Challenges Of New Molecular Format Development

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The storied rise of monoclonal antibodies (mAbs) in the pharmaceutical industry — full of challenges and triumphs — offers a compelling case for what is possible when science and technology come together to address development and manufacturing bottlenecks. Initially rife with immunogenic potential and efficacy issues and then costly and inefficient commercial manufacturing, mAbs eventually became one of the fastest-growing segments in today's market through a concerted effort to improve production processes.

Now, as we see more manufacturers pursuing next-generation therapeutics, the history of mAbs and, more importantly, the need for fit-for-purpose solutions and technologies to bring new and innovative products to fruition is more applicable than ever. This is due, in part, to the increasing prevalence of new molecular formats, such as recombinant proteins and fusion proteins in today's global biologics pipeline, a presence that is expected to grow at a rate two times faster than mAbs. These formats have the ability to be more precisely targeted and more potent, and potentially open up access to new therapeutic targets and biological mechanisms previously not accessible with mAbs. However, the operational and technical challenges of these molecules can complicate development and manufacturing and lead to delays and added costs.

To make sure their existing solutions and strategies are able to accommodate the needs of customers and their therapies, Lonza recently conducted a survey to confirm the challenges in upstream, downstream, and analytical development of new molecular formats, as well as identify any additional challenges in developing biologics based on them. The results, as outlined in this paper, highlight where the industry stands today with these exciting products and offer valuable insight into what can be done to ensure they reach the patients who need them.

SURVEY RESPONDENTS AND MOLECULE TYPE OVERVIEWS

To explore what specific challenges customers are facing with these therapeutics in upstream, downstream, and analytical development, Industry Standard Research (ISR), on behalf of Lonza, conducted a web-based quantitative survey with 100 participants. Of these respondents, 69% were from North America, while 30% were in Europe and about 1% in Asia. Company sizes varied, with 61% of respondents from a large pharma organization, 22% from midsize, and 17% from small pharma; the company size by production method is outlined in Figure 1.

On average, the respondents are focused on 2.45 types of molecules from the following types included in this report:

- Recombinant proteins
- Bispecific antibodies

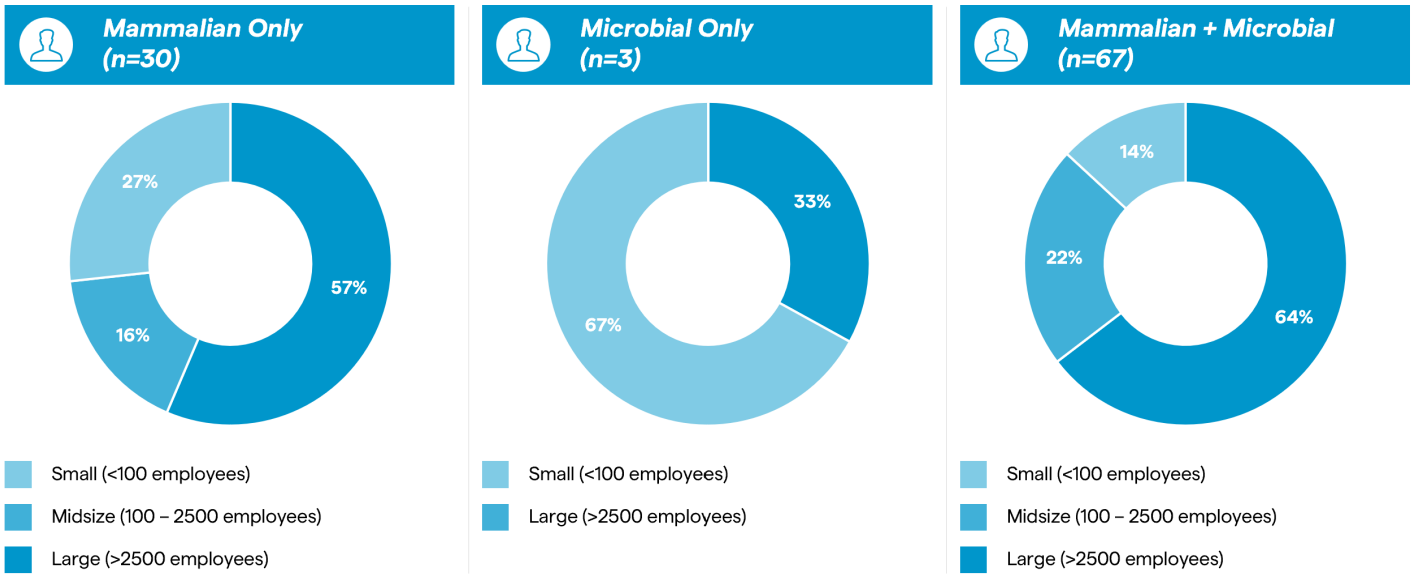

Figure 1: *Respondent company size by production method*

- Single-chain antibodies
- Fusion proteins
- Antigen-binding fragments (Fabs)
- Nanobodies
- Non-antibody scaffolds

