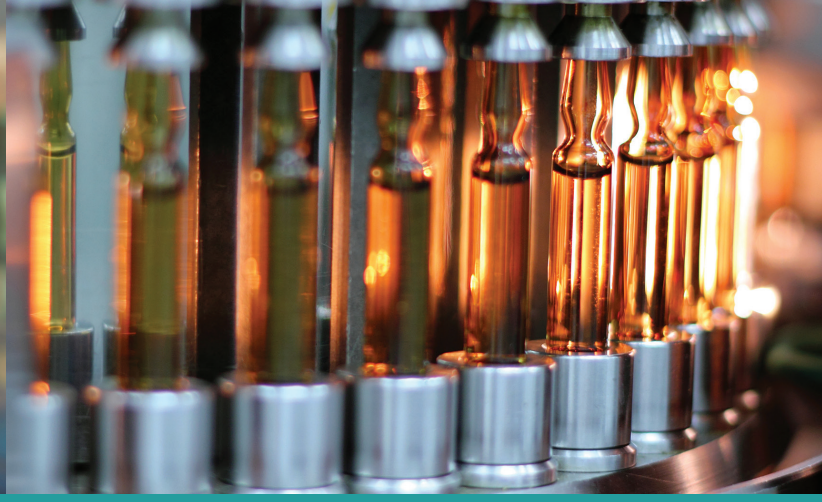

NIMBL

The National Institute for Innovation in Manufacturing Biopharmaceuticals

Antibody-Drug Conjugate and Bispecific Antibody Roadmap

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1.0 Executive Summary

The National Institute for Innovation in Manufacturing Biopharmaceuticals (NIIMBL) launched a technical roadmap process in 2017 to serve the needs of the biopharmaceutical manufacturing community in the US and worldwide. Subject matter experts representing major biopharmaceutical manufacturers, equipment vendors, suppliers, academic institutions, federal agencies and non-profits participated in a series of in-depth discussions focused on the technical needs and manufacturing challenges associated with biopharmaceutical products. These products are increasingly important for the treatment of patients with chronic and deadly diseases. We are grateful for the time individuals (from both NIIMBL member and non-member organizations) contributed to this activity.

The topics for this roadmap process were chosen to complement other technology roadmaps for biopharmaceutical processing that were recently published or are in progress. At a visioning conference held in November 2017 it was decided the first NIIMBL Roadmaps would focus on three different areas: vaccines, antibody-drug conjugates (ADC) and bispecific antibodies (bispecifics), and gene therapy. Many individuals contributed to this effort, facilitated by BioPhorum Operations Group and NIIMBL personnel, and we believe that the resulting roadmaps set the stage for numerous technical- and process-development efforts in the future. We look forward to NIIMBLs next set of roadmapping activities starting in late 2018.

This NIIMBL Roadmap on ADC/bispecifics addresses the market trends and business drivers influencing the discovery, development and manufacturing best practices for these biotherapeutics worldwide. It then discusses the future needs and challenges associated with the manufacturing of these products at various functional levels and proposes some potential solutions to these production barriers. There are numerous issues covered including; modality-specific active pharmaceutical ingredients (API) and product-related impurities, a specific focus on process optimization, modality-specific analytical approaches for in-process and drug substance (DS)/product release and characterization, raw materials, supply chain, modeling, control strategies, regulatory concerns during product filing.

Most of these issues cut across bioprocess manufacturing development, including cell-line development (CLD), upstream and downstream process development, analytical development and drug product (DP) formulation. Finally, there is a discussion on workforce development needs, including the skills and knowledge base required for the future of biopharmaceutical manufacturing of these important classes of drugs. As with all of the NIIMBL roadmaps, the writing team has worked collaboratively to connect its efforts to complementary areas in other roadmaps.

The roadmap highlights a number of goals that, if achieved, will enable the development and manufacture of more affordable ADC/bispecific drugs for patients with critical healthcare needs:

Drug substance goals

- efficient molecular design through protein engineering and new expression system development, including host-cell and vector construction, to improve the manufacturability of target drugs
- better process control and continuous processing to improve productivity and reduce costs
- flexible, automated, continuous manufacturing for multi-product manufacturing
- for ADCs, single-use and closed systems to minimize human contact

Drug product goals

- safely enable stable formulations (including liquid) and improve administration to patients of ADCs and bispecifics
- increase CMO capacity for manufacturing clinical and commercial ADCs

Analytics goals

- comprehensive characterization and analytical control strategies for molecular variants
- rapid analytical feedback to support real-time process control

Modeling goals

- molecular modeling of drug molecules to facilitate manufacturability and quality control
- provide guidance to improve overall process robustness and understanding

Regulatory goals

- understand the unique nature of the quality attributes of bispecific and ADC DPs
- control the drug-to-antibody ratio (DAR) and monitor the efficacy of ADC products
- control product-related variants

Workforce goals

- appropriate programs for operators/technicians and research scientists across academia and industry.

For the pharmaceutical industry, academia, regulatory agencies and healthcare providers to fully realize the value of ADCs and bispecifics, and to achieve the goals described in this roadmap, the following recommendations are considered appropriate:

- an interdisciplinary, open collaboration should be fostered between pharmaceutical and private industries and CMOs to drive innovation in the advanced manufacturing of ADCs and bispecifics
- open collaborations between industry and academia in process-modeling techniques are needed to improve overall manufacturability and prioritization of technology innovation
- safety should be an increased priority in facility design, training and handling of ADC development and production.

2.0 Introduction

Monoclonal antibodies (mAbs) are complete immunoglobulin gamma (IgG) molecules, which consist of two heavy chains (HC) and two light chains (LC) that fold into a complex quaternary Y-shaped structure. The mAbs are composed of a Y-shaped, two-armed molecule with an antigen binding domain, and a stalk, called the crystallizable fragments (Fc) region [1]. The Fc region functions to extend the half-life of mAbs in the human body. The identical antigen-binding fragment regions are responsible for antigen binding and have been extensively engineered for developing highly specific and synthetic antibodies for anti-inflammatory, anti-cancer and anti-viral applications, among others.

However, many of these diseases are typically multifactorial, with many signaling pathways implicated in pathogenesis, resulting in insufficient treatment using the single-target therapy offered by traditional mAbs. Recently, researchers have begun investigating new methods for modifying the mAb to improve treatment efficacy and reduce the necessary dosage of the therapeutic treatment. More specifically, the production of bispecific antibodies and ADCs present great opportunities for improved therapeutics. However, these modified mAbs create new manufacturing challenges that will require technology advancements to bring manufacturing efficiency to the level of recombinant proteins and thus improve the accessibility and affordability of these treatments.

Bispecifics are capable of simultaneously binding two different epitopes on the same or different antigens. They can serve as mediators to redirect immune effector cells (such as 'natural killer' cells, T-cells and tumor cells) to enhance their destruction, or to target two different receptors in combination on the same cell to modulate cell signaling pathways. There are many protein scaffolds in the bispecifics' field, these can be categorized into non-Fc-containing and Fc-containing scaffolds. Besides targeting two antigens simultaneously, these two types of molecule design have very different features and challenges in bioactivity, pharmacokinetics and manufacturability. The Fc-containing scaffolds are mostly bispecific antibodies, which are similar in structure to traditional mAbs.

Bispecific IgG molecules can be assembled from two different HCs and LCs expressed in the same producer cell. However, because of random assembly of the different chains, this results in a substantial number of non-functional molecules in respect to bispecificity. To improve the efficiency in producing bivalent, bispecific antibodies, HC-heterodimerization was forced by introducing different mutations into the two CH3 domains, resulting in asymmetric antibodies. Mutations have been introduced in the CH3 domains of different HCs of the antibody to ensure the formation of the heterodimer, instead of the homodimer, between the two different HCs. Besides HC-heterodimerization, LC shuffling, or the mispairing of LCs with the corresponding HC resulting in a mixture of species, is another critical issue. Mutations were also introduced into the CH1–CL and VH–VL interfaces of the antigen-binding fragments to enforce the correct pairing of the LCs with the corresponding HCs.

Even though all these various protein-engineering approaches have significantly improved the efficiency of producing bispecific antibodies from mammalian cells, the production process yield of API is still much lower when compared to conventional mAbs with various amounts of product-related impurities, such as homodimers or half-molecules. Also, developing appropriate methods for the identification, quantitation and removal of these impurities are all challenging for DS and DP manufacturing.

One unique anti-cancer therapeutic that combines the selectivity of mAbs with the efficacy of a targeted drug is the ADC. An ADC uses chemical linkers to attach a cytotoxic drug of interest to various locations on the mAb structure to provide a highly specific and targeted approach to cancer treatment that can discriminate between healthy cells and their cancerous counterparts. Typical anti-cancer agents used in these therapeutics are DNA-damaging agents and microtubule inhibitors that interfere with cell proliferation to prevent the growth and spread of cancer cells, such as doxorubicin, anthracyclines, auristatin and maytansine. Also, new cytotoxic agents for ADC applications are continually being developed and improved. Cleavable and non-cleavable linkers are available to connect the cytotoxic drug to the mAb. Cleavable linkers use hydrolytic enzymes found in the lysosome for drug release; whereas non-cleavable linkers do not allow for drug release from the mAb but are highly stable and minimize the early release of cytotoxic drugs. Current conjugation methods have been found to lack site-specificity, resulting in a significant variance in the DAR. This heterogeneity results in suboptimal ADC formulations that complicate DP processing of these anti-cancer treatments.

Opportunities for improving ADC production include efforts to improve their specific components, including the more efficient fermentative production and purification of antibodies. This includes the use of bispecifics where applicable, the synthesis of better cytotoxic drugs and the synthesis of better linkers for effective release of cytotoxic drugs. At the processing level, better conjugation chemistry and purification schemes, and advanced process monitoring and control can be used to minimize heterogeneity. Also, better process design and safety protocols can be critical to prevent the loss of cytotoxic drug containment.

