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ACHIEVING SAFETY, EFFICIENCY, AND FLEXIBILITY IN ADC MANUFACTURING

Antibody-drug conjugates (ADCs) have emerged as a powerful targeted therapy for cancers. By combining the specificity of monoclonal antibodies (mAbs) with the potency of a cytotoxic drug, ADCs function like "biological missiles" by delivering cell-killing drugs precisely to cancer cells.[1]

"If you look at the number of ADC programs, there are about 700 different clinical trials and over 250 drug candidates, including those in preclinical development. That is a very big portion of new biologic drugs," says Chor Sing Tan, global strategic account executive at Repligen. "[ADCs are] addressing broader challenges in oncology, like breast and colon cancer, and we can see [the field] evolving to replace some traditional antibody treatments."

By mid-2024, 15 ADCs received global regulatory approval across a range of cancer types.[2] ADCs are also being developed for new therapeutic areas, such as metabolic, autoimmune, and infectious diseases, and some candidates are already in clinical trials.[3]

Scientific momentum has been matched by growing commercial interest, with major investments now aimed at expanding manufacturing capacity. In 2023, Pfizer acquired ADC pioneer Seagen, gaining access to established technical expertise.[4] Contract development and manufacturing organizations (CDMOs), including Lonza and Samsung Biologics, have responded to the growing demand by expanding their dedicated ADC facilities.[5,6]

As investment in ADC manufacturing grows, organizations must confront the unique complexities and risks that preparing ADCs introduces compared to preparing conventional mAbs or small-molecule drugs. ADC manufacturing is a multistep process that begins with independently producing and purifying the mAb, a highly cytotoxic payload, and a linker. A controlled conjugation reaction

then links the biological and chemical components. Rigorous purification and formulation yield the final ADC product, which must meet stringent quality, efficacy, and safety standards.[7]

THE ANATOMY OF AN ADC

ADCs consist of three essential components (Figure 1). The mAb recognizes and binds selectively to antigens on the surface of tumor cells. The payload is a highly potent drug—often 100 to 1000 times more cytotoxic than traditional chemotherapeutics. The final component is a chemical linker that connects the antibody to the drug. A critical ADC design parameter is the drug-to-antibody ratio (DAR), which is defined as the average number of conjugated drugs on the antibody. This ratio is fine-tuned to maximize therapeutic efficacy and minimize toxicity.[8]

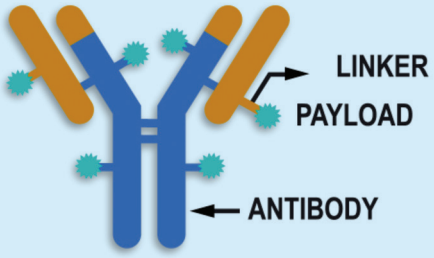

Figure 1: Antibody-drug conjugates consist of three main components: the antibody, a cytotoxic drug payload, and a chemical linker connecting the two.

Source: Santiago Escrivá-de-Romaní and Cristina Saura. "The Change of Paradigm in the Treatment of HER2-Positive Breast Cancer with the Development of New Generation Antibody-Drug Conjugates," *Cancer Drug Resist.* 6, no. 1 (Jan. 2023): 45–58, http://dx.doi.org/10.20517/cdr.2022.52.

CHALLENGES IN ADC MANUFACTURING: CONJUGATION AND PURIFICATION

Although standard mAb production is supported by decades of process optimization, ADC manufacturing introduces a host of additional challenges. For example, the antibody used in ADCs must withstand chemical modifications during the manufacturing process without compromising its stability or ability to target. Even minor variations, such as differences in the sugar molecules bound to the antibody or newly exposed reactive sites, can affect the ADC's robustness, solubility, and propensity to aggregate.[7] Aggregated species can disrupt downstream chromatography separation, resulting in inconsistent product profiles and, ultimately, batches that fail quality control.

