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Designing, Selecting, and Developing Bioconjugates for Clinical Success

Finding Design Solutions for ADCs with Abzena's Bioconjugation Toolbox

The clinical success of ADCs relies on their ability to demonstrate a sufficiently wide therapeutic index. Dose-limiting toxicities are commonly observed in the clinic, emphasizing the need for careful initial design and lead selection to ensure efficacy and minimize undesired toxic side effects.

While the efficacy and safety profile of an ADC is mainly driven by the combination of target receptor, antibody, and payload, linkers can also modulate their biological activity. Tuning linker stability can be key to finding an optimal balance between ADC safety and efficacy. Similarly, conjugating a linker-payload to an antibody can profoundly affect its PK properties, impacting both in vivo efficacy and toxicity profiles. Linker design can help mitigate hydrophobicity issues frequently seen with ADCs and restore favorable PK properties.

At Abzena, we have experience designing, producing, and evaluating a wide variety of bioconjugates. This includes peptide-drug conjugates, engineered antibody fragments conjugates, traditional ADCs, and nanoparticle conjugates, all of which are functionalized with various payloads to reflect the diversity of therapeutic applications of these types of biomolecules. These bioconjugates come with their own specific requirements with regards to linker design and conjugation strategy.

Exposure to a broad range of conjugates has led us to develop a bioconjugation toolbox to rapidly produce bioconjugate candidates with different linkers and conjugation chemistries. This allows us to explore various designs to choose from for the lead candidate. This depth of expertise means we can optimize the therapeutic profile of ADC candidates to find the best-suited conjugation approach and linker.

Introduction

Bioconjugates are comprised of therapeutic agents attached to biological targeting molecules such as antibodies to give a drug unprecedented targeting capability. Bioconjugates have contributed to significant advancements in different therapeutic areas, such as oncology with cytotoxic Antibody-Drug Conjugates (ADCs) or infectious diseases with conjugate vaccines, and are bound to deliver new, better drugs in many others. However, the successful development of bioconjugates also comes with challenges related to their design and manufacture. Here, we present Abzena's approach to support the rapid and successful development of the complex new therapeutic modalities that bioconjugates represent.

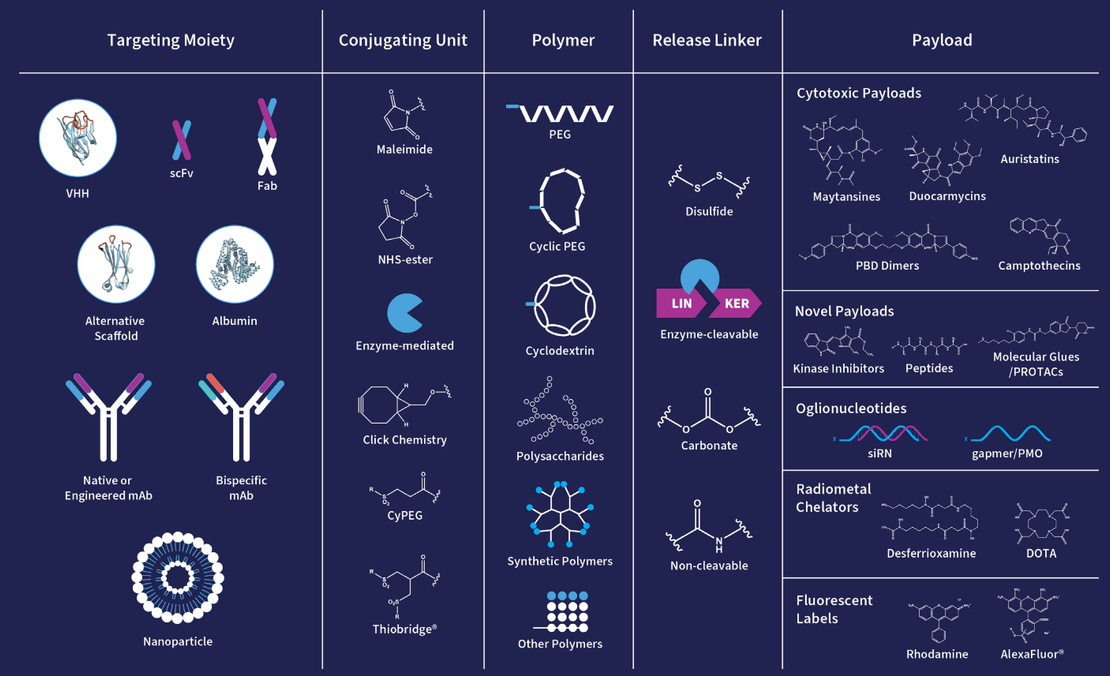

Figure 1: Abzena's extensive bioconjugation toolbox allows rapid ADC optimization.

Moving ADCs Into the Clinic Faster with Abzena's Technological Innovations

Extensive expertise in bioconjugation is key to the success of ADC programs. However, access to technological innovations can be just as crucial to both increasing the likelihood of clinical success and moving programs faster into clinical development. Abzena's scientists have developed technologies to help customers meet the demands of their projects and bring bioconjugate therapeutic innovations to patients faster.

A prime example of this is ThioBridge™, one of our proprietary technologies, which improves the stability and efficacy of ADCs through better conjugation and linker chemistry.

Currently, most ADCs approved for use are produced by coupling a maleimide moiety on the linker payload to cysteine residues on the antibody. This bioconjugation method leads to unstable ADCs, with linker-payload deconjugation occurring via a retro-Michael reaction and impacting efficacy as less payload gets delivered to the tumor. The deconjugated linker-payload also becomes a source of systemic toxicity[1]. Lysine conjugation is also another common approach, leading to indiscriminate conjugation of the linker-payload across the whole antibody, with the potential to interfere with antigen recognition and FcR functions. While these traditional bioconjugation chemistries have found clinical applications, better options are now available.

ThioBridge™, a site-specific conjugation technology covalently rebridging antibody interchain disulfides, overcomes some of the shortcomings of first-generation conjugation technologies[2]. ThioBridge™ delivers a uniform DAR profile compared to the multiple DAR species generated by maleimide and lysine conjugation (Figure 2). Different ThioBridge™ formats give access to conjugates with DAR 2, 4, or 8, providing flexibility to find an optimal drug loading. ThioBridge™ creates a stable attachment between the antibody and linker-payload with no deconjugation in systemic circulation. ThioBridge™ linkers also incorporate solubilizing elements as part of their design to produce hydrophilic conjugates with enhanced PK properties. These features typically lead to superior efficacy in animal models for ThioBridge™ ADCs compared to traditional conjugates and favorable tolerability[3].

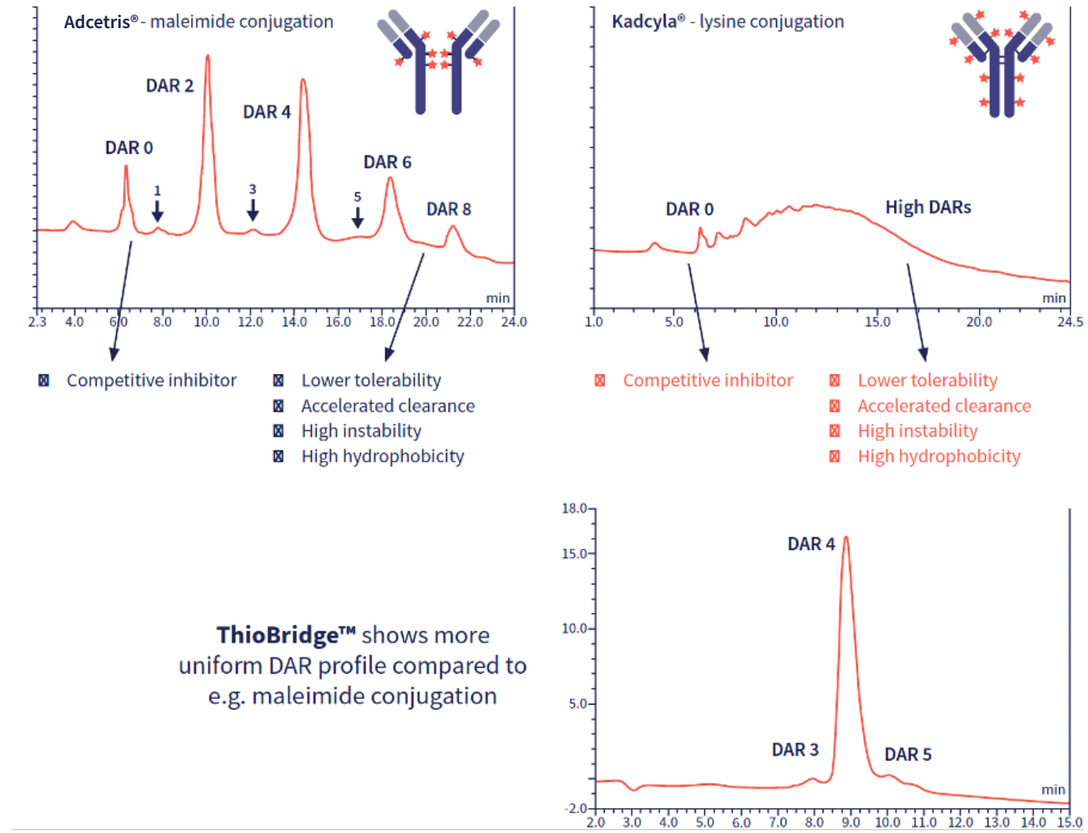

Figure 2: Drug-to-antibody ratio (DAR) distribution comparison of first-generation conjugation technologies assessed by hydrophobic interaction chromatography (HIC). Adcetris® represents maleimide conjugation, while Kadcyla® represents lysine conjugation. Both DAR profiles are far more heterogeneous than ADCs produced with ThioBridge™.

ThioBridge™ can be applied to native antibodies without needing prior modification via recombinant or enzymatic methods, allowing conversions to a conserved DAR ADC that are typically in the range of 70–90% with overall process yields of >70%. To date, three ThioBridge™ ADCs have been manufactured under GMP and are undergoing clinical investigation.

Overall, ThioBridge™ gives developers the power and control to generate homogeneous constructs with precise drug loading and predetermined, controlled attachment sites for improved therapeutic activity.

De-risking ADC Lead Selection with Developability Assessment

Successful development of an ADC requires significant expertise and tools for linker-payload synthesis and antibody conjugation to create and optimize ADC candidates. Overall, the success of an ADC program depends on the early identification of liabilities so that design alterations can be rapidly implemented to avoid much more costly modifications during the clinical stages of drug development. At Abzena, we use a developability assessment of ADCs to look extensively at the analytical and biological characterization of the candidates, focusing on the different components of the conjugate both individually and in the context of the conjugate as a whole.

We conduct a multi-pronged investigation to assess different characteristics of the conjugate and identify any property that could compromise its development or affect its performance. This can involve detecting instabilities, either antibody sequence liabilities (presence of deamidation, isomerization or oxidation sites) or biophysical instability and any propensity for aggregation of the conjugate, as well as liabilities related to degradation in the serum of species used for pre-clinical evaluation and in human serum. Assays also aim to demonstrate the functionality of the ADC, including specificity of target binding, internalization by target cells, and antigen-mediated cell killing. Looking ahead at the clinical stages, we assess – and attenuate, if necessary – any immunogenicity of the antibody component to lower the likelihood of anti-drug antibody generation that may affect the ADC's efficacy. This developability assessment approach front-loads a project with more data-rich assays to avoid more costly issues later in the drug development process.

Another key consideration to ensure the overall success of an ADC program is manufacturability assessment. This focuses on establishing the robustness of the production process and determining the conjugation efficiency, the ability to isolate the ADC with a suitable purity profile, and the overall process yield. Establishing a supply chain for the materials entering the ADC production process and mapping the associated cost of goods will also be critically important for the successful progression of the lead ADC candidate into clinical and commercial development.

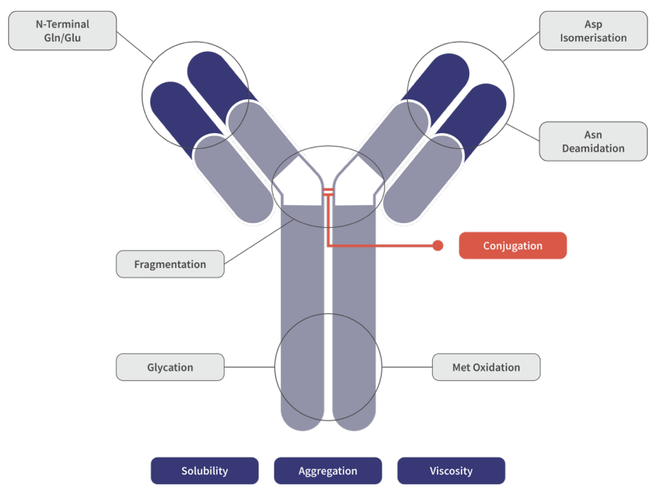

Figure 3: Identifying ADC candidates' liabilities early in the development process is the aim of developability assessment. An important aspect of developability assessment focuses on identification of physical and biophysical instabilities leading to aggregation, fragmentation or degradation of the antibody and linker-payload and ADC.

Building a Pre-Clinical Data Package with DRIVE-Biologics

Progressing an ADC lead candidate to clinical development fundamentally depends on data that demonstrates efficacy, safety, and favorable PK in translational in vivo models, which will be an integral part of the IND application.

Abzena, as a member of the DRIVE-Biologics consortium, can help generate these critical data packages through our partnership with Oncodesign Services, a recognized expert and leader in in vivo pre-clinical evaluation services. Typically, pre-clinical studies will need to demonstrate tumor growth inhibition and complete remissions upon repeat – or preferably single – dose administration in cell line-derived (CDX) and patient-derived xenograft (PDX) models representative of the target disease. PK studies in rodents and non-human primates (NHP) will be performed to predict the behavior of the ADC in humans. Toxicity studies in different species, including NHPs, are used as safety assessments and will inform the selection of a first-in-human dose and the design of the Phase I clinical trial dose escalation strategy.

An Integrated Approach from Concept to Commercialization

To simplify operational complexities and shorten timelines, Abzena provides integrated services covering the entire spectrum of bioconjugate program development, from lead discovery and candidate selection through to commercial manufacturing. This comprehensive capability allows us to maintain control over the quality and timing of each development phase, ensuring planned, predictable execution. This approach is customized to meet the unique needs of each program, balancing time, quality, and risk to optimize outcomes.

A typical process involves:

- **Bioconjugate design and lead candidate selection:** Incorporating leading antibody discovery, protein engineering, and synthetic and bioconjugation chemistry techniques, bioconjugate candidates are designed, synthesized, and evaluated as part of a detailed developability assessment aiming to select a de-risked lead candidate. Developability allows us to identify and manage risk early in development to reduce major cost, quality, and time issues at later stages of manufacturing and clinical evaluation.
- **Development and scale-up**: Focused on process and analytical development, optimized for robustness and scalability.
- **cGMP Manufacturing:** Carried out in facilities capable of handling everything from small-scale early-phase trials to large-scale commercial production.

Working with Abzena provides a tangible competitive advantage for ADC and bioconjugates development, as clients benefit from reduced scientific, financial, and operational risks alongside shortened timelines. Our standardized platforms and parallel workstreams enhance efficiency and minimize redundancy, which is often associated with technology transfers between phases of development, giving ADC programs the best chance of rapidly and successfully reaching the clinic.

Case Study – ACT-903 – A Novel AFP-Maytansinoid Conjugate with Promising Pre-Clinical Efficacy

ACT-903 is a novel bioconjugate developed with Abzena by Alpha Cancer Technologies, Inc. It targets the alpha-fetoprotein receptor (AFPR), an oncofetal antigen highly expressed on the surface of many cancers, and myeloid-derived suppressor cells (MDSCs) in the cancer microenvironment but not on normal adult cells[4].

Mechanism of Action

ACT-903 consists of recombinant human AFP (ACT-101) covalently linked to a novel maytansine toxin proprietary to Abzena (ABZ-981) through a glutathione-sensitive linker. This linker exploits the higher concentrations of glutathione in tumor cells compared to the bloodstream to promote the selective release of the toxin to cancer cells. This targeted approach aims to maximize tumor cell death while minimizing off-target effects.

Preclinical Studies

Researchers conducted a biodistribution study in COLO-205 tumor-bearing mice, using three different maytansine conjugates (DM1, DM4, and ABZ-981) to identify an optimal candidate[5]. The ACT-101-ABZ981 conjugate showed the highest toxin delivery to tumors with minimal accumulation in non-target tissues – notably, bone marrow was avoided, which is crucial for reducing chemotherapy-related toxicities.

The team determined the maximum tolerated dose (MTD) of four optimized ACT-101-maytansine conjugates. Mice received varying doses (5–40 mg/kg/day) for 10 days. The optimal dose for the efficacy study was set at 10 mg/kg/day or 20 mg/kg/day depending on the conjugate. Toxicities at higher doses included elevated liver enzymes and weight loss.

In in vivo efficacy experiments, COLO-205 human colon tumor cells were implanted in mice, and treatment with the ACT-101-maytansine conjugates began once tumors reached 100–200 mm^3. Tumor volume and survival rates were monitored over 60 days. The ACT-101-ABZ981 conjugate (designated ACT-903) exhibited superior efficacy, with significant tumor regression and survival benefits. In one group, tumors became non-palpable in 9 out of 10 mice by Day 38, with no treatment-related toxicities observed. A subsequent study in the ovarian xenograft model showed complete tumor elimination and no re-growth over a period of 60 days with no signs of toxicity[6].

Conclusions

ACT-903 demonstrated potent anti-tumor activity and excellent safety in preclinical models. Its ability to selectively target AFPR-expressing cancer cells, coupled with minimal off-target toxicity, positions it as a promising candidate for targeted cancer therapy. Further preclinical studies are underway to support an IND submission for clinical trials.

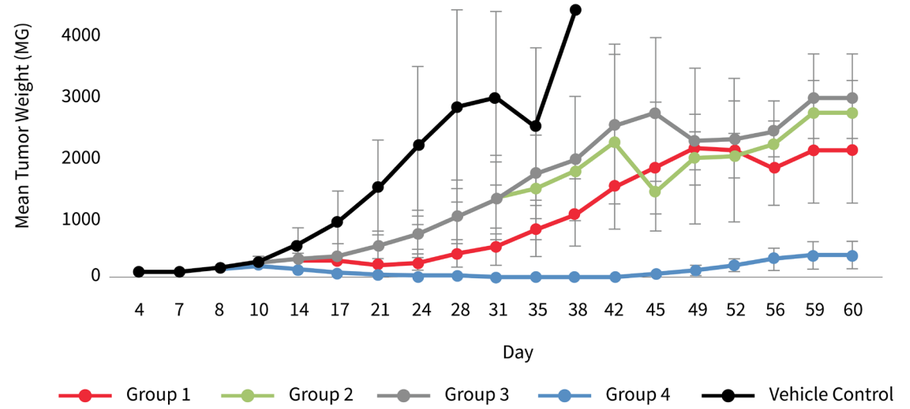

Figure 4: ACT-101-Maytansine conjugates efficacy studies. Mice implanted with COLO-205 human colon cancer cells were treated with different ACT-101-Maytansine conjugates at 10 mg/kg/day for Groups 2 and 3 and 20 mg/kg/day for Groups 1 and 4, with the same two 5-day treatment cycles. No treatment-related toxicities or deaths were observed in this study.

Looking Ahead to the Future of Bioconjugates

The field of ADCs and bioconjugates will continue to evolve, with technical innovations continually expanding the potential of these biopharmaceuticals. At Abzena, our commitment to advancing bioconjugate technologies is unwavering, and we'll strive to meet the complex demands of modern medicine.

The future of bioconjugates will be marked by the emergence of novel technologies that promise to enhance the specificity, efficacy, and safety of biotherapeutics. Innovations such as multi-specific antibodies, targeted nanoparticle delivery systems, and new linker chemistry pave the way for more sophisticated bioconjugate designs. These advancements are expected to address current limitations by offering finer control over drug release profiles and minimizing off-target effects.

Personalized and precision medicine has been a long-standing goal across the therapeutics industry, and we believe bioconjugates will play a central role in achieving this. By integrating biomarker research with bioconjugation, therapies can be specifically designed to target the unique molecular signatures of patients' diseases with the hopes of improving treatment outcomes and reducing side effects simultaneously.

With so much potential in the future of ADCs and bioconjugates, we are always expanding the extent of our collaborations with academic, biotechnology, and pharmaceutical organizations to drive R&D onward. These partnerships hinge on our combined strengths to speed up the development of new bioconjugate therapies and ensure they are accessible to the patients who need them most.

Let's turn ideas into action.

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