2.1 Vision

This roadmapping effort aims to identify potential solutions to the production barriers associated with ADCs and bispecifics to achieve products of defined quality, with lower manufacturing costs and reduced development timelines. This has the ultimate goal of improving affordability and accessibility of these biotherapeutics to patients. It is envisaged that ADCs and bispecifics will, in the future, show a high degree of similarity with mAb manufacturing, including manufacturing facilities with smaller footprints and hybrid equipment with continuous, connected and batch processes employed as appropriate. The facilities will need to be flexible with the ability to change product quickly using plug-and-play equipment and consumables.

It is also envisaged that manufacturing process yields will continue to improve in both upstream and downstream, and appropriate testing and controls will be in place to improve productivity and eliminate mid-run failures. The effectiveness of DS processes will be improved with respect to both operability and predictability. The roadmap also envisions the utilization of robotics and artificial intelligence methods to decrease costs and, due to the highly toxic nature of the compounds used in ADC manufacture, provide physical separation from operators. Finally, clear guidance will be needed for the safe manufacturability of drugs taking into account strict safety guidelines.

2.2 Market trends and business drivers

The market trends affecting the biopharmaceutical industry have been comprehensively described by other roadmaps including BioPhorum's Biomanufacturing Technology Roadmap [2] and can generally be summarized in terms of four major trends: the continued growth of the biopharmaceutical market, increasing numbers of new product classes (including ADCs and bispecifics), rising cost pressures and the uncertainty of product approvals and sales.

Market growth

ADCs and bispecifics present powerful opportunities to treat human disease and are important business drivers for value in the biopharmaceutical sector. The successful approvals by the US Food and Drug Administration of two ADCs, brentuximab vedotin in 2011 and ado-trastuzumab emtansine in 2013, have shown exciting possibilities when incorporating ADCs into cancer therapy. Also, 2017 saw the market re-approval of Mylotarg (gemtuzumab ozogamicin) and the approval of Besponsa (inotuzumab ozogamicin), which further prove the value of ADCs for oncology therapy. More than 80 ADCs have entered clinical evaluation over the last 15 years and the number of clinical-stage candidates has increased year on year across all clinical stages (phases 1 through 3) as shown in Figures 1 and 2.

Figure 1: Number of ADC clinical trials initiated per year [3]

Year	No. of trials	% growth
2010	20	(+25%)
2011	29	(+45%)
2012	36	(+24%)
2013	49	(+36%)
2014	56	(+14%)
2015	62	(+11%)
2016	57	(-8%)
2017	70	(+23%)
2018	64	

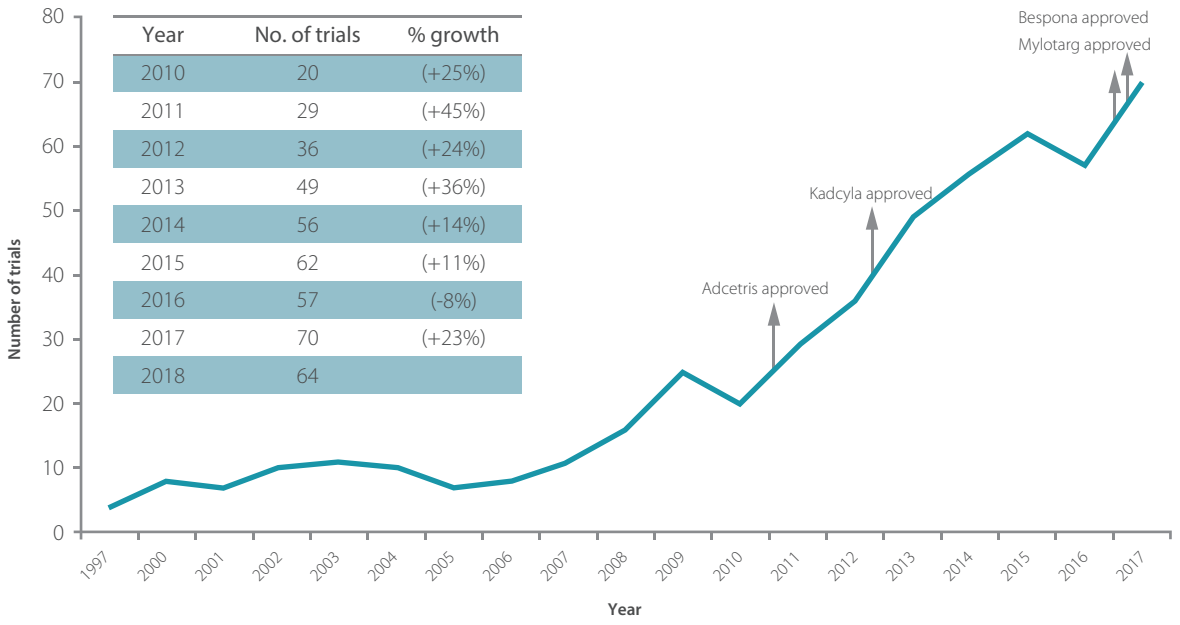
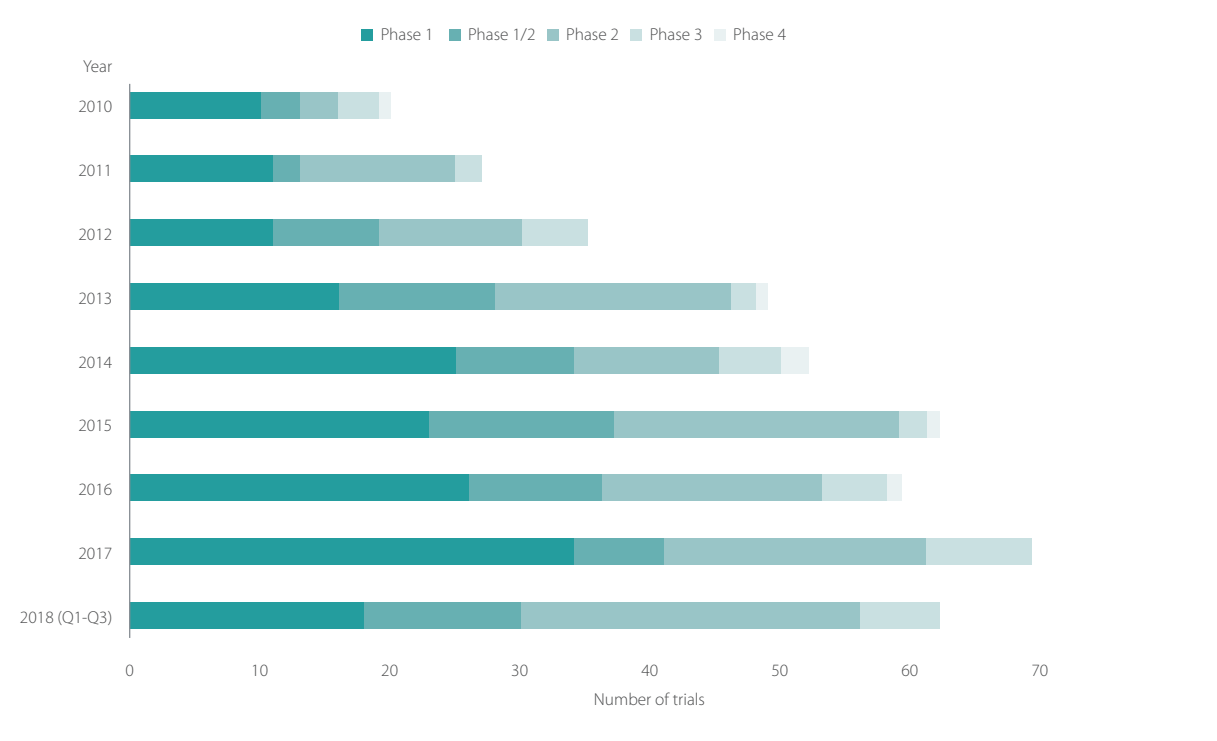


Figure 2: Number of ADC clinical trials initiated by phase per year [3]



Interest in bispecifics for therapeutic use has increased significantly in the last 10 years, especially after the approvals of two bispecifics, Catumaxomab in 2009 and Blinatumomab in 2014 in cancer therapy. So far, there have been more than 130 clinical trials of bispecifics in various disease areas and even diagnostics indicating bispecifics can play a key role in biologic therapeutic drug development in the near future.

Cost pressure

Pressures are increasing on healthcare costs while patient accessibility depends on affordability. ADCs and bispecifics are inherently more complex than traditional antibodies and more expensive to manufacture. To ensure the viability of these formats, cost-effective, consistent development efforts and manufacturing processes are required.

The stainless steel, fixed plants of the recent past are being retrofitted with new and single-use devices as they often lack the needed throughput and/or flexibility expected today. Newer plants are being developed that leverage more single-use technologies ultimately driving down capital expenditure and the time needed to build such facilities. Capital expenditure costs for these newer plants are currently in the region of $300m, while continued cost pressures are expected to drive those numbers down even further.

Operating expenditure is higher for these products due to smaller campaign demands in large facilities and, in the case of ADCs, containment requirements. Facilities that are flexible in scale (200–2000L reactors) to produce the right amount of drug needed at various stages of clinical development and commercialization are critical to reducing operating expenditure. Single-use systems that eliminate the need for cleaning and validation present a solution to lower operating expenditure and can provide additional safety in manufacturing these new formats. For ADCs, the efficiency of the conjugation process impacts on operating expenditure, therefore, highly specific conjugation sites are desired.

A general description of biopharmaceutical business drivers and their impact on market trends is outlined in BioPhorum's Biomanufacturing Technology Roadmap [2]. The drivers can be broadly summarized as speed, quality, cost reduction and facility flexibility. Given the toxic nature of the compounds used in ADC manufacture, this NIIMBL roadmap also considers environmental impact and process-related safety as important business drivers for the manufacture of these products. For each potential technology solution discussed in Section 3 an assessment has been made about the likely impact on key manufacturing business drivers.

