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White paper

Bringing novel ADC formats into the clinic

Process development for antibody-oligonucleotide conjugates

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Antibody-drug conjugates (ADCs) have become a substantial part of anticancer drug therapies. More than a dozen ADCs are already on the market, and over 200 are in various stages of (pre-)clinical development [1, 2]. Traditional ADCs consist of a targeted antibody linked to a small-molecule drug via a chemical linker.

In recent years, the concept of ADCs has evolved to include innovative formats with improved architecture and performance. These advancements include novel payloads, cutting-edge linker technologies, improved conjugation methods, and new antibody formats [3]. This diversification and expansion of the bioconjugate technology landscape has gained momentum, paving the way for applications beyond oncology and into new therapeutic areas.

Novel bioconjugate formats

Over the past two decades, steady technological advancements have played a pivotal role in the emergence of novel bioconjugate formats within the development pipeline (Figure 1). These innovations can be broadly categorized into three key areas[4]: First, new types of payloads have diversified the field beyond traditional cytotoxic agents and include a wide range of molecules such as oligonucleotides, polymers, chelators, peptides, or polysaccharides[5,6,7]. Second, new antibodies and antibody formats have been introduced to enable targeted delivery to new tissues and cell types[8,9]. Third, optimized linker technologies and conjugation techniques have been developed to refine the molecular architecture — enabling, for example, high drug-to-antibody ratio (DAR) ADCs or site-specific bioconjugates with improved efficacy and pharmacokinetic profiles[10].

This increasingly diverse technology landscape has unlocked new mechanisms of action across a broad range of therapeutic areas, including oncology, muscle disorders[11], ophthalmology[12], diabetes[13], or inflammatory diseases[14].

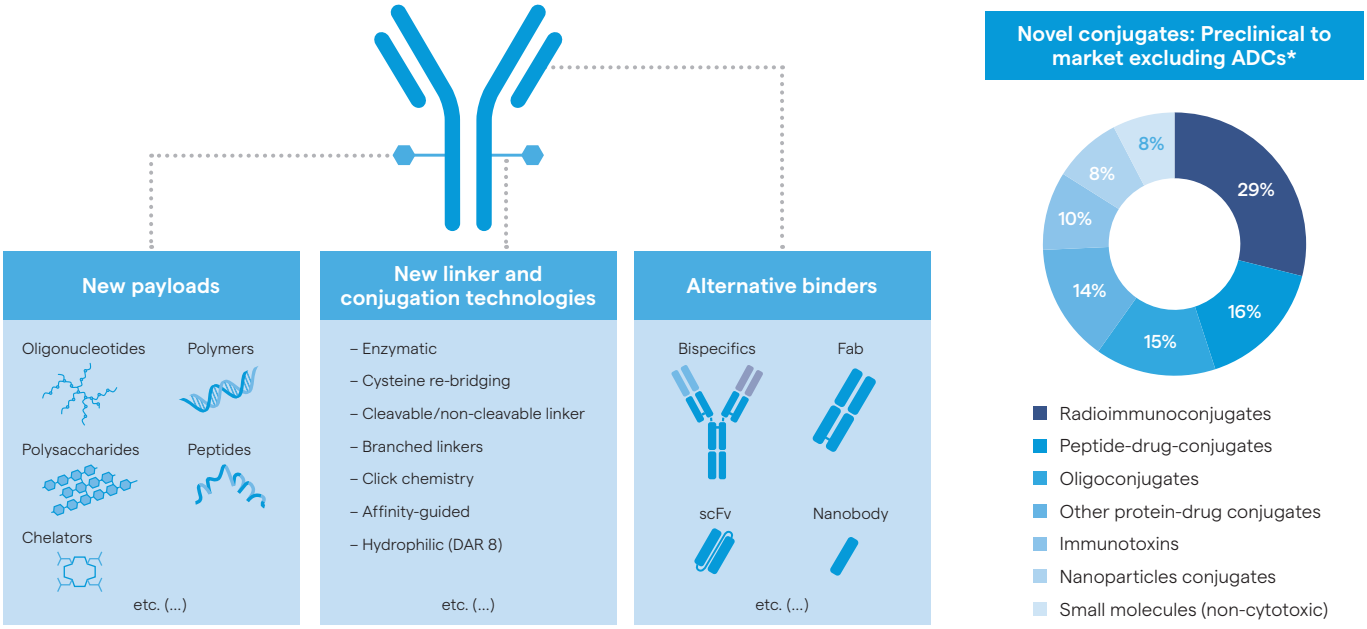

Figure 1
Emerging bioconjugate modalities combining novel payloads, alternative protein binders, and advanced linker and conjugation technologies. Pie chart generated with data from Beacon Intelligence databank[15].
*Excludes small-molecule drug conjugates and radioconjugates.