Overall, the majority of respondents (68%) are currently working with recombinant proteins. Just under half are working with bispecific antibodies (47%) and/or single-chain antibodies (42%). For early development of new molecular formats, respondents had experience in two of these three areas on average, with 64% of respondents possessing downstream experience, 58% having analytical development experience, and 57% with upstream experience. Less than 5% of respondents are working with other types of molecules that include cyclic peptides, gene therapies, oncolytic viruses, protein-drug conjugates, and vaccines.

UPSTREAM DEVELOPMENT CHALLENGES

Mitigating risk during the development of any biotherapeutic requires access to vital information about your molecule early in the process, particularly to monitor functionally critical product-quality attributes. Examining those as early as possible allows for better upstream optimization before selecting your lead candidate, ultimately ensuring your best chance at success during scale-up later.

To take a closer look at where the respondents with experience in upstream are facing the biggest bottlenecks with new molecular formats during this stage of production, ISR asked them to indicate which of the following four challenges they'd experienced during upstream process development or manufacture:

- Unable to reduce the development timelines for complex proteins
- Required analytics were not available at the "right time" during the development cycle
- Lack of a common platform for upstream development
- Unable to compile and use historic data to drive a systemic approach to upstream development

Figure 2 shows an overview of the challenges from which respondents could select and the distribution of their answers.

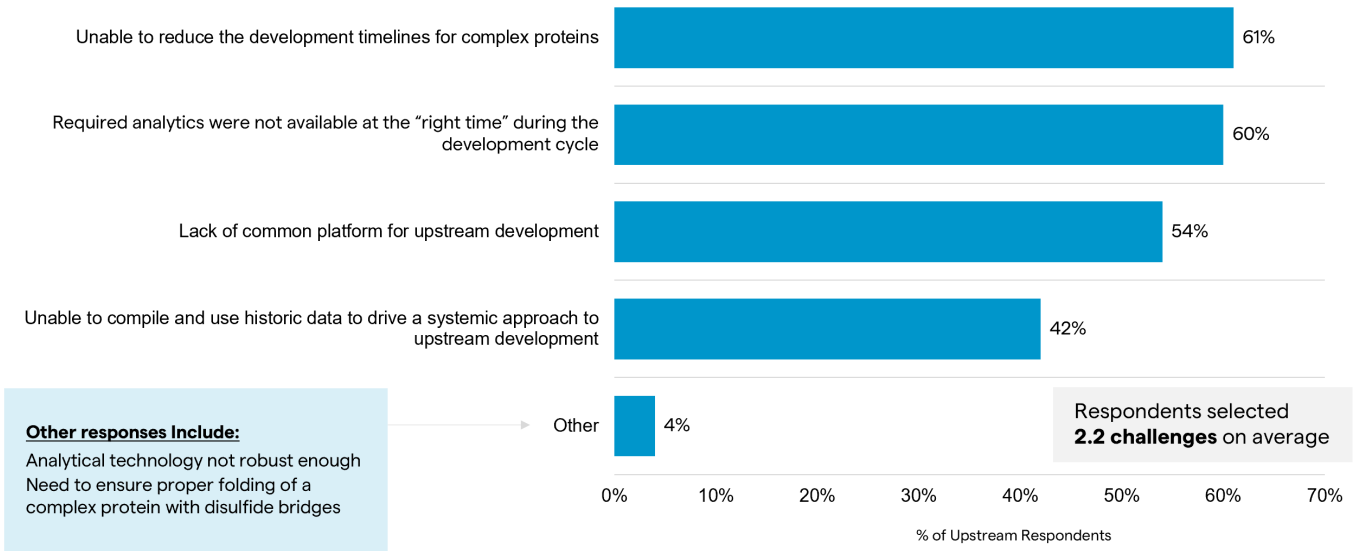

Figure 2: *Challenges experienced by survey respondents during upstream development*

The upstream respondents experienced an average of 2.2 challenges out of the four difficulties listed; few mentioned different challenges (4%). The most reported challenges among respondents with upstream development responsibilities are Unable to reduce the development timelines for complex proteins (61%) and Required analytics were not available at the "right time" during the development cycle (60%).