2.3 Scope and links to other roadmaps

This roadmap assesses the current state of manufacture, as well as future technology and capability needs, relating specifically to ADCs and bispecifics and covers the following topic areas:

- DS
- DP
- analytics
- modeling
- regulatory science and standards
- workforce development.

Technology considerations relating to general mAb manufacture are described by BioPhorum's Biomanufacturing Technology Roadmap [2] and are therefore not included here.

3.0 Future needs, challenges and potential solutions

3.1 Drug substance

3.1.1 Molecular design and cell-line development

ADCs and bispecifics present different challenges in both molecule design and cell-line development. For ADCs, the molecule design is focused on linker designs, including non-specific and site-specific linkers. In the non-specific approach, the drug payload is conjugated to any free cysteine or lysine residues in the antibody, resulting in product heterogeneity that could impact product quality consistency from batch to batch. Alternatively, ADC manufacturing through site-specific mutagenesis, or incorporation of unnatural amino acids into the antibody, will generate a site for controlled and stable attachment of the drug. This approach improves product quality, homogeneity and improved product quality controls during manufacturing.

Cell-line development plays a significant role in creating bispecific molecules. Bispecific IgG molecules can be assembled from two different HCs and LCs expressed in the same producer cell. However, because of the random assembly of the different chains, this approach results in a substantial number of non-functional molecules with respect to bispecificity.

As mentioned earlier, even though these protein engineering approaches have significantly improved the efficiency of producing bispecific antibodies from mammalian cells, the production process yield is still much lower when compared to conventional mAb production. This is because some of the product-related impurities, such as homodimers or half-molecules, are present at significant levels due to the imbalanced expression of each polypeptide. Different approaches have been developed to improve the heterodimer ratio for the generation of

production clones at expression vector design and clone screening steps. Optimum vector design can control the expression level of each polypeptide at a transcription level, whereas effective clone screening is critical to identifying a robust clone with efficient protein assembly capacity to produce high levels of heterodimer product.

Since balanced expression is not required in conventional mAb expressions, most of the stable expression vector designs cannot resolve this issue. So far, the common strategy across the industry is to significantly increase the effort during cloning and clone screening to identify the most robust clone; i.e. one with the highest ratio of bispecific heterodimer and lowest amount of impurity species (homodimers or half-molecules). The caveat of this strategy is the workload and timeline increase significantly with unpredictable and potentially poor results. Usually, after screening hundreds of clones, the heterodimer is only around 60–80% of the secreted product.

See Table 1 for a summary of the needs, challenges and potential solutions relevant to molecular design and cell line development.

Table 1: Drug substance – molecular design and cell-line development – needs

		Current	3yrs	5yrs	10yrs	Impact
Need	Appropriate expression systems to ensure a high % hetero-oligomer (bispecifics)					Increased % heterodimer, manufacturing yield and efficacy
Challenge	Imbalanced expression of individual chain to product-related impurity					
Potential solution	Vector design; efficient clone screening					
		●	●	●	●	
Need	Efficient molecule design to enable unique combinations and maximize product quality and product yield					Increased % heterodimer, manufacturing yield, efficacy and safety
Challenge	Expression of four chains					
Potential solution	Protein engineering					
		●	●	●	●	
Disruptive technology	Protein engineering, (e.g. chimeric Fc; cell-free technology; alternative host)					
		●	●	●	●	
Need	Efficient purification schemes; efficient separation and quantitation analytics (non-Fc)					Increased product quality and yield, decreased CoGs, development time
Challenge	Remove product-related impurity species (e.g. high molecular weight, half-antibodies, homodimers); DP/DS stability; non-standard Protein A purification					
Potential solution	Downstream process development; effective in-process analytics; formulation development; cell-free system; alternative host					
		●	●	●	●	
Disruptive technology	Effective analytical method to distinguish individual species					
		●	●	●	●	
Need	Efficient expression system for targeted conjugation of ADCs					Increased product quality/consistency, robust process and decreased development time, CoGs
Challenge	Design of both linker sequence and cell/vector system; needs high yield and consistent quality					
Potential solution	Specific antibody site-engineering for drug conjugation					
		●	●	●	●	

Manufacturing Readiness Level

●	●	●
Research	Development	Manufacturing

3.1.2 Upstream

ADCs

As ADCs are formed through the linkage between an antibody and small-molecule drugs, upstream processes for producing ADCs are similar to those used for producing standard mAb products. Ample summaries can be found in literature reflecting the advancements and challenges in upstream process development. At a high level, current upstream process development is pursuing the goals of higher cell density, product titer and total productivity, and better control of quality attributes (e.g. aggregates, N-linked glycosylation profiles and trisulphide levels). Specific quality attributes for ADCs, such as the site of modification and the DAR, are also targets for improved control. Those goals are often achieved by process intensification and process analytical technology (PAT) tools. Notably, perfusion-based process intensification has been demonstrated and adopted by major biopharmaceutical companies to maximize facility output and improve efficiency.

Bispecifics

Bispecifics are expressed in commonly used hosts such as Chinese hamster ovary cell lines; however, expression is generally regarded as more challenging than standard mAbs. Production of bispecifics follows the typical biologics platforms, where fed-batch cultures are widely used. Due to their specific characteristics, traditional cell-culture processes may yield bispecifics with high levels of impurities and aggregates. The following are the needs in the upstream area for process improvement and product quality assurance.

Process analytical technology and product attribute control (PAC) tools for process monitoring/control and quality attributes control

Currently, the manufacturing of bispecifics still predominately relies on traditional batch cultures in stirred-tank bioreactors. Better monitoring and control of the cell growth, product secretion and product quality is needed. Additionally, to modulate the product-quality attributes in the production process, advanced PAC is desired. Upstream developments in PAT tools (e.g. Raman and near-infrared spectroscopies, biomass probes) in typical cell-culture operations are expected to migrate to the production of bispecifics. For example, Raman spectroscopy is relatively mature in providing reliable cell-culture performance (e.g. nutrients/metabolites, cell density, titer), monitoring and controlling, and is expected to be further developed for the real-time control of critical quality attributes (CQAs), such as aggregates and glycoforms.

Specific needs, such as monitoring impurities, could be fulfilled through the development of PAT tools. Advanced sensors for dynamic monitoring of critical culture parameters (e.g. pH and dissolved oxygen) in large-scale bioreactor operations could also be valuable in understanding environmental heterogeneities at a large scale. Soft sensors (where on-line sensor signals are combined with mathematical models) used to derive additional process information and deliver more complex process control are also desired to drive overall process robustness.

PAC tools are expected to improve bioreactor performance via the ability to control quality attributes through feedback loops based on process and product understanding. PAC is typically achieved through the advancement in PAT tools and improved process understanding where certain culture levers could be applied for modulating quality attributes. Attributes such as specific glycoforms have been demonstrated to be well controlled using PAC tools. It is expected that further advances in PAC tools will enable a better controlled product quality from the upstream process.

Process efficiency and cost of goods reduction for bispecifics

Improving process efficiencies and reducing CoGs are critical for the accessibility of medicines. Due to the impurities and purification challenges, the overall yield is relatively low for bispecifics when compared to a standard mAb process. Purification improvement could lead to significant overall yield improvement. From an upstream perspective, improving the overall bioreactor and harvest yields could also contribute to process efficiency. Technologies leading to bioreactor yield improvement are desired. Process intensification, such as perfusion at both seed and production stages, is expected to lead to such improvements. Additionally, perfusion processes are necessary for producing products prone to aggregation or oxidation due to long residence times.

See Table 2 for a summary of the needs, challenges and potential solutions relevant to bispecifics drug substance upstream manufacturing.

Table 2: Bispecifics – drug substance upstream – needs

		Current	3yrs	5yrs	10yrs	Impact
Need	Improved process monitoring and control					Improved process yield, process economics
Challenge	Increase bioreactor titer while controlling impurities; assembly of bispecifics					
Potential solution	PAT and PAC tools for better process control					
		🟡	🟢	🟢	🟢	
Disruptive technology	Innovative PAT tools for advanced process control and product modulation					
		🔴	🟡	🟢	🟢	
Need	Improve process efficiencies and reduce CoGs					Continuous purification, perfusion for bispecifics
Challenge	New facility design; integrated processing implementation (connecting continuous upstream with downstream for streamlined processing) for bispecifics					
Potential solution	Develop continuous processing and end-to-end platforms for bispecific mAbs					
		🟡	🟢	🟢	🟢	
Disruptive technology	Alternative host systems with improved productivity and desired quality (refer to Table 1)					
		🔴	🟡	🟡	🟢	

Manufacturing Readiness Level

🔴	🟡	🟢
Research	Development	Manufacturing

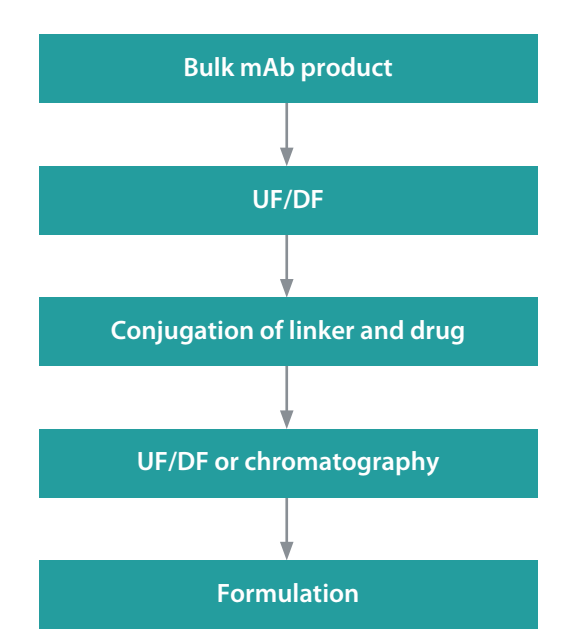
3.1.3 Downstream

ADCs

Monoclonal antibodies used in ADC production are typically manufactured according to traditional processes, including purification via protein A-platform processes. Figure 3 is an example workflow for manufacturing an ADC from a bulk mAb product [4]. The mAb is expressed in mammalian cells and purified by chromatography. The cytotoxic drug is conjugated onto the mAb through the linker in a solvent such as N, N-dimethylacetamide or dimethyl sulfoxide [4].