The development and manufacturing of these novel bioconjugate formats often come with unique requirements and challenges throughout the product life cycle—from (pre-) clinical process development to clinical and commercial manufacturing. At Lonza, our experts bring deep experience in navigating each stage of this journey.

Importantly, many process-related challenges are identified and addressed during early- and late-stage process development. In this article, we highlight antibody-oligonucleotide conjugates (AOCs) as a case study to demonstrate how our team supports customers in developing and optimizing manufacturing processes. This includes recommending conjugation strategies and downstream purification approaches tailored to the specific needs of each bioconjugate format.

Antibody-oligonucleotide conjugates: Challenges

Figure 2 compares the manufacturing workflows of standard ADCs—comprising a monoclonal antibody (mAb) and a small-molecule toxin—and AOCs. Although the overall manufacturing concept follows a similar process flow, AOCs require additional considerations and controls during conjugation and purification.

Oligonucleotide payloads are considerably larger (approximately 5 – 20 kDa) than the small molecules (< 1 kDa) of standard ADCs. Moreover, depending on their chemical backbone, oligonucleotides may be negatively charged. These characteristics may influence both the stoichiometry and reaction kinetics during conjugation, necessitating careful optimization and evaluation.

Analytical challenges may also arise due to the strong UV absorbance of oligonucleotides, which can interfere with accurate quantification during process development and optimization experiments.

From a purification standpoint, the large size of unconjugated oligonucleotide payloads makes them difficult to remove using tangential flow filtration (TFF) alone. Chromatography offers several advantages in this context: i. efficient removal of unconjugated payload; ii. separation of unconjugated antibody; and iii. the possibility of separating and isolating different oligonucleotide-to-antibody ratio (OAR) species.

Finally, since AOCs often require higher dosing, the final TFF step typically focuses on both the removal of residual impurities during buffer exchange and ultrafiltration to concentrate the drug substance to the desired level.

The following sections provide an overview of the strategies that Lonza employs to address these specific requirements and challenges—demonstrating how our expertise supports the successful development and manufacturing of complex bioconjugates like AOCs.

