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Taking A Targeted Approach To Cancer Treatment: Designing And Developing ADCs For Clinical Success

Antibody-drug conjugates (ADCs) are an emerging class of biopharmaceuticals with therapeutic applications in oncology and beyond. This class of biotherapeutics best illustrates the scientific concept of targeted drugs as a 'magic bullet', introduced over a century ago by the Nobel Prize-winning immunologist, Paul Ehrlich[1]. Essentially, cytotoxic drugs are attached via a linker to an antibody which directs them to the target cells, where they can be specifically released. Target cells are then killed without damaging the healthy tissue around them.

While the simplicity of the concept is attractive, ADCs are particularly complex constructs combining elements of both large molecules and small molecules. Their design requires careful consideration and great attention must be paid to their individual constituents, as well as to how they behave in combination. With this in mind, a high degree of coordination between biologics and small molecule manufacturing teams is also required to not only improve the chances of success but also achieve timely delivery of material for clinical trials. After three decades of explorative work resulting in relatively few approvals, the requirements for successful development of efficacious and safe ADCs are now starting to be better understood, leading to recent increases of ADC approvals, which have doubled over the last two years.

In this article, Nicolas Camper, Group Leader, Bioconjugation Chemistry at Abzena discusses the potential of these molecules if correctly harnessed. He also outlines the intensive work required from both small molecules and biologics teams to ensure both efficacy and safety.

ADCS: AN OVERVIEW

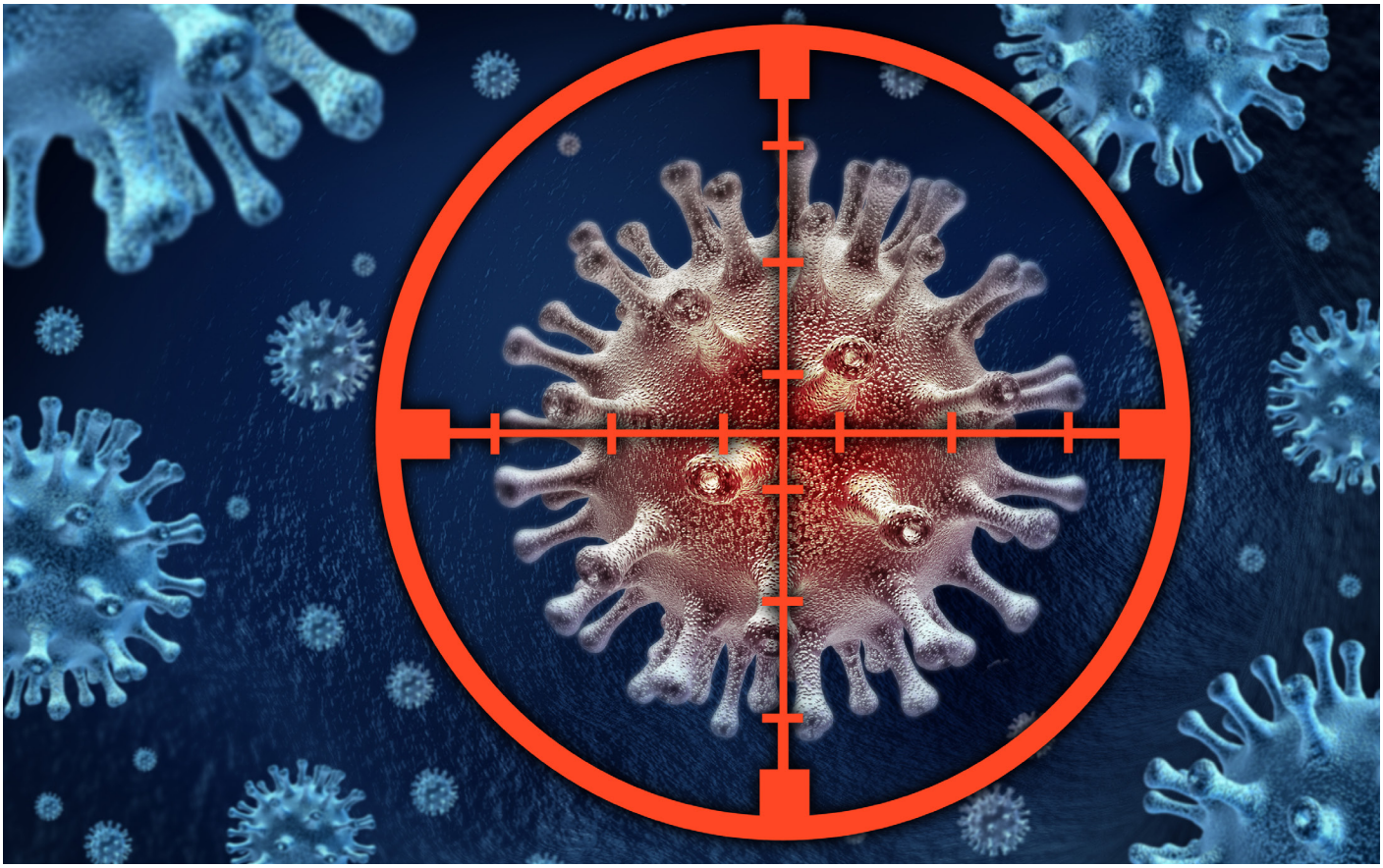
ADCs are constituted of three main elements: an antibody, a cytotoxic payload and a linker connecting the two. The main role of the antibody is to specifically target the cytotoxic payload to the diseased cells. This is done through the recognition of an antigen uniquely expressed by these cells. The key to the selection of a suitable antibody lead is in the evaluation of its specificity and affinity for the target antigen. Various techniques can be used to carry out this evaluation. For example, hybridoma technology combined with high throughput robotic colony picking and binding affinity evaluation by bio-layer interferometry allows for an extensive screening of the murine immune repertoires and the rapid identification of high affinity monoclonal antibodies. In addition, further enhancement of the antibody affinity to picomolar levels can be achieved using phage display technologies and evaluated by surface plasmon resonance or the ELISA method.