In addition to the site-specific, antibody-to-drug conjugation that is unique to each ADC platform, several aspects of conjugation conditions (such as the molar ratio of naked antibody to warhead, antibody concentration and conjugation times) are critical to reaching the final desired DAR. Following the conjugation reaction, another UF/DF and/or chromatography step is needed to remove the free cytotoxic and formulate the ADC into the final formulation.

Figure 3: Workflow for preparing an ADC from a bulk mAb [4]



The manufacturing of ADCs with mAbs and cytotoxin is a challenge. Because of the high-potent, small-molecule toxins, special handling is needed for each component. Single-use technology is well suited to ADC manufacturing because it minimizes operator exposure to toxins and in addition it does not require cleaning validation [4].

According to US Food and Drug Administration requirements, if a drug is occupationally potent (i.e. hazardous to operators) it needs to be present in the air at a level of not more than 10µg/m3 [5], which is about a 10th of the amount of dust that the eye can perceive in bright light. Although there is no regulatory definition of what makes a drug a highly potent API, it is essential to be cautious [5].

High potency APIs should be contained and powders handled within an isolator in a negative pressure environment to prevent the operator from being exposed to toxic materials. However, the final drug product should be handled in a positive pressure environment to prevent microbial and particle contamination. Post manufacturing, decontamination is vital and fully validated cleaning procedures must be implemented. Single-use and closed systems could address these issues and offer manufacturing advantages for ADC production [5].

See Table 3 for a summary of the needs, challenges and potential solutions relevant to ADC drug substance downstream manufacturing.

Table 3: ADCs – drug substance downstream – needs

		Current	3yrs	5yrs	10yrs	Impact
Need	Automation/disposable/PAT					Minimized risk of operator exposure to toxic compounds
Challenge	Handling highly potent compounds					
Potential solution	Single-use technology; high throughput process development					
		●	●	●	●	
Disruptive technology	Single-use technology; non-solvent-based conjugation					
		●	●	●	●	
Need	Site-specific conjugation with non-natural amino acid					Improved stability and improved precision of control payload
Challenge	Payload control; conjugation efficiency					
Potential solution	Molecular design; synthetic system, e.g. cell-free					
		●	●	●	●	

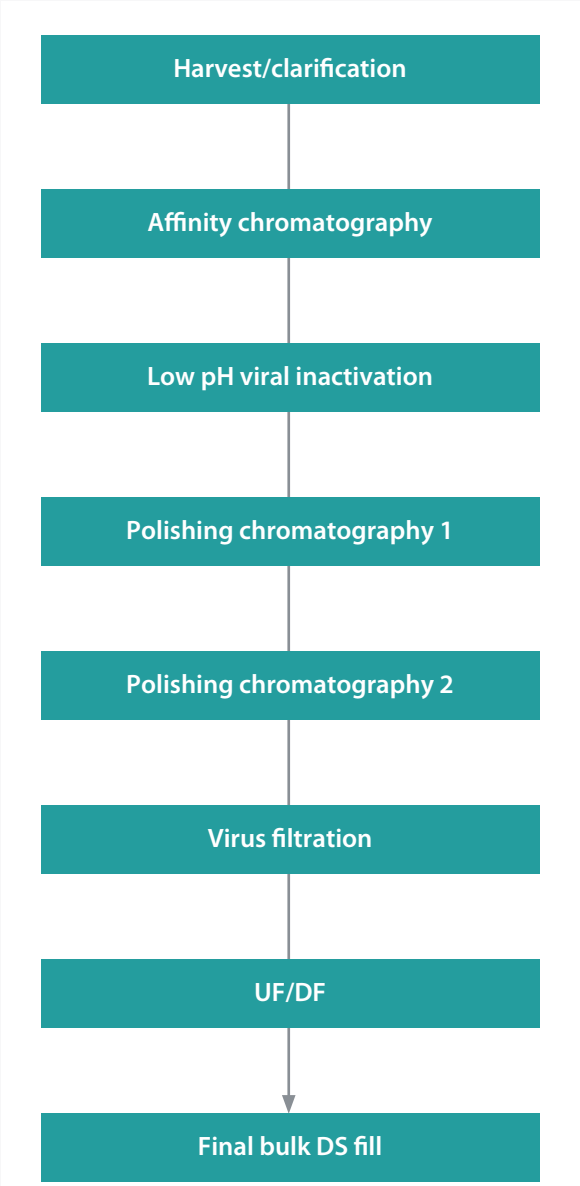
Manufacturing Readiness Level

●	●	●
Research	Development	Manufacturing

Bispecifics

The majority of bispecific antibodies are still produced in mammalian cell lines to reduce the risk of immunogenicity due to non-human glycosylation patterns. Centrifugation and/or depth filtration can be used for primary and secondary clarification steps. Depth filtration has also been shown to assist with the removal of impurities, such as host cell protein and DNA, and improve downstream filter and column capacities [7]. During large-scale, cell-culture processes (>2,000L), centrifugation is frequently used to isolate cells from their culture supernatant. If cell densities increase, the use of flocculation polymers and acid precipitation are becoming useful tools for clarification during the harvest step [6]. Figure 4 is an example downstream workflow for purifying a bispecific from a cell culture fluid.

Figure 4: Typical Downstream flow diagram for purifying a bispecific [8]



The primary challenges in bispecific antibody production include chemical manufacturing control issues, production yield, homogeneity and purity. Full-length bispecifics with the Fc region can be captured using a Protein A affinity column, as shown in Figure 4. As for traditional mAb purification, the HCP and DNA will be reduced to a level that is enough to perform the *in vitro* generation of the bispecific molecule. The product-related impurities will be further reduced by subsequent polishing column steps, e.g. ion exchange (IEX), hydrophobic interaction chromatography (HIC) or mixed-mode chromatography [8].

Capture of bispecific antibody molecules that do not contain the Fc region has been achieved using non-protein A-affinity chromatography. Some bispecifics of this type are engineered with a histidine tag that allows the use of immobilized, metal-affinity chromatography for the initial chromatography step. Other small, bispecific antibody molecules containing the variable region of the kappa LC can be captured using Protein L-affinity chromatography [9].

Aggregation and degradation may also occur at various stages of the bispecific antibody expression/purification through to the final DS formulation. IEX, HIC or mixed-mode columns are often able to remove these product-related impurities.

The final UF/DF (formulation) steps in bispecific-antibody processes can present unique challenges if a high concentration of drug substance is needed. Some new products, such as EMD Millipore's new 'D' screen device, allow for a successful formulation step while remaining within a customer's designated manufacturing process pressure range [8]. Polyethylene sulfate or cellulosic-based membranes are often used at this step for their low binding characteristics [8].

See Table 4 for a summary of the needs, challenges and potential solutions relevant to bispecifics drug substance downstream manufacturing.

Table 4: Bispecifics – drug substance downstream – needs

		Current	3yrs	5yrs	10yrs	Impact
Need	Improved technology for bispecific separation					Minimized process development cycle and reduced speed to market
Challenge	High process- and product-related impurities; low overall step yield					
Potential solution	High throughput technologies to speed up the process development cycle					
		🟡	🟢	🟢	🟢	
	Salt-tolerant membrane/resin chromatography with a high capacity and resolution; new tangential flow filtration (TFF) products, such as a high-performance TFF that has some degree of selectivity to remove impurities					Improved yield and cost
		🟡	🟢	🟢	🟢	
Disruptive technology	Multicolumn countercurrent solvent gradient purification [9]					Improved yield and purity
		🔴	🟡	🟢	🟢	
Need	Improve product yield and reduce cost					Improved yield and cost
Challenge	Reduce aggregate formation during in-process					
Potential solution	Continuous manufacturing to minimize in-process hold time					
		🟡	🟢	🟢	🟢	
Disruptive technology	New stabilizers					
		🔴	🟡	🟢	🟢	

Manufacturing Readiness Level

🔴	🟡	🟢
Research	Development	Manufacturing

Considerations for manufacturing facilities

Facilities making ADCs and bispecific antibodies share many similarities with mAb facilities. This means it should be relatively easy to adapt mAb facilities to make bispecific antibodies; however, this is not the case for ADCs. The reason for this is twofold: the need for chemical containment and the wider use of solvents. Looking to the future, BioPhorum's Biomanufacturing Technology Roadmap [2] will cover many of the facility needs for these two product classes, e.g. the introduction of continuous, high-titer processes; however, ADCs do add some specific needs as described in table 5.

Table 5: ADC facility – needs

		Current	3yrs	5yrs	10yrs	Impact
Need	Due to the high toxicity of the product, there is an increased need to reduce human contact with the process on safety grounds					Improved safety, reduced cost
Challenge	Undertake chemistry without human exposure					
Potential solution	Develop autonomous robotics, static robotics and automation solutions to provide continuous/connected processes and leave the system to operate itself					
		●	●	●	●	
Disruptive technologies	Robotics, artificial intelligence and sensor/visual systems					
		●	●	●	●	
	Develop cartridges that can be inserted by robots					
		●	●	●	●	
Need	To attract more CMOs into the ADC market					Reduced speed to market, reduced cost
Challenge	Containment is expensive requiring specialized facilities. A combination of CMOs is needed to manufacture the products					
Potential solution	Reduce the cost of containment through plug-and-play to minimize facility-fit challenges, process validation, training					
		●	●	●	●	
Disruptive technology	Robotics, miniaturization and plug-and-play software					
		●	●	●	●	

Manufacturing Readiness Level

● Research ● Development ● Manufacturing

3.2 Drug product

The formulation, DP attributes and requirements for ADCs are unique because they combine the small-molecule degradation liabilities (i.e. oxidation, hydrolysis and reactivity) of the cytotoxic payload with the macromolecule instabilities of mAbs and bispecifics. The addition of the small-molecule payload to the mAb increases the inherent instability of the mAb, making the ADC more prone to aggregation compared to the mAb alone. Due to these instabilities, all commercial ADCs are currently manufactured as lyophilized DPs to assure minimal degradation during shelf-life storage and distribution. Stability, storage container, temperature and manufacturing scale must be aligned between the DS and DP CMOs, if different. Depending on the potency of the warhead, the formulation concentrations can range from 1–20mg/mL and may require between 1–50mL of DP volume, to be diluted in an IV bag and administered via IV infusion.