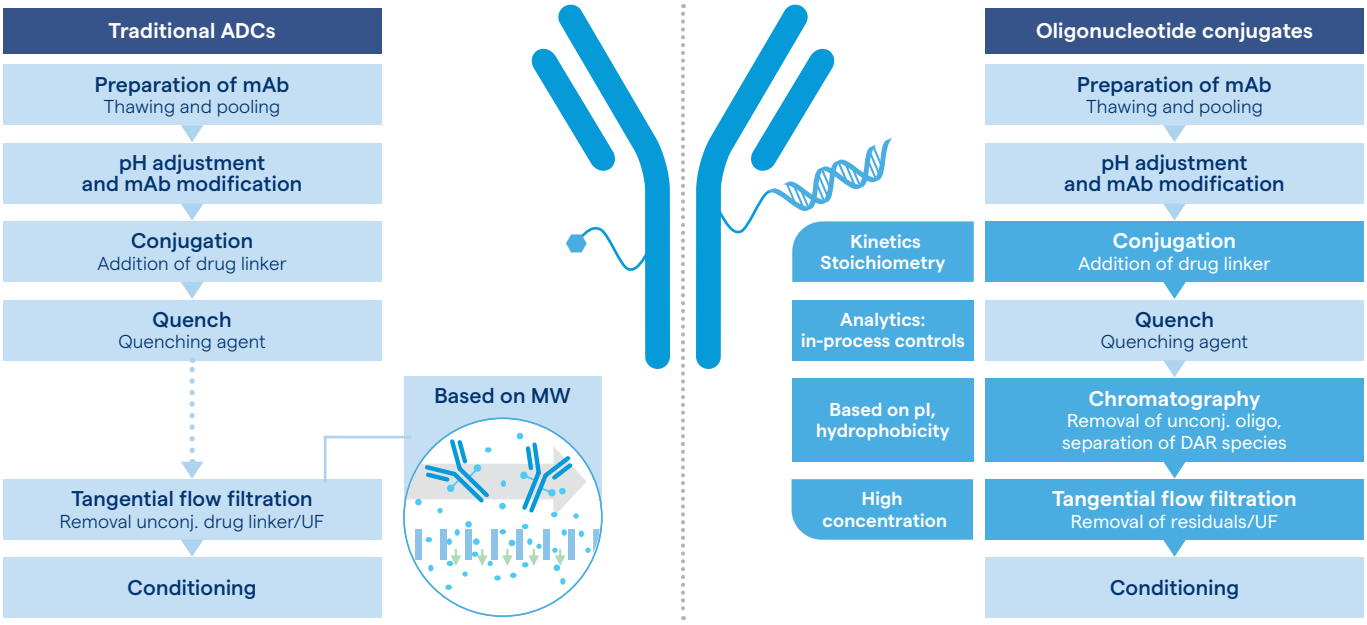

Figure 2
Comparison of the conjugation process workflow for traditional antibody-drug conjugates (ADCs) with small molecule payloads versus antibody-oligonucleotide conjugates (AOCs). The increased molecular weight and distinct physicochemical properties of oligonucleotide payloads necessitate modified conjugation and purification strategies that need to be carefully addressed during process development.

Conjugation reaction: Development and optimization

Process development often prioritizes optimizing conjugation efficiency, as it directly influences both product quality and manufacturing costs. Two parameters routinely evaluated are reaction time and reactant stoichiometry.

Fine-tuning the reaction time is crucial for balancing efficient conjugation—ensuring the reaction reaches completion—with minimizing prolonged hold times, i.e. running the conjugation only as long as necessary. Reaction completion is essential for process robustness, while extended hold times can negatively impact product quality and increase overall batch cycle time, ultimately affecting manufacturing costs.

In the case of AOCs, the oligonucleotide payload plays a significant role in reaction kinetics. It is often the most expensive raw material in the conjugation process making the use of large excesses undesirable. Additionally, the oligonucleotide's size and charge can influence both reaction efficiency and kinetics.

To determine the optimal conjugation time, we conduct automated time-course experiments using Ultra-High Performance Liquid Chromatography-Size Exclusion Chromatography (UP-LC-SEC). Reaction completion is assessed at predefined time intervals by integrating the peak areas corresponding to the conjugated product and the unconjugated antibody. The time point at which the conjugate peak area reaches saturation defines the optimal reaction duration (Figure 3).

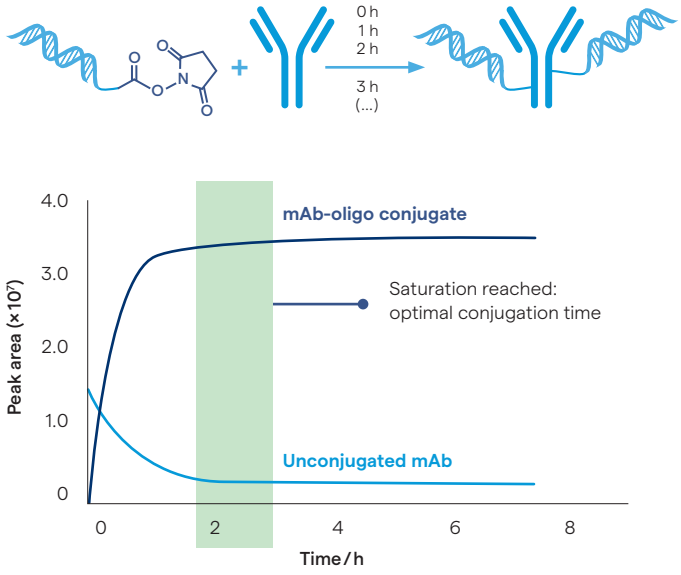

Figure 3
Evaluation of reaction completion via automated time-course analysis using Ultra-High Performance Liquid Chromatography-Size Exclusion Chromatography (UP-LC-SEC).

Beyond reaction time, the stoichiometry between payload-linker and the antibody is a critical parameter that must be carefully optimized to achieve the desired average OAR. In standard ADC processes, DARs and their distribution can be assessed in crude reaction mixtures using techniques such as Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC) or Hydrophobic Interaction (HIC). When the buffer matrix of crude reaction mixtures is incompatible with analytical columns or sample preparation protocols, gravity-flow desalting columns provide a simple and effective means for rapid buffer exchange.