One key requirement for antibodies intended for targeted drug delivery is their ability to induce receptor-mediated internalization upon binding to the target cells. The efficacy of an ADC directly depends on the efficient internalization of the ADC-antigen complex and subsequent trafficking to subcellular compartments. These compartments provide suitable conditions for payload release such as endosomes and lysosomes. Labelling of antibodies with fluorescent probes and co-staining of subcellular compartments allows the study of the intracellular trafficking of the antibody and quantitative measurement of antibody internalization by flow cytometry and fluorescence microscopy. Specific targeting of the diseased cells and effective internalization of the ADC are a prerequisite for the payload to specifically deliver its cytotoxic effect.

The payload attached to the antibody also needs careful selection to maximize its cytotoxic effect. Microtubule polymerization inhibitors such as auristatins and maytansines have been widely adopted as payloads for ADCs due to their potent anti-mitotic activity on proliferating cancer cells. These inhibitors are present in more than half of approved ADCs. Payloads interacting with DNA in various ways, either causing DNA strand breaks (calicheamicin), interfering with DNA replication through the inhibition of topoisomerases I and II (camptothecin derivatives and anthracyclines) or alkylating DNA strands (PBD dimers, duocarmycins), have also been used extensively as payloads for ADCs.

THE CHALLENGES OF ADC DEVELOPMENT

As cancer cells often evolve to evade the action of cytotoxic drugs, attention should be given to finding a payload with a



mechanism of action adapted to the biology of the target cancer cells. Payloads with other intracellular targets such as amatoxins, which potently inhibit RNA polymerase II and interfere with mRNA synthesis, microcystins, eukaryotic protein phosphatase inhibitors produced by cyanobacteria with multimodal cytotoxic activity[2], are worth exploring, along with immune system activating payloads such as TLR7/8 or STING agonists. This offers opportunities to widen the options available to efficiently kill resistant cancer cells. Beside its mechanism of action, another factor to consider during the selection of a payload is its ability to kill neighboring cells in addition to the ones targeted by the ADC. This 'bystander effect' is particularly useful to increase the cytotoxic potency of ADCs against tumor cells displaying heterogenous levels of expression of the target antigen.

If the intrinsic physicochemical properties of payloads and in particular their cross-membrane permeability dictates their ability to display bystander activity, the type of linker connecting the payload to the antibody can modulate this activity. A whole suite of cell-based assays allows for comprehensive evaluation and ranking of the potency of different payloads and release mechanisms combinations, from standard 2D cell viability assays, to limited exposure assays and 3D cells viability assays with finer mimicking of the tumor environment. In addition, it is also possible to assess bystander effect in co-culture in vitro assays.

Linkers not only attach antibody and payload and possibly incorporate cleavable moieties to enable the release of payloads in an active form, they also include other design elements to further potentiate ADCs. Hydrophilic elements such as polyethylene glycol units, carbohydrates or hydrophilic amino acids are often introduced in the linker to compensate for the hydrophobic nature of many cytotoxic payloads and impart higher solubility and stability to the ADC. Introduction of these hydrophilic moieties may not only be a physicochemical necessity but can reveal an effective approach to improving ADC efficacy. Enhanced hydrophilicity improves the pharmacokinetic properties of the ADC, reducing hydrophobic clearance and increasing tumor exposure to the ADC.

Linker-payload attachment sites on the antibody and respective positioning of the different linker components can also be key parameters to investigate in order to tune the pharmacokinetics of the ADC and improve its overall in vivo efficacy[3]. Chemistry toolboxes allowing rapid assembly of reagents with different payloads, release moieties, solubility enhancing elements and attachment moieties combined with conjugation platforms to attach these reagents to antibodies can greatly streamline the generation of a matrix of ADCs[4]. This facilitates the identification of the best combination of antibody, linker and payload to take into development.