The high potency and toxicity of the small-drug payload require special handling and containment, limiting the number of facilities that are capable of manufacturing, lyophilization and final packaging and storage, thereby increasing the cost and time to market. The cytotoxic payloads are often hydrophobic, which make it difficult to reconstitute lyophilized powders and increases the potential for protein aggregation. Alternative DP formulation excipients are currently of limited benefit with respect to stabilization of ADCs, partly due to the poor chemical stability and reactivity of payloads, and the ability of traditional excipients to stabilize only the protein component of the ADC. Additionally, the safety challenges of a commercial liquid vial fill step at a large scale are not easily addressed.

Developing an alternate dosage form for ADCs (e.g. spray-dried powder) is also difficult because of the containment challenges when spray drying and vial filling a toxic substance. Furthermore, the poor stability of the ADC combined with high storage volumes in the form of low concentration liquids pose additional challenges for storage and container selection for commercial manufacturing. More facilities that can handle high potency, toxic DPs and excipients that can increase solubility and/or stability are needed. Due to the narrow therapeutic window of ADCs, IV infusion is currently the only option for delivery to the patient, reducing patient satisfaction and the ease of delivery. Even a modest loss of protein in the IV bag can lead to variable dosing. Improved construction materials for IV bags will reduce binding to the bag and improve reproducibility of drug dosing. High-potency bispecific molecules and products will have similar manufacturing and delivery issues as ADCs.

By increasing stability with new excipients and/or more soluble payloads, then developing higher concentration products could be achieved. This would be beneficial for ADC DP manufacturing to better optimize scale, storage requirements and DP batch sizes and reduce the CoGs. These lower-volume liquid formulations could lead to easier and more efficient dose preparation by the healthcare administrator and potentially reduced infusion times/volumes during administration.

See Table 6 for a summary of the needs, challenges and potential solutions relevant to ADC and bispecifics drug product.

Table 6: Drug product – needs

		Current	3yrs	5yrs	10yrs	Impact
Need	Due to the high toxicity of the DP, there are only a limited number of CMOs that can handle the cytotoxics					Reduced CoGs, improved speed to market, continuous processing
Challenge	Increase the number of CMOs in this space that can safely handle toxic substances					
Potential solution	Increase the number of CMOs in this space					
		🟡	🟡	🟡	🟢	
Disruptive technology	Develop new technology for freeze drying: more scalable, in-line, continuous instead of batch (e.g. spray drying). Currently, no one is spray drying cytotoxic compounds due to safety concerns					
		🔴	🔴	🟡	🟢	
Need	Improved solubility and hydrophilicity					Improved speed to market, reduced capital expenditure, improved end-user acceptance through easier dose preparation in a clinical setting, reduced environmental impact (reduced solvents with more soluble drugs)
Challenge	Reduce instability of hydrophobic drugs with effective reconstitution					
Potential solution	More stable and soluable payloads (design of warheads)					
		🔴	🟡	🟢	🟢	
	Develop excipients to prevent drug-drug interactions, particularly related to cytotoxic payload					
		🔴	🔴	🟡	🟢	
Disruptive technologies	Screen technology for warheads and drugs, particularly in a solid state					
		🔴	🟡	🟢	🟢	
	New manufacturing technology that supports stability (e.g. spray drying)					
		🔴	🔴	🟡	🟢	
	New excipients to protect warheads in the lyophilized state through reconstitution; keep soluble, reduce drug-drug interactions					
		🔴	🔴	🟡	🟢	
Need	Improve delivery to patient in IV bag (ADC and bispecific non-Fc)					Improved patient and pharmacist acceptance through simpler dose preparation and administration procedures
Challenge	Low concentration and poor compatibility in IV bag leads to instability and adsorption					
Potential solution	Improve material of construction for IV bag (i.e. less sticky); reduce drug adsorption to and aggregation in bags					
		🔴	🔴	🟡	🟢	
Disruptive technologies	Manufacture bags from new materials					
		🔴	🔴	🟡	🟢	
	New excipients to reduce drug adsorption and aggregation (reduce the amount of surfactant in IV bag)					
		🔴	🔴	🟡	🟢	
	Delivery via a wearable IV or subcutaneous infusion device; requires formulation for subcutaneous administration and suitable device					
		🔴	🔴	🟡	🟢	

Manufacturing Readiness Level

🔴	🟡	🟢
Research	Development	Manufacturing

Table 6: Drug product – needs (continued)

		Current	3yrs	5yrs	10yrs	Impact
Need	Enable liquid formulation (ADC and non-Fc bispecific)					Easier and safer dose preparation by a healthcare provider, improved safety of ADC by reduced aggregation, reduced CoGs
Challenge	Reduce aggregation and chemical degradation (e.g. oxidation, reactivity of payload, hydrolysis, acid/base); reduce the volume of low-concentration drugs; need to increase protein concentration					
Potential solution	More stable payloads (design of warheads)					
		●	●	●	●	
	Better excipients					
		●	●	●	●	
Disruptive technologies	New manufacturing technology (e.g. materials of construction, pumps, reduction of shear) that supports stability					
		●	●	●	●	
	Rational design of excipients that do not interact with the payload					
		●	●	●	●	
Need	Be able to safely work with toxic ADCs during development					Reduced environmental impact, increased speed to market
Challenge	Safe, surrogate ADCs for process development and formulation R&D					
Potential solution	Synthesize small molecules with similar physical and chemical properties to toxic molecule without toxic effects					
		●	●	●	●	
Disruptive technology	Computational modeling of toxic drugs to design non-toxic surrogates					
		●	●	●	●	
Need	Ability to handle toxic waste for rejected lots					Reduced environmental impact, reduced CoGs
Challenge	Handling and disposal of cytotoxic waste					
Potential solution	Design cytotoxic compounds that can be safely and easily neutralized (e.g. chemically deactivated)					
		●	●	●	●	
Disruptive technologies	Build universal chemical inactivation trigger into the molecule					
		●	●	●	●	
	Process engineering component; intelligent design to build in inactivation stream (e.g. heat, neutralization, etc.)					
		●	●	●	●	

Manufacturing Readiness Level

●	●	●
Research	Development	Manufacturing

3.3 Analytics

Analytical development and GXP testing are essential components of biologics process development, and clinical and commercial manufacturing. Analytical development focuses on developing appropriate assays to support the manufacturing of DS and DP, while GXP testing provides assurance of DS and DP manufacturing robustness and product quality. The GXP testing is less variable and mainly depends on the achievements of analytical development; thus, it is not covered in this roadmap.

It should be emphasized molecular design, process capability and analytical support work hand-in-hand with process development for biologics. This is even more so for ADCs and bispecifics due to their increased complexity of molecular structures, manufacturing processes and quality control measures compared to mAb drugs. It always requires a concerted effort involving all functional areas to develop a highly efficient manufacturing process with an appropriate control strategy. For example, the design of more homogeneous mAbs and more hydrophilic ADCs can significantly lessen the burden of downstream processing steps, and will also reduce the complexity of molecular variants and lower the hurdles for method development. In the meantime, information obtained during process and analytical development can, in turn, provide feedback to guide molecular designs to optimize drug candidates entering the drug development pipelines.

Similar to biologics development, the analytical development of ADCs and bispecifics is centered around identifying CQAs for these molecules, followed by establishing appropriate process control strategies including developing suitable analytical methods for release testing, stability monitoring and characterization. The challenge is the list of CQAs for these areas is quite different compared to standard mAbs due to the nature of the molecule design as mentioned previously, i.e. homodimer, half molecules. Developing appropriate assays to identify these specific impurity species is not only critical to release and characterization of the DS or DP, but is also important to assist in the development of robust processes during manufacturing. In addition, the impact of the major impurities on drug safety and efficacy needs to be thoroughly evaluated. An appropriate control strategy needs to be established to ensure manufacturing consistency and to properly control product-related impurities.

Based on the CQA assignment and corresponding analytical development activities required, the following have been identified as the challenges/gaps facing analytics for ADCs and bispecifics:

- the molecular variants resulting from the incorrect pairing of HCs and LCs in bispecifics, i.e. homodimers, half molecules, cross-pairing
- DAR measurement and control
- Charge profiles of APIs
- identifying and quantifying high molecular weight and low molecular weight species, especially those barely separated from the monomer by size exclusion chromatography (SEC) and/or capillary electrophoresis sodium dodecyl sulfate (CE-SDS)
- the structure-function relationship for ADCs and bispecifics
- degradation pathway identification for ADCs and bispecifics
- the dynamic secondary, tertiary and quaternary structures of ADCs and bispecifics, and their impact on efficacy, safety and stability.

See Table 7 for a summary of the needs, challenges and potential solutions relevant to ADC and bispecific analytics.