However, for AOCs, analyzing crude reaction mixtures is often not feasible for accurate OAR determination, regardless of buffer composition. This limitation arises from the strong absorbance of the oligonucleotide payload, which peaks at 260 nm and significantly overlaps with the protein absorbance at 280 nm. As a result, absorbance-based methods at 280 nm may yield only relative or qualitative data and are unsuitable for absolute quantification — especially at higher OARs, where oligonucleotide interference becomes more pronounced.

While purified drug substances can be analyzed by UV/Vis spectroscopy using the extinction coefficients for the payload and antibody, this approach is not applicable to crude reaction mixtures containing free (residual) oligonucleotide. Therefore, a non-absorbance-based method is required for accurate OAR screening in crude samples.

To address this, we employ Sodium Dodecyl Sulfate-Polyacrylamid Gel Electrophoresis (SDS-PAGE) combined with densitometry. This method enables the separation of OAR species within a gel matrix, followed by the staining and quantification of the antibody. The staining intensity of the corresponding OAR species correlates with the OAR of the sample (Figure 4). Because multiple samples can be processed in parallel in under two hours, this approach offers a practical and efficient solution for screening and optimizing conjugation parameters. These include stoichiometry, buffer composition, starting material quality, reactant concentrations, organic solvent content, and conjugation temperature.