Cytotoxic payloads used in ADCs are among the most potent agents known, adding another layer of complexity. Many of these compounds, such as the tubulin inhibitor maytansine, display extraordinary cell growth inhibition but also cause severe side effects when administered on their own.[9] Owing to their extreme potency, manufacturers use closed-system processing and specialized containment measures insteps involving the payload to protect personnel and to prevent environmental contamination.[7]

These challenges—the stability of the mAb and the cytotoxicity of the payload—have outsized effects on two stages of ADC manufacturing: conjugation and purification.

Conjugation

After the mAb and drug-linker complex have been prepared, they're linked via a conjugation reaction. To accommodate the hydrophilic mAb and the hydrophobic drug–linker complex, this reaction typically involves a mix of water and organic solvents.[10]

But exposing mAbs to organic solvents can introduce stability issues. "During conjugation, the challenge is really understanding the optimum way of solubilizing high-potency drugs and controlling the mixing efficiency," says Tan. "However, excess organic solvents can lead to aggregation and denaturation of the antibody."

The conjugation step also often results in a spectrum of DAR across the ADC population. For each ADC, an optimal DAR is defined to maximize therapeutic efficacy while maintaining safety. Variability in the DAR can introduce inconsistencies in potency, stability, and pharmacokinetics.

Purification

Once conjugation is complete, the mixture contains conjugated and unconjugated antibodies, antibody fragments, aggregates, unreacted payload-linker complexes, free payload, linker derivatives, and residual organic solvents.

"Aggregated ADCs can lead to immunogenicity and reduced efficacy. Separating these species and ensuring a consistent DAR calls for optimum

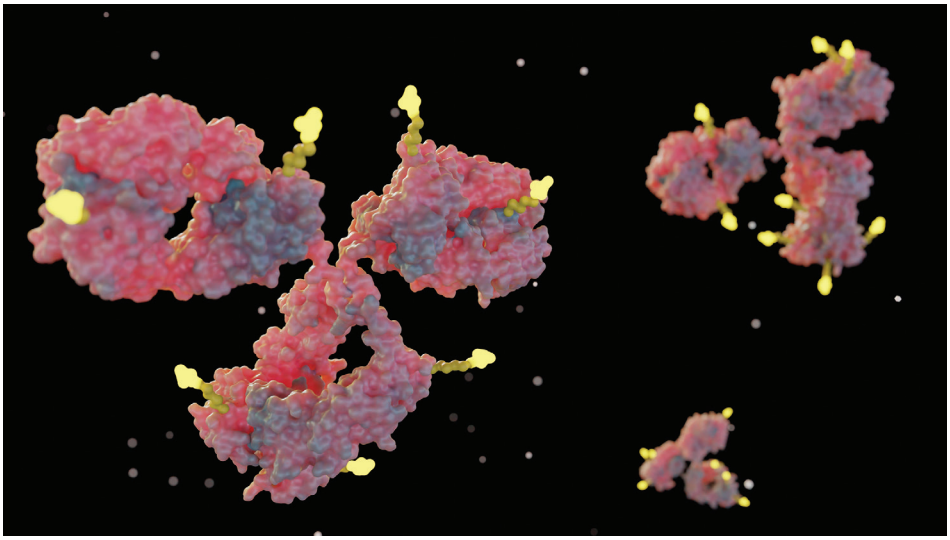

Figure 2. Although ADCs appear simple in concept, 3D structures demonstrate their complex composition.

Credit: Shutterstock

filtration and advanced chromatography methods that are both selective and gentle," says Tan. In practice, the purification of the conjugated antibody is achieved by combining tangential flow filtration (TFF) techniques with chromatography methods. At manufacturing scales, TFF systems are used to separate impurities from conjugated antibodies, followed by chromatography to remove antibody aggregates and concentrate the ADC to the target final formulation.[7]

STREAMLINING ADC PRODUCTION WITH SINGLE-USE SYSTEMS

Safely handling the cytotoxic payloads during conjugation and purification is one of the most significant challenges in ADC manufacturing. One way ADC manufacturers can maintain cGMP-compliant aseptic conditions while significantly reducing worker exposure to these high-potency compounds is to incorporate single-use systems (SUS). SUS are used for filtration and purification in various bioprocessing applications and often consist of tubing, membranes, reservoirs, sensors, prepacked chromatography columns, and flow paths. These components are designed to be disposable after each use.