Table 7: Analytical – needs

		Current	3yrs	5yrs	10yrs	Impact
Need	Characterization and quantification of poorly separated API and other major impurity species, i.e. homodimer, half molecule, high molecular weight and low molecular weight species					Reduced timeline and cost
Challenge	Current/conventional separation techniques (e.g. SEC, CE-SDS) may not provide adequate size variant separation for ADCs and bispecifics					
Potential solution	New separation modes/technologies; simpler chromatography profiles resulting from enhanced molecular design, improved stability, site-specific conjugation and HC-LC pairing					
		●	●	●	●	
Disruptive technology	Rational molecular design; protein engineering and appropriate expression system design					
		●	●	●	●	
Need	Robust and simplified analytical approach for charge variants characterization, quantification and control					Reduced timeline and cost
Challenge	The complexity of molecular variants from ADCs and bispecifics is significantly higher than traditional mAbs which challenges characterization, method development and process control strategies					
Potential solution	Reducing molecular heterogeneity through optimized molecular design, (e.g. site-specific conjugation for ADCs and specific HC-LC pairing for bispecifics) Improving analytical performance through high-resolution separation techniques and have a better understanding of conjugation and labeling chemistry					
		●	●	●	●	
Disruptive technology	Optimized platforms that generate more homogeneous ADCs and bispecifics					
		●	●	●	●	
Need	Understanding of structure-function relationship for ADCs and bispecifics, and their degradation pathways for enhanced quality control					Reduced timeline and cost
Challenge	Limited analytical capability in characterizing and quantifying molecular variants, especially in isolating molecular variants for structure-function relationship studies. Technical difficulties in developing appropriate cell-based bioassays that mimic the biological functions of bispecifics and are capable of differentiating bispecifics from combination therapies					
Potential solution	More learning and experience in molecular design, process control and dynamics, and advanced analytical capabilities					
		●	●	●	●	
Disruptive technology	New ADC and bispecific platforms and innovative analytical tools					
		●	●	●	●	

Manufacturing Readiness Level

● Research ● Development ● Manufacturing

Table 7: Analytical – needs (continued)

		Current	3yrs	5yrs	10yrs	Impact
Need	More reliable and quality control-friendly analytical methods for DAR determination					Reduced timeline and cost, more robust process
Challenge	Current analytical methods based on dual-wavelength UV absorption, HIC separation or mass spectrometry, all have limitations. The most challenging ones are non-specific conjugations at primary amine sites leading to random payload attachment					
Potential solution	Technologically, liquid chromatography-mass spectrometry may face the lowest hurdle in terms of establishing a platform approach suitable for DAR measurement for most ADCs. The issue can be effectively tackled by rational molecular design, e.g. if the conjugation is specific, or partially specific, the heterogeneity can be reduced significantly, which translates to significantly simplified analytical methods. Nonetheless, at the present time orthogonal analytical methods are needed to support characterization and release					
		●	●	●	●	
Disruptive technology	A quality control-friendly, mass spectrometry-based analytical technology or a conjugation process that significantly simplifies conjugation chemistry and product heterogeneity					
		●	●	●	●	
Need	Better understanding of the dynamic secondary, tertiary and quaternary structures of ADCs and bispecifics, their impact on efficacy and safety, and their respective quality control					Reduced timeline and cost, more robust process
Challenge	Limited studies in this area due to lack of access to ADCs by academic institutions					
Potential solution	More collaboration between research institutions and industry					
Disruptive technology	Combination techniques such as nuclear magnetic resonance, optical spectroscopy or mass spectrometry may provide critical information and analytical capabilities					
		●	●	●	●	
Need	Quantification of mispaired components in bispecifics					Reduced timeline and cost, improved product quality
Challenge	The characterization and quantification of mispaired HC and LC components can be very challenging for certain molecular platforms					
Potential solution	Optimized molecular design facilitating intended pairing vs. random pairing, such as a knob-in-hole approach					
		●	●	●	●	
Disruptive technology	Advanced mass spectrometry or chromatography methods to separate and quantify mispaired species					
		●	●	●	●	

Manufacturing Readiness Level

● Research ● Development ● Manufacturing

3.4 Modeling

The investigation of ADCs and bispecific antibodies has, to this point, been mostly focused on the experimental discovery of new products and the associated evaluation of their efficacy. As these novel platforms gain momentum and show their wide applicability across various purposes, more quantitative methods are needed to improve the understanding of new product developments, improve the success of clinical studies and facilitate the cost-effective, large-scale production after clinical trial completion. This section discusses the modeling needs and challenges associated with the modeling of ADCs and bispecifics.

Modeling of bispecifics

Current modeling needs in the area of bispecific antibodies range from new product development tools at the atomistic level to novel process development at the unit operation level. Efforts in this area should focus on developing novel bispecifics for a variety of applications, including a wide variety of multifactorial health problems with many signaling pathways implicated in pathogenesis. Facilitating the cost-effective manufacture of these and existing products to maximize their availability and affordability should be a focus.

However, due to the variable nature of bispecific antibody composition, specifically the asymmetric pairing of HCs and LCs, the production of these products is a challenge. Additional work is needed at the atomistic level using computational methods in predictive protein engineering to determine the structure of new bispecifics for specific applications. Developing novel bispecific structures that can be easily produced using current manufacturing practices for mAbs will increase their speed to market.

Furthermore, atomistic studies focusing on the interaction between specific bispecific structures and their target pathogens can be instrumental in understanding the side effects of treatment and help elucidate dosage requirements that can inform product finishing strategies in a manufacturing environment.

Current investigations on the manufacture of bispecific antibodies have been experimental and focus on the development of novel cell lines to produce specific types of products. Even with advanced bispecific designs such as knob-in-hole, charge pairing and other engineered modalities, non-bispecific impurities resulting from mis-pairing of HCs and LCs still cannot be avoided, which can lead to increased costs and a higher risk of contamination. As such, gaining an understanding of new vectors for bispecific antibody production that can guarantee a high selectivity for the desired chain pairing in a single production step is critical to the success of the bispecific platform. Additionally, mathematical models characterizing bispecific production at the cellular level can lead to discovering advances in existing production methods that minimize impurity levels while maximizing product selectivity and titers.

Beyond the challenges at the cellular level, modeling can also be utilized to overcome manufacturing challenges at the unit operation level. First-principles models describing the culture kinetics of currently available or novel cell lines that produce mAbs can be used to gain a deeper understanding of the effect of various reactor parameters on production rates. Moreover, optimizing the reactor parameters that influence stable bispecific production in batch, fed-batch and continuous strategies can elucidate cost-effective solutions to manufacturing challenges at the laboratory scale.

Furthermore, scale-up studies examining the effects of parameters that depend on reactor scale are needed to develop a high level of process knowledge at scales applicable to current market needs. After the development of reliable production methods, process control and fault detection strategies can be developed that take advantage of process models and real-time data collection to ensure product quality, minimize contamination concerns and mitigate the effect of production disturbances on production levels.

In conjunction with improved upstream production methods, models describing current and novel downstream separation and finishing methods need to be explored. Understanding the adsorption/desorption kinetics for various chromatography resins can help identify key parameters of chromatography and facilitate improved methods for antibody purification. Moreover, first-principles models can be developed for crystallization that take advantage of the properties associated with the bispecific antibody structure.

Process intensification strategies that utilize the combined modeling knowledge of bispecific production and purification can be investigated to further reduce the CoGs. Product characterization models capable of predicting the stability of bispecific products during lyophilization and formulation steps can elucidate novel product formulations that maximize the shelf-life of these products. Further development and understanding of reliable continuous strategies that facilitate downstream separation, purification and finishing are critical to reducing the costs of bispecific production.

Modeling of ADCs

The effective use of fundamental modeling has the potential to revolutionize the manufacture of ADCs. Similar to bispecific antibody production, modeling strategies are needed at all scales of product development, from atomistic studies to process-level understanding, to drive cost-effective manufacturing. ADCs are composed of three sections including the antibody, the linker and the drug of interest, each of which influences the efficacy, stability and manufacturability of the product.

Models that can facilitate the decisions between using highly stable, non-cleavable linkers and cleavable linkers using hydrolytic enzymes in lysosome to release cytotoxic drugs can minimize the early release of these drugs while maximizing the effectiveness of the ADC product. Models that can predict the cytotoxicity of available drugs and can predict improved linkers to effectively release identified drugs when conjugated with an antibody can lead to more effective drug-conjugate products. Moreover, models capable of determining the optimal conjugation chemistry of these three components that can minimize heterogeneity, optimize drug loading, guarantee effective drug release and minimize production costs are of great interest.

Knowledge of the various combinations of antibody, linker and drug can influence the design of the large-scale processes needed for their cost-effective production. Current challenges in this area include a lack of site specificity for current conjugation methods, the significant variance of DAR and the formation of suboptimal heterogeneous ADC products. Reactor-scale studies investigating the optimal methods of combining the key pieces of ADCs must show the preferred site-specificity of the linker and drug, as well as achieve the desired DAR to maximize the efficacy of the product.

Once correctly configured, the ADCs with the desired structure must be purified from the bulk-reactor effluent in a cost-effective and efficient manner. Models that can accurately link the performance of these unit operations to specific performance criteria, such as increased titer or separation efficiency, can be used to design novel processes, study the optimal performance of these processes and apply state-of-the-art control methodologies to ensure stable operations. Finally, models that can predict the efficiency and stability of product-finishing techniques for ADCs can be used alongside the other models mentioned in this section to develop an overall CoGs analysis to gain an understanding of the economic bottlenecks of the ADC production process.

ADC and bispecific antibody process and cost modeling will require coordinated efforts to map the process space. It would be beneficial to solicit projects that would look at various scenarios for these modalities and compare/contrast them on process efficiency and CoGs bases. For example, for ADCs it was found that various linker chemistries and conjugation strategies could cause significant variations in process efficiency and yield [10]. Facility utilization and capital costs for ADCs, as well as additional purification strategies for bispecifics, can also cause significant variations. Also, safety concerns for potent toxins used in ADC manufacturing place significant burdens on the manufacturers to design appropriate facilities with significant benefits from closed-processing and single-use systems. Novel work recently published shows continuous processing for ADC production is also feasible [11].

The impact of these variations on facility design, logistics, labor utilization and CoGs has not been adequately addressed in the industry. Being able to model these various scenarios will assist end-users in making informed decisions about the path towards implementation. The scenarios described in the following paragraphs could be used as a good start in this direction.

For both ADCs and bispecifics, it would be useful to establish some typical baseline processes and use them as the basis for a process economic evaluation. By careful selection of 'typical' processes, together with defining the sensitivities around key parameters, an understanding of the variations of manufacturing costs can be established as a function of scale. This becomes a baseline reference whose objectives are to understand the key contributors to process costs and to use this understanding to assess the potential impact process improvement strategies may have on manufacturing costs.

In the case of ADCs, there would be a better understanding of the relative contributions of mAb, linker, toxin and conjugation to the overall cost as well as an assessment of how these relative contributions change with scale. This information would provide insights into the factors that are currently important and provide guidance on where to focus future efforts. Further approaches to future technology/process choices can be developed by ranking the most favorable in terms of potential benefits over the 3, 5 and 10-year periods. For bispecifics, it is more important to assess the impact of the different product classes, the degree to which they can fit into existing platforms and the cost of not being able to do so.

In the case of bispecifics, it is important to map a range of 'typical' processes to understand the variability in processing options and economics, then determine the baseline and the range of cost outcomes and their drivers. If this can link to the factors that drive the use of non-standard mAb purification routes then, for the baseline, one can quantify the manufacturing cost penalties for deviating from a standard mAb platform and the extent of that deviation.

Also, it may be possible to determine those product characteristics that enable the product to fit a standard platform. This would then feed into the 3-, 5- and 10-year goals where not only the potential of new technologies could be assessed, but it could also evaluate the potential of a standardized, flexible/configurable manufacturing approach for bispecifics.

There is already a strong foundation for CoGs modeling for the production of traditional mAbs, including newer efforts that have begun to explore issues involving single-use and continuous processing. There are certainly opportunities to refine/improve these efforts to focus on the need to understand the unique issues associated with bispecifics and ADCs and how these can be best addressed by modeling efforts. Some of the examples of these unique issues, where there is a need for the development of CoGs models for both bispecifics and ADCs, include:

- **product stability:** there are likely to be significant issues with the stability of both bispecifics and ADCs that may be very different than those of traditional mAb products. These include aggregation, fragmentation, chemical modification, novel formulations. There is a need for models to understand how the molecular structure affects stability and how this might impact processing (e.g. the need to eliminate hold-times, which would tend to drive towards continuous processing)
- **ADCs**: there is a need to reduce free drug/linker to very low concentrations. This is not a required step in mAb processing but is likely to be critical in the production of ADCs. CoGs models are needed to evaluate the technology options relating to issues associated with the very high toxicity of the free drug (in most applications)
- **variant removal:** although this is an issue for all products, it may be a particular challenge when it comes to bispecifics and ADCs given the nature of the variants (which will likely depend, at least in part, on the details of the processes). Developing CoGs models that allow one to compare different process options could be critical in this area.

As for mAbs, the product-related immunogenicity of bispecific molecules can be sequence-, structure- and target-related. The immunogenicity risk of a bispecific mAb cannot be higher than the conventional mAb unless a foreign sequence or molecular structure is introduced into the bispecific molecule, or the synergy of the dual targets has the potential to activate the immune system. Process-related immunogenicity risks need to be managed and controlled during the manufacturing process of bispecifics. Currently, there is no *in vitro* model or animal study that can accurately predict immunogenicity in humans.

3.5 Regulatory science and standards

To date, four ADCs have been approved in the US. The approval of these molecules has paved the way for the clinical development and evaluation of other ADCs for oncology indications, including the treatment of solid tumors. The new generation of ADCs is aimed at not only oncology targets but also non-oncology indications. There are constant efforts to optimize the target antigen, alternative scaffolds and new payloads to improve drug efficacy and reduce cytotoxicity. Meanwhile, the selection and engineering of antibodies for site-specific drug conjugation, which will result in decreased DARs with a higher homogeneity and increased stability while preventing potential aggregations, are priorities in ADC-manufacturing research.

Biologics, including ADCs, bi- or multi-specific antibodies and T-cell-based approaches are rapidly changing the landscape of biotherapeutics, especially cancer therapeutics. Currently, there are more than 30 different bispecific formats being tested clinically or preclinically including BiTEs, dual-affinity re-targeting antibodies, knob-in-hole and trifunctional bispecifics in both liquid and solid tumors. The future design and format of bispecifics are likely to incorporate multiple functionalities to target two or more tumor antigens along with bringing both T-cells and accessory cells to the immunological synapse. Although substantial manufacturing improvements have been made to produce bispecific antibodies from mammalian cells, high-yielding and cost-effective manufacturing are challenging as described previously in Section 3.1.

The regulatory principles for the development of novel antibody-related products have previously been described by Zhou and Shapiro in an American Pharmaceutical Review article published in 2016 [12] from which the following text has been adapted.

The principles for regulating the quality of biologics including ADC and bispecific products are largely dependent on the understanding of the structure, function, manufacturing process and control strategy of the products. Typical quality attributes for biologics include intrinsic (product-related) and extrinsic (process-related) characteristics.

The product-related characteristics include, but are not limited to, color, clarity, osmolarity, visible and sub-visible particulates, pH, product concentration, potency, size variants (aggregates and fragments), charge variants (acidic, main and basic isoforms), glycosylation, oxidation, deamidation, free thiol, primary amino acid sequences, and secondary and tertiary structures.

In general, the process-related characteristics for a mAb include, but are not limited to, HCP, host cell DNA, residual Protein A (if Protein A chromatography is used in the purification process), cell culture-medium components, purification-buffer components, selective agents (if used in production), and viral and microbiological purity.

Based on the intrinsic and extrinsic characteristics, the general regulatory considerations are to control potential contamination in the bioprocess, and to minimize process (e.g. HCP, host DNA, methotrexate, soy hydrolysate, recombinant insulin) and product-related impurities (e.g. size, charge and glycoform variants), all of which could potentially impede clinical outcomes (e.g. efficacy, pharmacokinetic/pharmacodynamic (PK/PD), immunogenicity, allergenicity, adverse events). For ADC and bispecific molecules, the general regulatory principles regarding product- and process-related characteristics apply; however, specific considerations are necessary due to the unique characteristics of these molecules.

Antibody-drug conjugates

The conjugation of mAbs with cytotoxic entities or radioactive isotopes is aimed at enhancing the efficacy of mAb- based therapy. Antibody conjugation can be generated via natural (lysine or cysteine) or engineered amino acid residues (e.g. site-specific engineered cysteine, non-natural amino acids, aldehyde tagging), carbohydrates, and small-molecule or peptide linkers. The structure of an ADC is complex and consists of three components: the naked antibody, the linker and the drug. Therefore, the regulatory considerations for ADCs are derived from the chemistry, manufacturing and control (CMC) considerations associated with the individual components (mAb, linker and drug) and the ADC DS and DP as a whole molecule. Generally, the CMC expectations for the mAb intermediate are the same as those for a typical mAb DS.

In addition to the control of the identity, purity, potency and stability of the individual components, other unique characteristics that could contribute to the safety and efficacy of the ADC (including drug to antibody ratio (DAR), free drug/mAb and potential conjugation sites), should be characterized throughout development and might need to be controlled for release and stability. The CQAs of the DP should be related back to the intermediates so appropriate control strategies, including end-of-shelf-life criteria, can be established.

For example, the small molecule drug is typically hydrophobic and may increase mAb aggregates once conjugated. Therefore, the release and end-of-shelf-life criteria for aggregates of the mAb intermediate and DS should be narrower to support DP release and end-of-shelf-life criteria where the data shows increases over the expiry period for each. The impact of conjugation on the mAb, such as potential interference with its binding to target and Fc receptors, and possible changes in purity (e.g. size and charge variants), should be assessed during development and controlled as needed.

With respect to developing an appropriate method to control potency, multiple assays may be needed to ensure that all aspects of the mechanism of action (MOA) are properly controlled. The small-molecule impurity profile is important as some may have their own toxicities. It should also be determined if these impurities are conjugatable or non-conjugatable.

Bispecifics

Bispecific manufacturing processes can be challenging, especially when there is a need to isolate the desired bispecific product from all the possible variants. For those bispecifics targeting two soluble antigens, simultaneous binding may not be necessary. Bispecific molecules vary in their design and structurally, can be categorized into five major classes: IgG with additional antigen-binding domains, bispecific IgGs, fragments, fusion proteins and antibody conjugates. Additional bispecific formats include bispecific antibody fusion proteins and conjugates, and multivalent constructs. Due to the dual or multivalent binding nature of these constructs, the binding affinity of each domain to its individual target should be characterized and optimized at the Research and Development (R&D) stage to achieve a desirable safety, pharmacokinetic and efficacy profile.

Multiple potency assays may be needed to demonstrate the binding of the bispecifics to both targets simultaneously and the applicable effector functions that could be involved in the mechanism of action. The potential for success of the candidate molecule is also a concern and may be unique for each type of bispecific platform. For some bispecific constructs, stability could be an issue with the formation of aggregates over time during storage; therefore, extensive formulation studies may be needed at early stages to avoid the derailment of product development. For small bispecific constructs, typically without the Fc region, microbial control during prolonged administration (which is necessary due to the short half-life of these constructs), could be problematic.

The impact of the antimicrobial agent (if added) on the stability of the bispecific should be studied, in addition to the toxicity and effectiveness of the antimicrobial agent. Strategies such as the addition of antibiotics in the intravenous injection solution at the time of administration may be considered under certain circumstances to ensure patient safety.

In general, the regulatory consideration of CMC is to assure the identification, quality, purity and strength of the drug.

3.6 Workforce development

The biopharmaceutical industry has been growing continuously and it is expected that it will require a large number of trained technicians and professionals. But there is a pipeline problem in connecting the industry with a skilled workforce. Various surveys indicate that the top priorities for the industry include the recruitment of experienced technical staff and retaining experienced graduates.

The major disciplines from which the industry draws its workforce include chemical and biological engineering, microbiology, chemistry, pharmaceutical science, biochemistry, biology and various biotechnology programs. Graduates from universities and community colleges generally lack a solid understanding of biopharmaceutical processing and operations in a regulated environment and hands-on lab experiences.

NIIMBL surveys have identified the lack of hands-on experience as a major deficiency of graduates with Bachelor of Science or associate degrees for employment in the biopharmaceutical and manufacturing industry [13]. Although some college courses offer hands-on experience where students are exposed to current analytical methods and basic unit operations, their extent and relevance are not adequate to support the growing workforce needs in terms of the quality and quantity of graduates.

One way to address the current challenges is for academic institutions and industry to initiate and/or expand workforce development partnerships through internships and certificate programs that would provide relevant training for post-graduation employment. To fill that gap, regional centers should consider developing multifaceted, integrated workforce development programs. These could be constructed as a broad portfolio of online, hands-on and instructor-led training to serve multiple audiences—from community college and university students to biopharmaceutical technicians and operators.

The proposed actions relevant to workforce development are:

1. develop training in the chemistry, biology and engineering of ADCs, for example those covering:
    - new biomanufacturing course modules with hands-on experience in ADCs
    - conjugation chemistry, e.g. unpaired Cysteine, unnatural amino acid, transglutaminases and chemical conjugation, etc.
    - linker chemistry
    - ADC heterogeneity with respect to the number of cytotoxins per molecule and conjugation positions
    - novel resin chemistries and purification methods for bispecifics
    - training in process safety for drug conjugates
2. develop targeted internship/co-operative programs with appropriate industries as training venues
3. develop Bachelor of Science engineering certificate programs aimed at the development and manufacturing of ADC and bispecifics
4. develop relevant courses and specialized training programs to address operator/technician training deficiencies, for example those covering:
    - good manufacturing practice regulations (e.g. Code of Federal Regulations, International Council for Harmonisation guidelines and guidelines for the CMC sections of regulatory filings, etc.)
    - a general understanding of the CMC lifecycle of a process
    - the basics of cell culture, filtration and chromatography, ideally with laboratory courses designed for hands-on experience
    - an understanding of technology transfer (basic principles and strategy for outsourcing) and process scale-up
    - the principles of viral clearance
    - PAT, especially in the context of continuous manufacturing but also potentially as a hands-on experience to enable students to design/conceptualize a project, create project models and feedback control loops, and understand how to implement a good manufacturing practice environment
    - advanced Excel skills (e.g. pivot tables, etc.)
    - 'design of experiment' training with practical application in the industry, e.g. understanding regulatory agency requirements for data and how these translate into experimental studies and connect to 'quality by design'
    - non-clinical statistical applications to the industry (e.g. tolerance intervals, comparability, process performance index)
    - data science/data modeling best practices (e.g. clean data principles, data storage, data management, data modeling, database architecture, 21 CFR Part 11/regulatory implications, etc.).

4.0 Conclusions and recommendations

ADCs and bispecifics present powerful opportunities to treat human disease and are an important business driver for value in the biopharmaceutical sector. These novel formats are inherently more complicated (and more expensive) to manufacture than traditional mAbs. Therefore, to ensure the viability of these formats, cost-effective, consistent development efforts and manufacturing processes are required.

A number of goals have been highlighted in this roadmap that, if achieved, will enable the development and manufacture of more affordable drugs for patients with critical healthcare needs:

Drug substance goals
- efficient molecular design through protein engineering and new expression system development, including host-cell and vector construction, to improve the manufacturability of target drugs
- better process control and continuous processing to improve productivity and reduce costs
- flexible, automated, continuous manufacturing for multi-product manufacturing
- for ADCs, single-use and closed systems to minimize human contact

Drug product goals
- safely enable stable formulations (including liquid) and improve administration to patients of ADCs and bispecifics
- increase CMO capacity for manufacturing clinical and commercial ADCs

Analytics goals
- comprehensive characterization and analytical control strategies for molecular variants
- rapid analytical feedback to support real-time process control

Modeling goals
- molecular modeling of drug molecules to facilitate manufacturability and quality control
- provide guidance to improve overall process robustness and understanding

Regulatory goals
- understand the unique nature of the quality attributes of bispecific and ADC DPs
- control the drug-to-antibody ratio (DAR) and monitor the efficacy of ADC products
- control product-related variants

Workforce goals
- appropriate programs for operators/technicians and research scientists across academia and industry.

For the pharmaceutical industry, academia, regulatory agencies and healthcare providers to fully realize the value of ADCs and bispecifics, and to achieve the goals described in this roadmap, the following recommendations are considered appropriate:

- an interdisciplinary, open collaboration should be fostered between pharmaceutical and private industries and CMOs to drive innovation in the advanced manufacturing of ADCs and bispecifics
- open collaborations between industry and academia in process-modeling techniques are needed to improve overall manufacturability and prioritization of technology innovation
- safety should be an increased priority in facility design, training and handling of ADC development and production.

References

1 Liu, H., Saxena, A., Sidhu, S.S., Wu, D., *Fc Engineering for Developing Therapeutic Bispecific Antibodies and Novel Scaffolds*, Front Immunol, 2017. 8: p. 38

2 Biomanufacturing Technology Roadmap, August 2017, BioPhorum Operations Group, www.biophorum.com/executive-summary

3 ADC Trial Landscape Update, July 2018, Beacon Trials Intelligence

4 Isaksson, K., "Extractables studies for single-use systems used in antibody-drug conjugate manufacturing" in Enabling Antibody-drug conjugate manufacturing using single-use systems in downstream – Extractables study demonstrates a good fit, Downstream Column blog, https://downstreamcolumn.com/downstream-bioprocessing/enabling-antibody-drug-conjugate-manufacturing-using-single-use-systems-in-downstream-extractables-study-demonstrates-a-good-fit

5 Wooge, C., *Process Challenges of Antibody–Drug Conjugates*, BioProcess International, June 1, 2014

6 Millipore Application Note: pDADMAC Flocculant Reagent for Use with Clarisolve Depth Filters http://www.merckmillipore.com/GB/en/search/pDADMAC%20flocculant%20reagent%20for%20use%20with%20clarisolve%20depth%20filters%20?search

7 Yigzaw Y. et al., *Exploitation of the Adsorptive Properties of Depth Filters for Host Cell Protein Removal during Monoclonal Antibody Purification*, Biotechnology Progress, V22, Issue1, 2006

8 Scanlan S., Gousseinov E., Becerra-Artega A., Waghmare R., *Challenges and Strategies for the Downstream Processing of BiSpecific Antibodies (BsAbs)*, https://adcreview.com/about-us/permission

9 Müller-Späth T., Ulmer N., Aumann L., Strohlein G., Bavand M., Hendriks L., de Kruif J., Throsby M., Bakker A., *Purifying Common Light-Chain Bispecific Antibodies*, BioProcess International, 11(5) 36–44. May 1, 2013

10 Vasalou, C., Helmlinger, G., Gomes, A., *Mechanistic Tumor Penetration Model to Guide Antibody Drug Conjugate Design*. PLOS One, 2015. 10(3)

11 Shukla, A., Wolfe, L., Mostafa, s., Norman, C., *Evolving trends in mAb production processes*, Bioengineering & Translational Medicine, 2017: 2: 58-69

12 Zhou, Q., Shapiro, M., *Regulatory Considerations for the Development of Novel Antibody-Related Products*, American Pharmaceutical Review, April 2016

13 NIIMBL Quick Start Project (QSP) Call 1.0, Request for Proposals, v07132017, https://niimbl.my.salesforce.com/sfc/p/#6A000000tfmF/a/6A000000GwKP/RAnpAFopaIek3.8yJQK7Y95LPLOIgsfx2RG7ObmiMbA

Acronyms/abbreviations

ADC	Antibody-drug conjugate
API	Active pharmaceutical ingredient
BiTE	Bispecific T-cell engager antibody
CLD	Cell-line development
CMC	Chemistry, manufacturing and control
CMO	Contract manufacturing organization
CoGs	Cost of goods
CQA	Critical quality attribute
DAR	Drug-to-antibody ratio
DNA	Deoxyribonucleic acid
DP	Drug product
DS	Drug substance
Fc	Crystallizable fragment
GXP	A general term meaning Good Clinical, Manufacturing, Distribution Practice
HC	Heavy chain
HIC	Hydrophobic interaction chromatography
IEX	Ion exchange
IgG	Immunoglobulin gamma
IV	Intravenous
LC	Light chain
mAb	Monoclonal antibody
PAC	Product attribute control
PAT	Process analytical technology
UF/DF	Ultrafiltration/diafiltration

Collaboration Statement

This document is the result of a collaboration between the National Institute for Innovation in Manufacturing Biopharmaceuticals (NIIMBL) and BioPhorum Operations Group (BioPhorum) to develop a NIIMBL roadmap for the biopharmaceutical manufacturing industry, which complements the existing Biomanufacturing technology roadmap and other industry roadmaps.

https://www.biophorum.com/executive-summary/

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Since its inception in 2004, the BioPhorum Operations Group (BioPhorum) has become a trusted environment where senior leaders of the biopharma industry come together to openly share and discuss the emerging trends and challenges facing their industry. The strong cross-company relationships built through BioPhorum have provided the solid foundation from which highly effective collaborations have grown, each focusing on key operational challenges, improving competitiveness and reducing patient risk. As well as the Technology Roadmap, BioPhorum runs six other Phorums – Drug Substance, The Development Group, Fill Finish, IT, Supply Partner and Cell & Gene Therapy. You can find out more at www.biophorum.com.

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This roadmap is created and intended in good faith as an industry assessment and guideline only, without regard to any particular commercial applications, individual products, equipment and/or materials.

Our hope is that it presents areas of opportunity for potential solutions facing the industry and encourages innovation and research and development for the biopharmaceutical industry community to continue to evolve successfully to serve our future patient populations.

Disclaimer

Roadmap team members were lead contributors to the content of this document, writing sections, editing and liaising with colleagues to ensure that the messages it contains are representative of current thinking across the biopharmaceutical industry. This document represents a consensus view, and as such it does not fully represent the internal policies of the contributing companies. Neither NIIMBL nor any of the contributing companies accept any liability to any person arising from their use of this document.