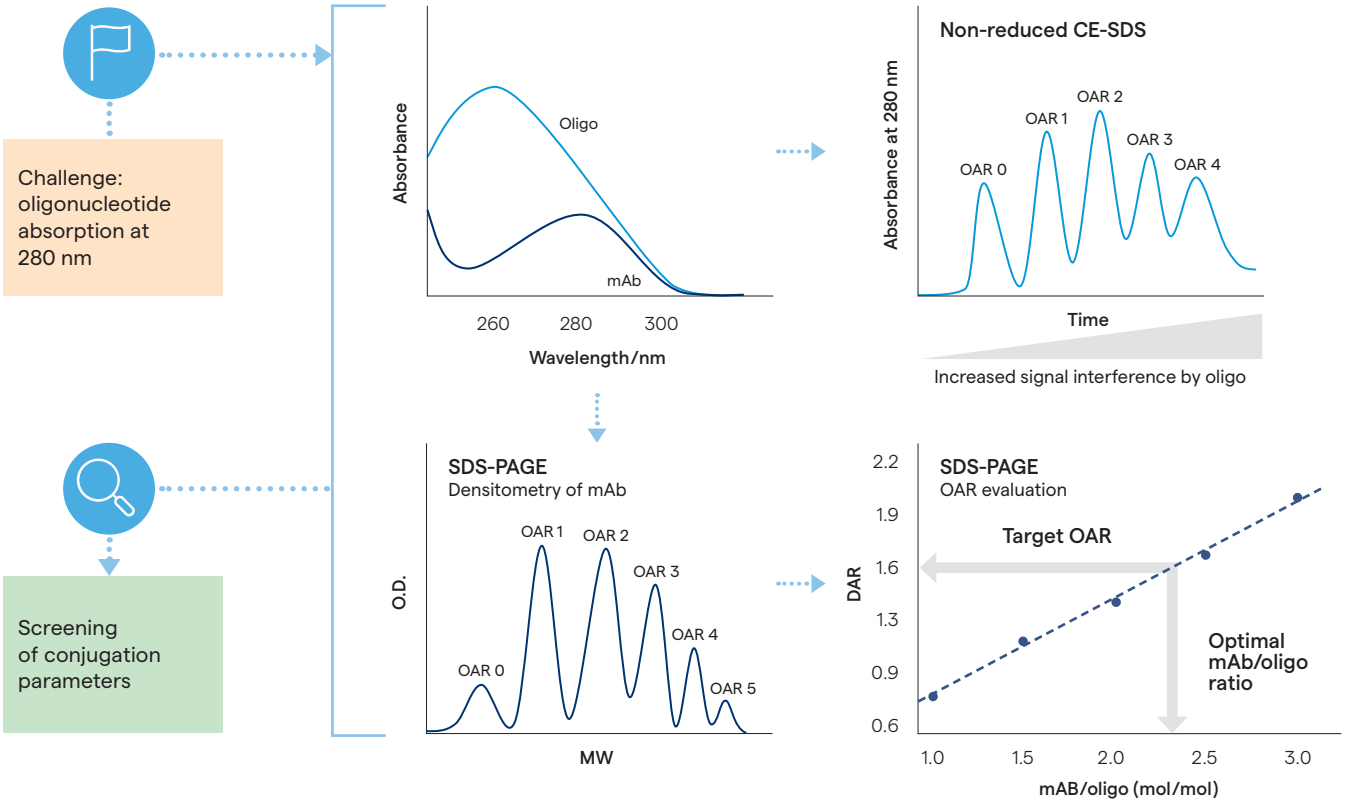

Figure 4
Overlapping absorbance of oligonucleotide payloads (oligo) and proteins at 280 nm requires tailored analytical strategies for accurate determination of the oligo-to-antibody ratio (OAR), particularly in crude reaction mixtures containing free oligo. Methods relying solely on 280 nm absorbance, such as non-reduced CE-SDS, may yield inaccurate OAR values due to signal interference from oligos, especially at higher OAR. In contrast, SDS-PAGE enables the staining and quantification of the mAb content per OAR species, thereby facilitating accurate determination of the average OAR without oligo interference.

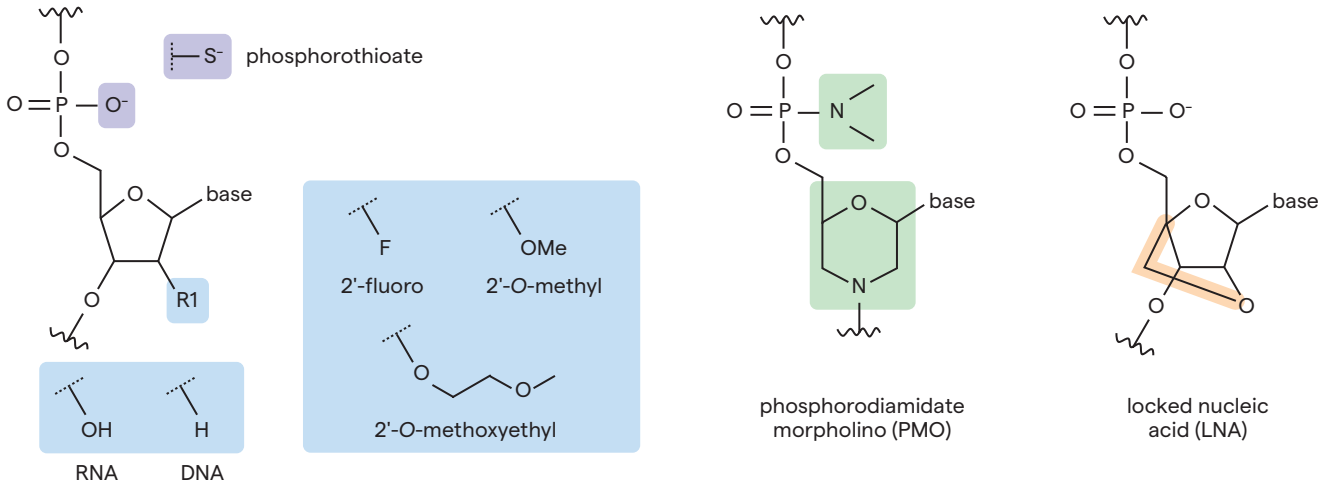

Figure 5
Common chemical modifications of oligonucleotide payloads. These modifications impact the physicochemical properties of the oligonucleotide, which must be carefully considered during the development of purification strategies for the resulting conjugates.

Purification
Once the reaction is complete, the AOC must be purified. For standard ADCs with small molecule payloads, TFF is typically sufficient to remove impurities such as unconjugated payload. However, when dealing with higher molecular weight payloads—such as oligonucleotides—TFF alone is often inadequate for efficient separation from the bioconjugate. In such cases, chromatography serves as a powerful purification method, offering the ability to i. remove unconjugated payload, ii. remove unconjugated antibody, and, if necessary, iii. separate and isolate distinct AOR species.