As these therapeutics increase in complexity, improvements in gene expression and early-stage screening are necessary, especially for multichain products, which are an extremely diverse group of proteins. They include fusion proteins, various antibody formats, such as IgA or IgM, as well as multispecific molecules containing three or four chains, resulting in molecules with two or more sites that bind to multiple target antigens. Multichain proteins are benefited by a vector system that allows the expression of multiple genes in one single vector. Optimizing vector design early allows the development team to determine whether gene order and number within a single vector influence the titer and product assembly. It also allows for product quality screening to de-risk process development. With this additional activity in mind, the inability to reduce development timelines is becoming indicative of complex protein development.

In order to support development and make the best decisions about achieving the right cell line with the right balance between product quality and titer, the rapid development of adequate analytical methods is essential. Hence, the latter challenge listed for this survey question, i.e., not having analytics at the right time, is likely a result of bespoke analytics that are required for these complex programs but often not available in an existing upstream development toolbox. Engineers must then wait for them to be developed, potentially leading to delays.

To overcome these early-stage challenges, Lonza has a suite of expression technology platforms, beginning with the GS Xceed® Mammalian Expression System, which is used for the optimal expression and production of monoclonal antibodies and next-generation recombinant proteins. This system offers a toolbox from which the team at Lonza can select the best vector system and expression host for the molecule

type. It includes the GS® base vector system; the GS® multi-gene vector system for the expression of multi-chain molecules; the GS piggyBac® technology for difficult-to-express molecules; and the vector system for IgG site-specific conjugation. For mammalian expression hosts, Lonza offers a standard GS knockout cell line, its POTELLIGENT® cell line for enhanced effector function, and the NSO cell line for biosimilars.

As discussed, difficult-to-express proteins or more complex molecular formats increase the risks associated with accelerating early drug development, making it even more essential to assess the potential for these risks early in the process. To address this, Lonza also offers its Epibase® platform, which comprises in silico and in vitro immunogenicity assessments; their suite of early development and de-risking services covers the late discovery to FIH phase of development. Taking advantage of technologies such as these early in the process will enhance the chances of your drug being successful by reducing the likelihood of issues later when the costs are higher and the consequences of delay are more harmful to your program.

DOWNSTREAM DEVELOPMENT CHALLENGES

Many of the challenges experienced in the upstream also impact downstream development, limiting the ability to generate large amounts of protein to work with and from which processes are developed. For this reason, many of the answers from downstream respondents are likely related to the challenges experienced during upstream development.

The four downstream challenges ISR gave respondents to select from are:

- Unable to reduce the development timelines for complex proteins
- Required analytics were not available at the "right time" during the development cycle
- Lack of platform process makes development more challenging
- Development and optimization of non-affinity capture from scratch

Figure 3 shows an overview of the challenges from which respondents could select and the distribution of their answers.