In contrast to stainless-steel systems, which require extensive cleaning and validation after each use, SUS enable rapid deployment. By discarding single-use components after a campaign, users can eliminate cross-contamination risks and the need for extensive clean-in-place (CIP) procedures, which are labor-intensive and time-consuming. Single-use components also minimize operators' exposure to hazardous materials by avoiding the disassembly of components. Additionally, the use of single-use membranes fitted with aseptic connections provides a fully closed assembly that further reduces these risks.

Another benefit of SUS is the built-in automation capabilities that aid in minimizing hands-on exposure by operators. SUS, which are often standard and off-the-shelf systems, have evolved to the point where they can provide users with end-to-end process automation.

"KrosFlo® RS TFF Systems and KRM™ Chromatography Systems by Repligen enable operators to automate their entire process remotely from start to finish. This limits operator exposure to hazardous materials while ensuring effective processing of ADCs," says Tan. "The KrosFlo® RS 10, in particular, is ideal for use in isolators (enclosures that fully isolate a process or product from its environment) due to its size and separate control unit. The system can be operated from outside the isolator without a network connection. All RS and KRM™ systems can also be controlled remotely via network connections."

The use of SUS offers multiple operational advantages to ADC manufacturers.[11]

Safety: Closed, disposable flow paths help create a contained environment, minimizing operators' direct contact with cytotoxins and reducing environmental contamination risks.

Figure 3. KrosFlo® RS TFF Systems (left) and KRM™ Chromatography Systems from Repligen minimize users' exposure to hazardous materials during manufacturing processes.

Credit: Repligen

Productivity: Each component is single-use and arrives presterilized, so there is no need for operators to clean equipment between ADC batches, eliminating the time and resources spent validating cleaning protocols. Removing the cleaning step accelerates batch turnaround and enables more agile scheduling, which is particularly valuable for multiproduct ADC facilities.

Cost: SUS avoid the significant capital expense of building and maintaining stainless-steel infrastructure. The lower initial investment and reduced utility consumption offer long-term cost advantages.

Flexibility: The modular and scalable nature of SUS supports adaptive manufacturing, which is ideal for early phase ADC programs or rapid scale-up. Facilities can quickly reconfigure for new molecules or campaigns simply by swapping out disposable flow paths.

But a key concern when implementing SUS in ADC processes is the risk of extractables and leachables (E&L), substances that may be released from plastic components under certain conditions. Extractables are chemical entities that can be released from plastic components when subjected to aggressive extraction methods designed to simulate worst-case conditions. Leachables are substances that migrate into the product under normal manufacturing, storage, or use conditions and may include secondary leachables that form when extractables interact with the drug product or packaging materials.

This risk of introducing E&L when using SUS is most significant when using organic solvents such as dimethyl sulfoxide (DMSO) or dimethyl acetamide (DMAC) during the conjugation reaction between the mAb and the payload linker.

AN EXTRACTABLES STUDY OF SINGLE-USE TUBING AND FILTER COMPONENTS USED IN ADC MANUFACTURING

Repligen researchers assessed extractables from single-use flow kits and filter components using aggressive extraction solvents, such as reverse osmosis deionized (RODI) water, 20% DMSO, and 20% DMAC to simulate typical conditions. They quantified compounds released from the plastic single-use materials under these conditions to provide data for early risk assessments and inform material selection for ADC production.