Development of the chromatography unit operation
The development of a chromatography unit operation typically begins with an evaluation of the physicochemical properties of the AOC to guide the selection of a suitable resin type. This selection is heavily influenced by the oligonucleotide payload, which is often chemically modified to enhance the AOC's stability, pharmacokinetics, pharmacodynamics and biodistribution[16]. Depending on the intended mode of action, the payload may be RNA- or DNA-based and can be either single- or double-stranded. These variations and modifications affect key properties such as charge (pI), hydrophobicity, and molecular size, which in turn guide the choice of resins to be screened (Figure 5).

For instance, a charged molecule may be best suited for ion exchange chromatography, while a hydrophobic payload would require a resin that leverages hydrophobic interactions for effective separation. In addition to separation performance, factors such as the resin's theoretical binding capacity and cost of goods should be considered to maximize step yield and minimize manufacturing costs.

Once the chromatography type is selected, small-scale screenings are conducted using various resin types and process conditions, including pH, conductivity and buffer composition. As noted earlier, the product specifications must guide this development. A step elution can be a robust approach for removing unconjugated oligonucleotide and, if necessary, unconjugated antibody from the product pool containing all OAR species. Conversely, if the goal is to isolate a specific OAR species, a gradient elution is typically employed (Figure 6).

Following these screenings, the resin demonstrating the best performance is selected for further optimization. This includes refining chromatography parameters such as flow rate, pressure limits, separation efficiency, binding capacity, yield, and pooling criteria. Importantly, these parameters must be aligned with the material and equipment capabilities intended for large-scale GMP manufacturing, as they are expected to remain consistent during scale-up.

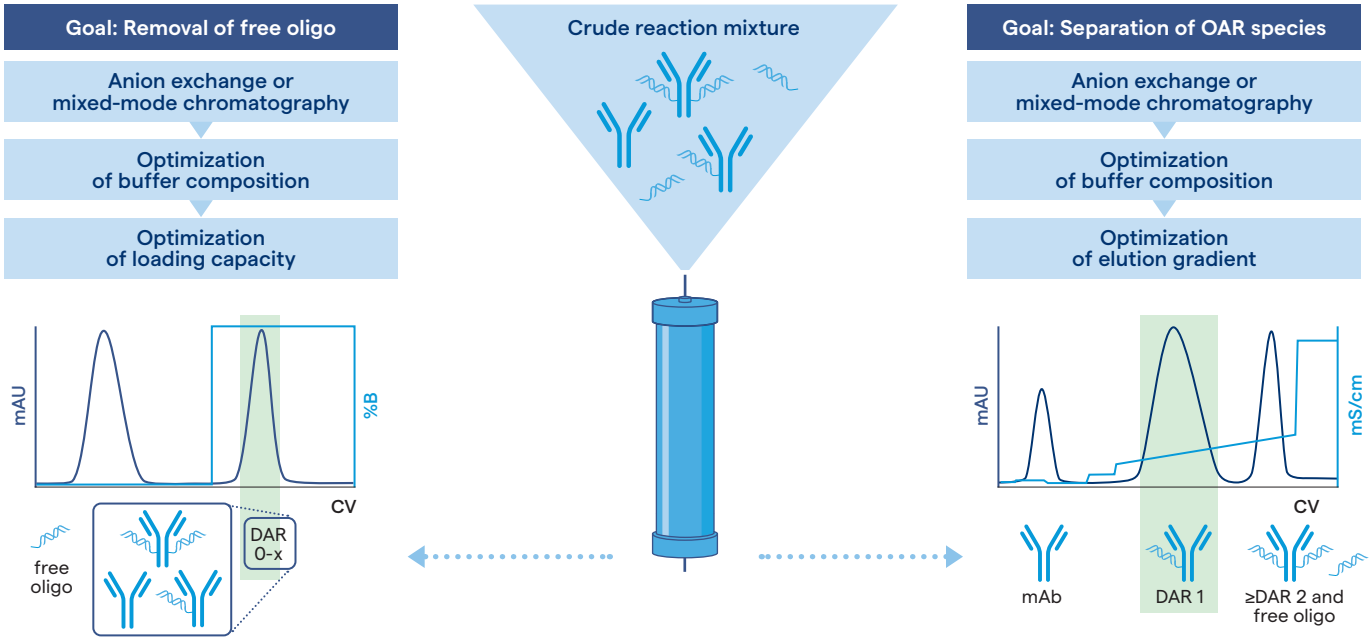

Figure 6
Chromatography is often a key step in the manufacturing process of antibody-oligonucleotide conjugates (AOCs). Depending on the purification objective, either step or gradient elution strategies may be applied.