REALIZING THE FULL POTENTIAL OF ADCS: THE KEY CONSIDERATIONS

When efficacy is often the primary focus in the early stages of development of an ADC, clinical failures, particularly in Phase I, have often come from safety issues, for example with dose limiting toxicities preventing administration of optimal doses.

To avoid late stage failures and de-risk ADC programs, safety assessment by an experienced team from the early stages of pre-clinical development is imperative.

ADC related toxicities are multifactorial in nature and can involve each of the different constitutive elements of the ADC. Instability of the linker-payload attachment to the antibody causes linker-payload release in systemic circulation, thereby increasing the risks of broad non-specific toxicities and reducing the amount of payload delivered to the tumor. Maleimide chemistry is commonly used for conjugation due to the high reactivity and selectivity of maleimides for thiols present in cysteine interchain disulfides. The resulting thioether linkages typically undergo retro-Michael reactions in serum leading to linker-payload shedding, and in addition, undertake cross-conjugation to proteins with endogenous free cysteines, such as albumin.

It has been suggested that systemic circulation of free linker-payload and linker-payload cross-conjugated to albumin can cause neutropenia, an abnormally low concentration of neutrophil white blood cells. This is a side-effect which is particularly prevalent with vedotin (mc-vcPAB-MMAE) ADCs[5]. Other conjugation chemistries such as disulfide rebridging technologies provide alternatives with much higher serum stability, meaning they could potentially reduce these side-effects[6]. Ex vivo serum stability evaluation is a useful tool when eliminating ADC candidates at an early stage of development that are identified as having this type of liability.

Payload choice also influences the type and severity of clinical side-effects. After a few years where extremely potent payloads with low picomolar activity, such as PBD dimers, were in the spotlight there is now a trend towards payloads with reduced potency. For example, the camptothecin derivative SN-38 has gained attention due to reduced severity of side-effects that decreases with the lower payload potency. Rapid transformation of the payload into inactive metabolites could also help reduce payload related side-effects.

Some ADC toxicities are related to antibody effector functions. Immune system cells and hepatocytes internalize antibodies via Fcγ receptors and mannose receptor binding. These Fc mediated interactions are suspected to be involved in off-target uptake of ADCs by those cells leading to thrombocytopenia and liver toxicities. These are the two main toxicities observed in patients treated with Kadcyla®[5]. Binding of ADCs to Fcγ receptor panels and the potential impact of conjugation on Fcγ receptor binding can be investigated using surface plasmon resonance and cell-based reporter assays.

Finally, expression of the ADC target antigen on healthy tissue can be another source of toxic side-effects even when differences in target antigen accessibility and expression levels between tumor cells and healthy cells can limit the severity of on-target, off-site toxicity. Thorough assessment of the target antigen expression levels in various healthy tissues should therefore be performed before the start of ADC development programs to minimize risks of toxicity caused by healthy tissue expression of the target antigen.

FINAL THOUGHTS

Rapid generation of ADC libraries and assessment of their efficacy and safety characteristics in pre-clinical assays is a powerful approach when selecting lead ADC candidates with a wide therapeutic index to avoid failure of ADC programs at the clinical stage. Delivery of a successful ADC pre-clinical development program requires a high level of expertise across a broad group of disciplines ranging from chemistry, molecular biology to analysis and biology and the involvement of discovery, design and development teams of scientists.

The same degree of coordination between the process development and manufacturing teams producing the different constitutive elements of the ADC and putting them together is also necessary at the CMC and clinical stages of ADC programs. Companies providing fully integrated R&D and manufacturing services for both small molecules and biologics should therefore be the first choice when outsourcing ADC programs.

REFERENCES

[1] Bosch, F. & Rosich, L. The contributions of Paul Ehrlich to pharmacology: A tribute on the occasion of the centenary of his Nobel prize. Pharmacology vol. 82 171–179 (2008).

[2] Niedermeyer, T. H. J., Daily, A., Swiatecka-Hagenbruch, M. & Moscow, J. A. Selectivity and potency of microcystin congeners against OATP1B1 and OATP1B3 expressing cancer cells. PLoS One 9, 1–7 (2014).

[3] Pabst, M. et al. Modulation of drug-linker design to enhance in vivo potency of homogeneous antibody-drug conjugates. J. Control. Release 253, 160–164 (2017).

[4] Frigerio, M. & Kyle, A. F. The Chemical Design and Synthesis of Linkers Used in Antibody Drug Conjugates. Curr. Top. Med. Chem. 17, 3393–3424 (2018).

[5] Hinrichs, M. J. M. & Dixit, R. Antibody Drug Conjugates: Nonclinical Safety Considerations. AAPS J. 17, 1055–1064 (2015).

[6] Badescu, G. et al. Bridging Disulfides for Stable and Defined Antibody Drug Conjugates. Bioconjugate Chem. 25, 1124–1136 (2014).

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