumoral degradation of the conjugate and premature drug release contributed to systemic toxicity. Development in this arena has particularly focused on selection of a linker length and attachment sites that retain antibody activity as well as on design of functional linker groups that are only degraded by intracellular enzymes such as cathepsin. This latter improvement in target cell activity has also allowed the employment of cytotoxic agents vastly more potent than the earlier drugs used. Today, ADCs commonly contain uristatin or maytansinoid derivatives with potent microtubulin disruption, or DNA-damaging agents like calicheamicin or duocarmycin analogs. Even more recently, derivatives of pyrrolobenzodiazepines antibiotics such as abechymycin and tomaycycin are being introduced.

Nonetheless, conjugation problems remain (Figure 1) two commonly used conjugation chemistries involve alkylation of reduced cysteine disulfides or acylation of lysines. Natural IgG1 antibodies contain eight interchain cysteines and up to 100 lysines. While not all of these are readily accessible for conjugation, it has proven extremely difficult to control both the site of conjugation and the final stoichiometry of conjugation to either of these amino acids. This lack of control is expressed in two parameters that are problematic from a manufacturing viewpoint—a heterogeneous product with an unpredictable (optimally two to four) number of drug moieties attached to various sites (Figure 1) and interbatch variability. Indeed, a study by Bross and colleagues on Gentuzumab Ozogamicin ADC (Mylotarg) showed that while on average the antibody bound four to six drug molecules, some 50% of the antibody was completely unbound. Product heterogeneity has additional important biological consequence in that the suboptimally conjugated antibody may actually interfere with the target binding of the ADC, thus reducing drug efficacy. This problem needs further study.

Tumour penetration

The treatment doses and protocols used in the initial applications of ADCs were also factors that resulted in limited product penetration into solid tumours. This area is also now receiving attention. For example, results from mathematical studies stress the negative relationship between overexpression of surface antigen, diffusion kinetics and antibody penetration into the tumour mass, particularly into the necrotic interior. These results are supported by biological studies demonstrating that the higher interstitial pressure within the tumour mass affects the rate of mass transfer of macromolecules such as antibodies. Interestingly, other investigators reported that high antibody affinity for the target molecule can also adversely affect penetration. So a better understanding of both antibody and target antigen dynamics within the tumour microenvironment should lead to better selection of antibody candidates for ADCs.

Production of ADCs

Therapeutic antibodies require significant quantities of product over multiple treatments to be effective (200–350mg/m² single dose). The therapeutic dose of ADCs should be lower than that of naked Therapeutic Monoclonal Antibodies (TMAs), but they are still significant. The recently approved Kadcyla® (TDM1) is used at 160 mg per single dose. Currently, these products are approved for manufacture in mammalian expression systems and bioreactor systems, both which require expensive upstream and downstream processes. Furthermore, ADC construction involves additional chemical conjugation steps that only complicate production streams. These factors, together with additional marketing issues, result in expensive drugs. For example, whereas treatment with the Trastumumab antibody alone (Herceptin®) is reported to cost $4500 per month, its incorporation into Kadcyla® more than doubles the price. To make these drugs more affordable, efforts are being made to reduce production costs, thus improving efficiency in both upstream and downstream processes involved in antibody manufacture (reviewed in) and use of cheaper protein expression systems such as algae. TMAs have already proven their clinical effectiveness, and it is predicted that the coming two years will see one to two ADCs receiving FDA approval, at least one of which will be for haematological cancers. Nonetheless,
the points raised above indicate that both TMAs and ADCs have some inherent limitations that are not easily overcome by technology. In order to broaden the scope of effective TDD therapies, it is therefore prudent to search in parallel for additional strategies that would complement the use of ADCs for cancer therapy.

Understanding the past can improve the future

The persistent and dedicated work of scientists, clinicians and technologists from various disciplines over the past three decades or so has resulted in groundbreaking development of target drug delivery systems based on the binding specificity of antibodies. The two ADCs currently in clinical use not only save the lives of cancer patients but provide proof-of-concept that this strategy is viable. While a series of additional ADCs are in clinical assessment, it is clear that limitations remain with these products in terms of absolute target specificity, tumour penetration, manufacturing efficiency and cost. Given the profound potential significance of ADCs to treat cancer as well as other diseases\textsuperscript{30,49}, it is not surprising that these issues are receiving both industrial and basic research attention.

The future for the clinical application of ADCs in cancer therapy is bright. However, as we move forward in the development of this field, it will be pertinent to carefully select shorter-term goals in order to demonstrate to control the variables. Several examples come to mind. Many of the limitations in diffusion and pharmacodynamics through solid tumour masses of ADCs carrying full-length antibodies can be bypassed by aiming at haematological cancers, and it should therefore be of no surprise that the ADCs currently in advanced clinical development indeed target leukemias and lymphomas\textsuperscript{5}. In this regard, an additional area to focus on would be the treatment of micrometastases. Finally, single-chain antibody fragments (scFv) are being used in next-generation ADCs\textsuperscript{31,50}. Being much smaller proteins and lacking an Fc region, these are cleared more rapidly from the circulation; however it has been known for some time that they show much higher accumulation into solid tumours\textsuperscript{51}. Learning to extend the plasma half-life of scFv-based ADCs or to develop a trade-off strategy between clearance and penetration therefore becomes a priority.

Abbreviations list

ADC, antibody-drug conjugate; ADCC, antibody-dependent cellular cytotoxicity; FDA, USA Food and Drug Administration; OEA, overexpressed antigen; TAA, tumour-associated cell surface antigen; Therapeutic monoclonal Antibodies (TMAs)

References

19. McLarty K, Cornelissen B, Scollard DA, Done SJ, Chun K, Reilly RM. Associations between the uptake of 111In-DTPA-trastuzumab, HER2 density and response to trastuzumab (Herceptin) in athymic mice bearing subcutaneous human tumour

For citation purposes:


