

The Evolution of Antibody-Drug Manufacturing

How the evolution of ADCs has changed manufacturing requirements

Even before the 1980s, the notion of attaching a cytotoxic agent to a tumorspecific antibody captured the collective imagination of the international drug development community. Were antibody-drug conjugates (ADCs) the magic bullets everyone was looking for that could, among other things, cure cancer? The evolution of ADCs is a stunning example of how global research can sculpt a new technology, gradually refining it in ways no one initially imagined, often spurred by other discoveries that surface along the way. For ADCs, years of advances in molecular and cell biology, conjugation chemistry and immunology were needed before concept could become practice¹. And ever since the initial approval of MYLOTARG™ in 2000, ADCs have changed significantly. Conjugation, linker

and toxin chemistries have all evolved, as has the field of bioprocess manufacturing. Today, with over a dozen commercially approved drugs on the market and more than 200 programs active in clinical trials, simplifying the complex supply chain to make manufacturing efficient is a necessity. With approximately 70% of ADC projects outsourced to contract development and manufacturing organizations (CDMOs)², a transparent and integrated supply chain is critical for success. This paper will discuss how ADC chemistry and manufacturing have evolved over the past fifteen years, present the challenges this dynamic growth has created and describe how CDMOs are adapting to these changes to meet customer needs, now and in the future.

The standard ADC supply chain is highly complex

Because of the numerous, specialized processes in their production and the logistical alignment involved, the typical ADC supply chain is elaborate. As separate steps, often in separate locations, custom linker and payload raw materials are made and then joined to produce the linker payload. Meanwhile, the monoclonal antibody (mAb) is produced elsewhere. Linker payloads and mAb are shipped to conjugation sites and, once

prepared, the conjugated material is shipped to a drug fill/finish site. Lastly, vials are often shipped for labeling and packaging to yet another facility. Customers often produce contingency batches requiring cold storage. Typically, a total of five to ten CDMOs, spread across the globe, are involved in the supply chain (see **Figure 1**).

Supply Chain Complexity

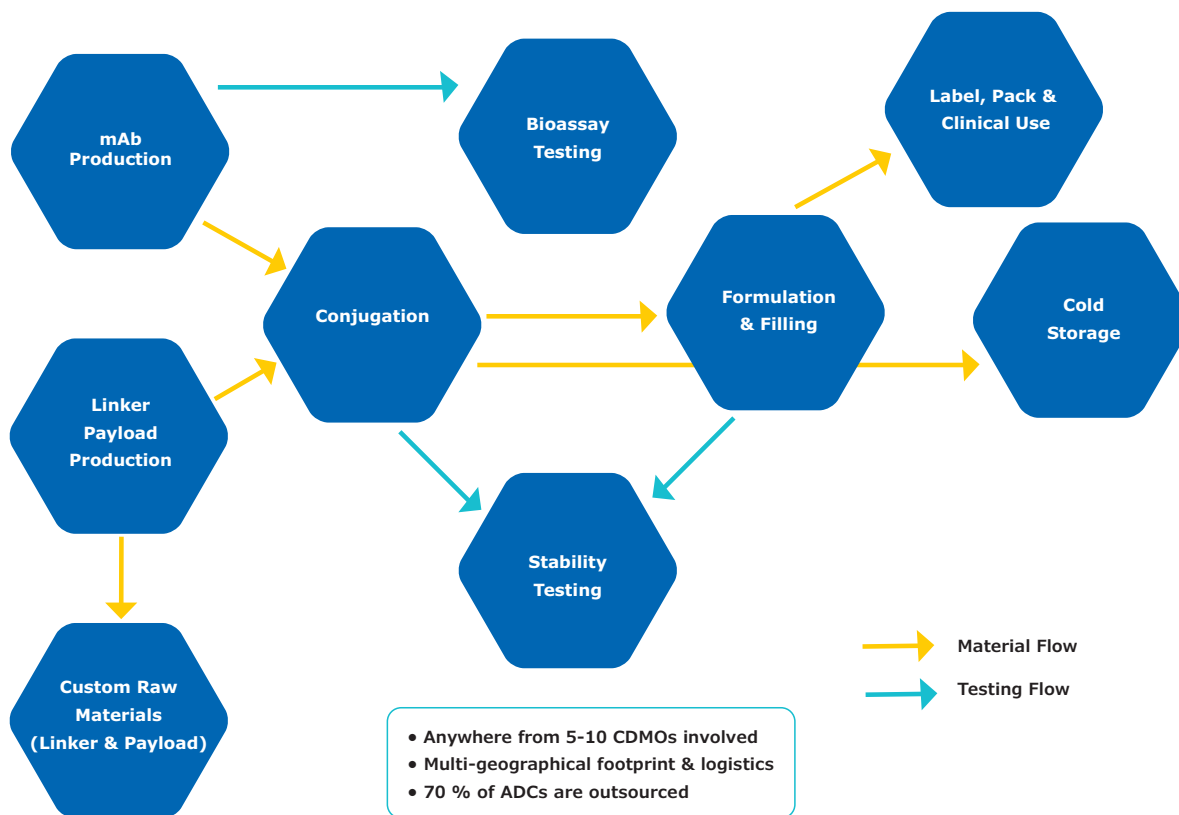


Figure 1: Schematic of a typical ADC supply chain

Interspersed with the above manufacturing steps, requisite quality-control measures— such as mAb bioassays and conjugate stability testing—compound an already complicated pathway. Clearly, manufacturers that can simplify this supply chain are doing

customers a great service. Is there relief in sight? To understand the trajectory of clinical to commercial ADC manufacturing, it's helpful to take a step back and look at what these processes entailed initially and how they have evolved since then.

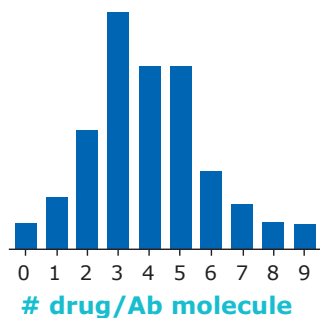
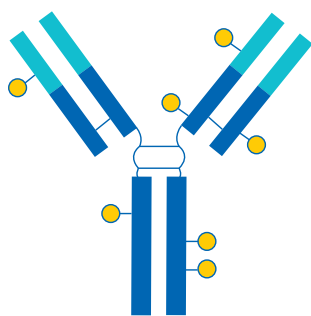
ADC manufacturing then: small scale, high variability

Fifteen years ago, ADC manufacturing took place on a much smaller scale than today. Raw materials such as mAbs were only available in clinical rather than commercial-sized batches. Manufacturing was performed as an add-on to other procedures rather than as optimized, stand-alone processes, and few sites were capable of handling high-potency payloads. Full-length mAbs were humanized IgGs, most often IgG1, conjugated randomly at cysteine or lysine sites, as shown in **Figure 2**.

In these constructs, the payload could bond to the antibody in multiple locations, potentially affecting its activity. With an IgG scaffold containing over 80 lysines³, conjugation resulted in very heterogeneous

ADCs with variable drug-to-antibody ratios (DARs). High DAR species in the final product can impact stability and solubility and can cause problems in manufacturing unit operations, such as tangential flow filtration (TFF). Furthermore, so much variability can lead to inconsistent pharmacological activity. First-generation payloads included DNA disrupting agents such as calicheamicin, SN-38, duocarmycin and doxorubicin. These were linked to the mAb via monovalent, non-cleavable bonds or by acid-labile linkers, which weren't reliably stable. Beyond functional properties, stability is a key consideration in selecting therapeutics. While these agents were an impressive first step, they had a much narrower therapeutic index than many had hoped.

Lysine conjugation



Cysteine conjugation

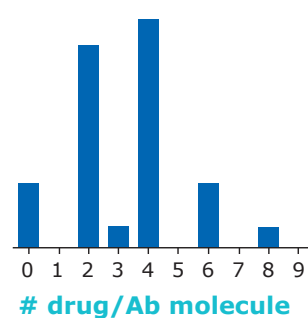
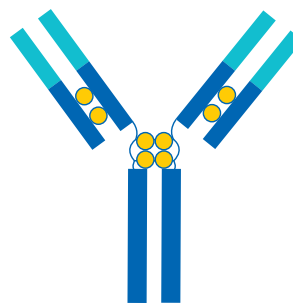


Figure 2: Drug-antibody ratio distributions for ADCs with standard lysine- and cysteine-based conjugation. Own representation based on "Sitespecific conjugation of a cytotoxic drug to an antibody improves the therapeutic index" by J. R. Junutula et al, 2008, Nat Biotechnol, 26, 925-932. Copyright 2008, Springer Nature

Second-generation ADC development: site-directed conjugation

Armed with more tools and more understanding, researchers looked to solve the heterogeneity problem, moving from native IgGs toward site-specific engineered mAbs. These have included mAbs with engineered cysteines, non-natural amino acids and sequence tags, all of which could be reacted to form a more homogeneous product. The goal was to manipulate the antibody so that had exact chemistries in specific, limited locations where the toxins were to bond. For example, monoclonal antibodies containing engineered cysteine moieties limit conjugation to positions that do not disturb immunoglobulin folding or assembly or alter antigen binding⁴. The structural representation for an ADC made with such an antibody is shown in **Figure 3**.

This type of construct first came to the clinic in 2013. Its tighter DAR distribution reflects a more homogeneous ADC final product with greater process control.⁵ Other second-generation developments included modulation of ADC hydrophobicity by using hydrophilic linkers; structure-activity relationship (SAR) design relating a molecule's structure to its function; and enhanced analytical tools such as chromatography, which is useful in characterizing ADC purity. Cytotoxic payloads with greater potency, such as auristatin and maytansine microtubule disruptors, also came into play. All of these developments helped to improve the usefulness of these agents.

More sophisticated, specialized manufacturing techniques and special facilities were also required to handle these powerful toxins safely. Second-generation linkers had slightly more functionality than earlier linkers. They were also monovalent, but some were cleavable, either enzymatically or via acid exposure, inside cells or in lysosomes. Examples include linkers based on proteases, hydrazone, polyethylene glycol (PEG) and disulfides. These linkers were expected to help the antibody release the toxin at the right place and the right time and also stabilize the ADC during preparation, storage and systemic circulation.

Cysteine conjugation

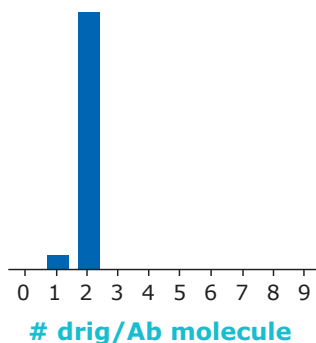
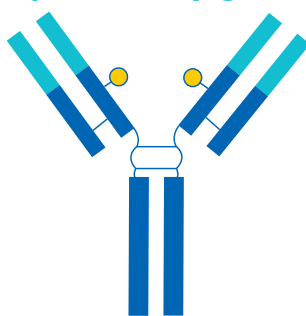


Figure 3: Controlled drug-antibody ratio with controlled, 2- and 4-species distribution. Own representation based on "Site-specific conjugation of a cytotoxic drug to an antibody improves the therapeutic index" by J. R. Junutula et al, 2008, Nat Biotechnol, 26, 925-932. Copyright 2008, Springer Nature.

ADC Building Blocks

Evolution of Technology / Experience Footprint

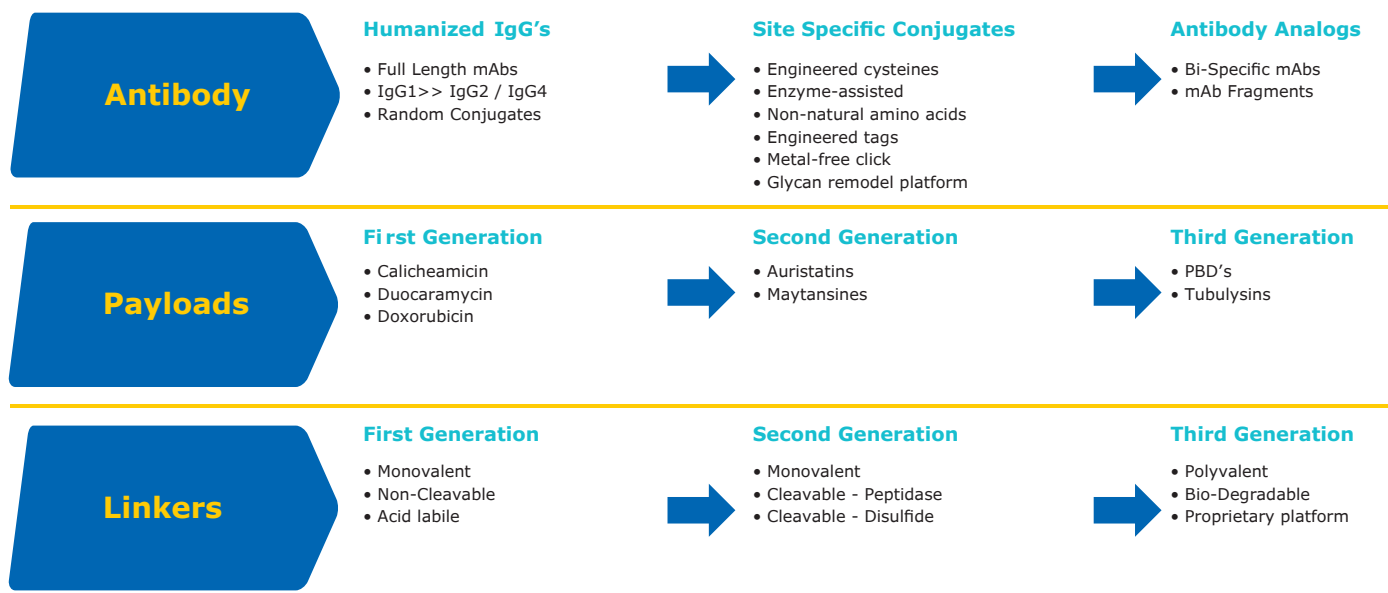


Figure 4: Summary of the evolution of ADC antibody, payload and linker technologies over the past fifteen years

Third-generation ADC development: new expectations for linkers

Like the second generation, third-generation ADCs utilize site-specific conjugation technologies leading to highly homogeneous DARs. However, today's designers are exploring additional modes of action and ways to increase activity and specificity. Bi-specific mAbs, both IgG-like and non-IgG-like, contain two dissimilar binding sites. For example, a single ADC may deliver a toxin AND activate natural killer cells. One recently constructed agent had four mechanisms of action. Obviously, the conjugation processes and analytics for these agents are non-trivial.

Another up-and-coming technology is utilizing Fabs (antigen-binding fragments) in place of intact mAbs. These are sections of antibodies that include sites for antigen binding and linkage. These antibody analog fragments are very stable, may be internalized more readily, are relatively easy to purify, and tend to be less immunogenic than larger ADCs.

Third-generation payloads—potent cytotoxins such as PBDs and tubulysin, which require special facilities

and special handling—are not that different from second-generation payloads.

The most unexpected aspect of third-generation ADC development has been a revolution in the general understanding of what linkers can do. SAR studies show that linkers change antibody properties, including changes in toxicity and pharmacokinetic profiles. It is now known that if a linker is altered, these parameters must be re-evaluated.

Newer linker categories still include cleavable and non-cleavable technology design with advancements in regard to payload release and to deliver at the target site. Additionally, hydrophilic linker modulation such as pegylation can mask a larger molecule from the immune system and decrease renal clearance to increase longevity in the circulation. This is a very useful concept as PBDs are very hydrophobic and, once conjugated, are prone to aggregation.

Manufacturing today: a litany of challenges

The evolution of ADCs from a relatively simple idea to the complex, multifaceted product they are today places substantial demands on drug developers and manufacturers that are trying to study these drugs and make them commercially viable. These challenges are compounded by the sense of urgency created by a crowded field and by the powerful need for life-saving therapies.

- Diverse strategies for engineering mAbs and fragments for site-specific conjugation to achieve controlled DAR distributions, stability and consistent pharmacokinetic (PK) and toxicity behavior necessitate numerous, sophisticated laboratory procedures.
- Aggregation must be controlled for drug safety. Many trials are failing because of aggregation. Greater species uniformity and improved process design helps, but the widespread use of hydrophobic PBD payloads complicates this issue.
- Highly toxic payloads such as PBD require special facilities and diligent safe handling.

- Linker chemistries are becoming more and more important and, if hydrophilic, can help with the aggregation problem. On the other hand, they complicate what was originally thought of as a very simple function, necessitating more steps, more R&D and more expertise.
- Additional drug mechanisms complicate manufacturing exponentially and demand increasingly sophisticated testing.
- SAR drug design, now *de rigueur*, requires a completely new category of expertise to help discover relationships between ADC structure, mechanism of action and efficacy.
- The above products and processes must be analyzed with increasingly sophisticated tools, such as automated surface plasmon resonance (SPR) immunoassays, capillary electrophoresis (CE) and liquid chromatography/mass spectrometry (LCMS). These advanced methods of characterization can also help with routine lot release and stability testing.

How CDMOs must rise to the occasion

Established CDMOs in the ADC business will need to keep pace with future technological advances in this fast-growing industry. Companies that will be successful in the bioconjugation space are providing dedicated facilities for high-potency biologicals, establishing platform operations and developing a workforce with the advanced and specialized expertise to meet the expectations of customers and regulatory agencies. Next-generation bioconjugation will not only be challenged by new and novel chemical unit operations, but will also require novel analytical technologies to provide a more granular understanding at the molecular level. Techniques and tools will need to provide answers for the control strategy of complex products and will need to evolve to support sophisticated release strategies for on-line and at-line in-process testing. Along with comprehensive laboratory services and expert assistance, the following technologies are particularly helpful for successful ADC manufacturing:

• Single-use systems (SUS)

SUS add simplicity. In this space, maximizing the deployment of SUS makes sense, and SUS technologies are available for steps throughout the ADC manufacturing process. Single-use GMP

manufactures of ADC succeeded in 2017. Having a complete, single-use process in place can make a big difference: no cleaning studies are needed. The components are designed to be scalable. Extractables and leachables documentation may be available from the manufacturer to meet regulatory requirements.

• Process and analytical technology (PAT)

PAT allows real-time testing during a GMP process to gather rich, real-time data during active processes. It can be used to ensure the process is going as planned and it can also be used to monitor trends in process iterations.

• Chromatography

Purification strategies have been expanded to include large-scale chromatography. Chromatography is used to clear lipophilic drugs that are not amenable to TFF clearance, to remove aggregates and to refine conjugated species distribution, as in removing unconjugated mAb. Chromatography can also help ensure the best possible ADC therapeutic index and specificity, an important function since 60% of constructs require this type of purification.

Complexity is the new normal

Fifteen years ago, antibody-drug conjugates were still a relatively simple concept: Use an antibody to target a cell and precisely deliver a biologically active agent. Now the mission is much more complex. CDMOs with long-range plans for ADC manufacturing are setting up processes to handle challenging supply chains and investing in facilities and processes to ensure efficiency, quality and security for their customers. Companies that can help deliver multiple constructs to enable a well-designed clinical program are providing customers the opportunity to advance in the field at a fast pace. CDMOs are uniquely positioned to see a wide variety of best practices and can provide solutions based on what has been observed in the industry. In addition, as

more commercial products reach the market, there is an acute need for companies that understand how to execute late-stage studies to support a filing strategy. The growth of ADCs in the clinical and commercial API space is a testimony to the ability of manufacturers to continue to evolve the technologies required to handle complex molecules. Whether it is understanding the structureactivity relationship around the antibody, linker and drug, or managing a complex supply chain, a CDMO with experience should have the skills and tools to help ADC developers navigate these challenges. Since complexity is the new normal, partnering with an experienced CDMO is a strategy that will enable you to take your product to the next level.

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