Study overview

Samples of ProConnex® tubing sets (used on KrosFlo® RS systems) and TangenX® SIUS® Gamma flat sheet cassettes from Repligen underwent 24-hour extractions using specified solvents under controlled conditions at ambient temperature.[12] The resulting extracts were stored in Teflon tubes at −20 °C and subsequently analyzed using headspace GC/MS for volatile organic extractables, direct injection GC/MS for semivolatile species, UPLC-PDA-Q-ToF for nonvolatile organic extractables, and ICP-MS for inorganic extractables. Reporting thresholds of 0.100 µg/mL for organic extractables and 0.020 µg/mL for inorganic extractables were applied to ensure that only the contaminants present at significant levels were flagged.

Conclusions

Under the specified study conditions, the extractables from the SIUS® Gamma cassette and ProConnex® flow kit indicated no significant toxicological concerns. Extractables exceeding 0.050 µg/cm² detected in samples containing 20% organic solvent were also present in control samples extracted with RODI water. These results support the use of the KrosFlo® RS system for ADC manufacturing processes.

		Concentration Extracted (µg/cm²)		
	Extractables	RODI	DMAC	DMSO
VOC	Trimethylsilanol		0.012	
Semi-VOC	α,α-Dimethyl-benzenemethanol		0.033	0.014
	Ribonic acid		0.039	
	3-(4-Methylbenzoyl) propionic acid	0.058		
	Hexanoic acid		0.015	
	3-Phenylphenol			0.021
	Vulculic acid		0.009	
Metals	Potassium	0.330		
	Silicon	0.106		

Table 1. Extracts measured from ProConnex® tubing sets after 24-hour extractions in reverse osmosis deionized (RODI) water, 20% aqueous dimethyl acetamide (DMAC), and 20% aqueous dimethyl sulfoxide (DMSO). All values are reported in µg/cm². VOC: volatile organic compound.[12]

Credit: Repligen

		Concentration Extracted (µg/cm²)		
	Extractables	RODI	DMAC	DMSO
VOC	Trimethylsilanol	0.154	0.206	0.269
	Benzene		0.016	0.009
	Unknown siloxane		0.006	
Semi-VOC	Unknown amide		0.009	
	1-Methyl-2-pyrrolidinone	0.701	0.664	0.967
	Acetophenone		0.008	
	α,α-Dimethyl-benzenemethanol		0.005	
	Benzothiazole		0.116	
Non-VOC	3-(4-Methylbenzoyl) propionic acid		0.020	
	3,3´-Iminobispropane-1,2-diol	0.029		
	2-Mercaptobenzothiazole			0.022
	Triethanolamine	0.110		0.106
	Bisphenol A bis(2,3-dihydroxypropyl) ether		0.028	
	6-Di-*tert*-butyl-4-methylphenol		0.030	0.006
Metals	Potassium		0.001	
	Zinc		0.002	0.001
	Sodium	3.183	2.015	2.003
	Silicon	1.480	4.500	3.857

Table 2. Extractable compounds measured from TangenX® SIUS® Gamma flat sheet cassettes after 24-hour extractions in reverse osmosis deionized (RODI) water, 20% aqueous dimethyl acetamide (DMAC), and 20% aqueous dimethyl sulfoxide (DMSO). All values are reported in µg/cm². VOC: volatile organic compound.[12]

To mitigate this risk, manufacturers conduct thorough E&L profiling and toxicological risk assessments according to industry and regulatory standards. Suppliers of single-use systems and components aid these studies by providing validated E&L data packages.

SUPPORTING THE NEXT PHASE OF ADC MANUFACTURING

Despite the clinical and commercial success of ADCs, the field continues to face significant challenges. Late-stage failures—often due to dose-limiting toxicities, off-target effects, or insufficient therapeutic efficacy—highlight the urgent need for improved selectivity and stability in ADC design and production. Significant research efforts are underway to improve control over ADC conjugation and DAR to provide a more uniform drug product with improved pharmacokinetics.[13]

As ADC chemistry and site-specific conjugation technologies continue to advance, the manufacturing process must evolve to support these innovations. Efficient, scalable, and flexible production platforms that incorporate SUS are critical to translating these scientific breakthroughs into high-quality, commercially viable therapies.

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