Development of the TFF unit operation
The final process steps after chromatography involve a TFF-based buffer exchange, concentration adjustment and (optionally) final conditioning—such as the addition of excipients—to yield the final bulk drug substance (BDS). As previously mentioned, AOCs often require higher BDS concentrations. Therefore, the development of the TFF unit operation focuses not only on the clearance of residual impurities but also on ultrafiltration to achieve the desired drug substance concentration.

TFF development includes the evaluation of optimal diafiltration concentration, the feasibility of ultrafiltration to high concentrations, and the identification of suitable transmembrane pressures (TMP) and feed flow rate combinations to ensure efficient permeate flux. Additionally, impurity clearance—such as the removal of residual solvents or linkers—is closely monitored to determine the number of diavolumes required for effective diafiltration.

Transfer into GMP manufacturing

The development and optimization of conjugation and purification unit operations, as described above, are designed to establish robust, reproducible, and scalable manufacturing processes that consistently deliver high-quality drug substance. Data generated at small scale provides a critical foundation for evaluating readiness for scale-up and transfer into GMP manufacturing facilities. This evaluation includes risk assessments and the implementation of mitigation strategies to address challenges associated with upscaling and technology transfer. Common considerations include the transfer accuracy of critical process solutions, equipment compatibility, and the anticipation of potential upscaling effects.

Furthermore, a final proof-of-concept (POC) run is typically performed at a scale of ≤10L using fixed process parameters and representative consumables and raw materials. This POC batch serves to assess both process performance and the quality of the resulting drug substance prior to GMP manufacturing.

Given the variability in dosing regimens and administration frequency for these novel therapies, flexible supply solutions are essential throughout clinical phases and later commercialization. Lonza's bioconjugate manufacturing capabilities span a wide range of scales—from small scale (10 – 60L) and small-mid scale (50 – 600L) to large scale, with volumes up to 1500L. For early-phase clinical GMP drug substance supply, the manufacturing scale typically ranges from 50L to maximal 600L using single-use equipment. Accordingly, the initial technology transfer is tailored to the specific requirements of the intended production scale within these facilities.

As programs advance toward late-stage manufacturing, further scale-up may be required to meet increasing clinical or commercial demand. In such cases, enhanced process understanding becomes essential to identify and address potential limitations—such as maximum filter loadings, acceptable hold times for multiple chromatography cycles, reuse of TFF membranes or chromatography resins, and other key process parameters. This data can be generated flexibly, either during the early program phases of development if scale-up is anticipated, or at a later stage as needed.

Lonza's services are tailored to meet individual customer needs. Clients can engage with Lonza at any project phase and scale—whether for specific steps such as intermediate, drug substance, or drug product manufacturing, or through fully integrated programs that encompass the entire manufacturing supply chain under one roof.

This integrated approach helps de-risk, streamline and accelerate development and manufacturing by simplifying supply chain management, aligning analytical strategies, improving communication, and consolidating contractual arrangements.

For oligonucleotide-antibody conjugates, customers can also benefit from Lonza's Bioconjugates Toolbox, including the state-of-the-art Synaffix GlycoConnect™ platform, which enables site-specific conjugation. This technology has recently been successfully applied to generate AOCs, offering a powerful tool for advancing the development and manufacturing of AOC programs.

Conclusion

The growing diversity and complexity of ADCs, particularly with the emergence of AOCs, is reshaping the landscape of bioconjugate therapeutics. These next-generation modalities hold exciting potential across a broad range of disease areas beyond oncology. However, they also introduce new technical challenges—from conjugation chemistry to purification strategies—that must be carefully addressed for successful development and commercialization.

Lonza has invested in capabilities to develop and manufacture the entire range of bioconjugates inclusive of AOCs. We offer the flexibility to scale processes efficiently and robustly as molecules progress through their lifecycle. By leveraging advanced conjugation technologies, tailored analytical methods, in-depth process understanding, and optimized purification methods, we help overcome the unique hurdles posed by complex bioconjugates like AOCs.

As the bioconjugate field continues to expand, Lonza remains committed to innovating alongside our partners—translating cutting-edge science into the transformative medicines of tomorrow.

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