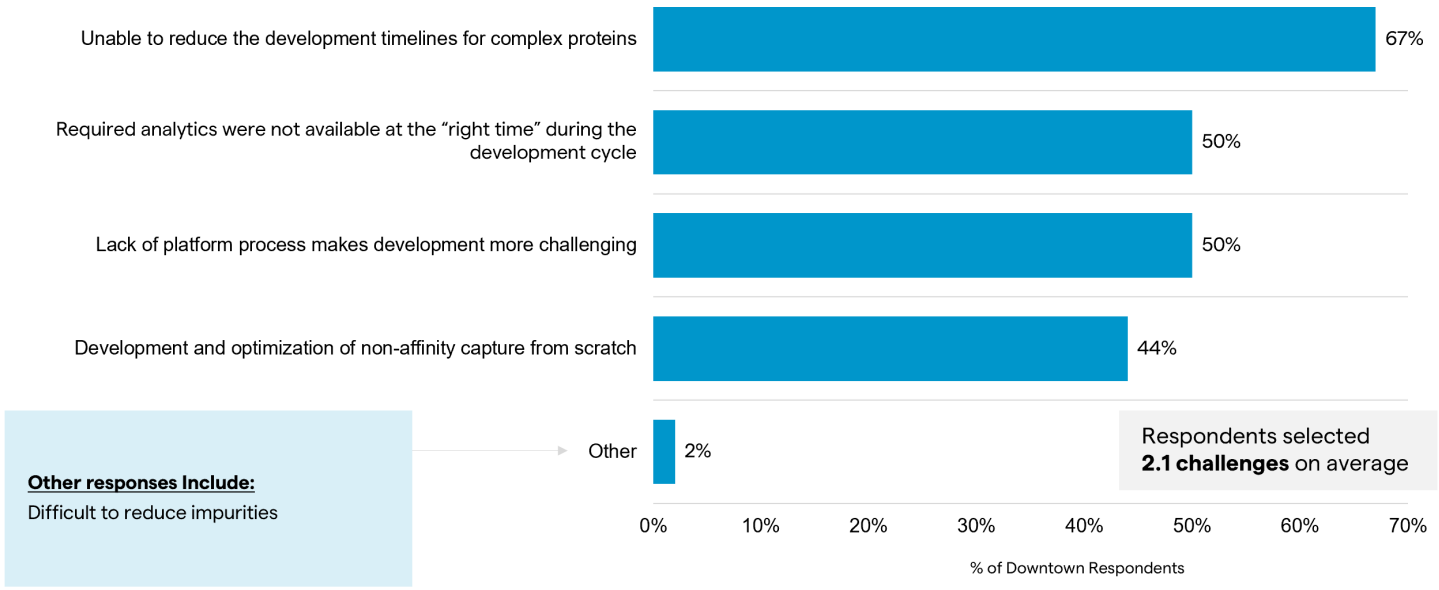

Figure 3: *Challenges experienced by survey respondents during downstream development*

The downstream respondents experienced an average of 2.1 challenges out of the four difficulties listed. Similar to upstream respondents, Unable to reduce the development timelines for complex proteins is the most frequently reported challenge among downstream respondents. Half also confirmed that Required analytics were not available at the "right time" during the development cycle and Lack of platform process makes development more challenging were difficulties they encounter. Finally, 44% of downstream respondents confirmed that they have encountered challenges with Development and optimization of non-affinity capture from scratch.

The challenge of non-affinity capture relates mainly to new molecular formats with no Fc region, which renders traditional capture methods (based on Protein-A affinity chromatography) ineffective. The need to find an alternative option can lead to the need for specialized resins and, likely, longer lead times. Overall, the advanced engineering required for new molecular formats moves development further away from platform technologies, which can cause several issues such as those listed in the survey.

Respondents who indicated they experienced challenges related to the non-affinity capture from scratch were asked a follow-up question to better understand how this obstacle impacts downstream development activities. Two-thirds of respondents with non-affinity capture challenges (64%) reported Difficulties with determining the capture steps rapidly and early in the development cycle. Half of these respondents reported Challenges with changes to feed streams; managing these changes in upstream while continuously optimizing downstream (54%) and Limited material available early on and its impact on developing the capture step (50%).

In addition to its technologies for early de-risking, Lonza can also help its customers define a modular workflow to develop the sequence of downstream unit operations. Lonza's downstream purification offering includes a wide range of services, including high-throughput resin screening to identify the optimal resin for your complex protein.

ANALYTICAL DEVELOPMENT CHALLENGES

Standard analytical processes are designed around mAbs, which have a molecular weight of about 150 kilodaltons, while multichain molecules can be more complex and, as discussed previously, are also highly engineered. This creates risks for aggregation and post-translational modifications, which can have implications for immunogenicity and immunotoxicity. Expression of multiple chains can also lead to unwanted product-related byproducts and an increase in chain mispairing and fragmentation; therefore, considerations must be made to ensure proper assembly, and robust analytical tools should be utilized to detect other potential issues.

When surveying respondents about their analytical development experience with complex proteins, ISR asked them to indicate which of the following five challenges they'd encountered:

- Deciding appropriate non-platform analytical methods for attributes, such as purity and potency
- Delivering relevant analytical data to support downstream decisions under compressed timelines
- Transferring the analytical methods between companies
- Developing potency assays for bispecific or multispecific molecules that have more than one target
- Forward processing decisions

Figure 4 shows an overview of the challenges from which they could select and the distribution of their answers.

The analytical development respondents experienced an average of 2.5 challenges out of the five difficulties listed. The challenge impacting most respondents with analytical development (66%) is in Deciding appropriate non-platform analytical methods for attributes, such as purity and potency. New molecular formats, unlike mAbs, can have multiple targets they may need to bind to and not all functions may be related, making it difficult to identify the right analytical method for measuring potency. The increased risk of impurities with complex

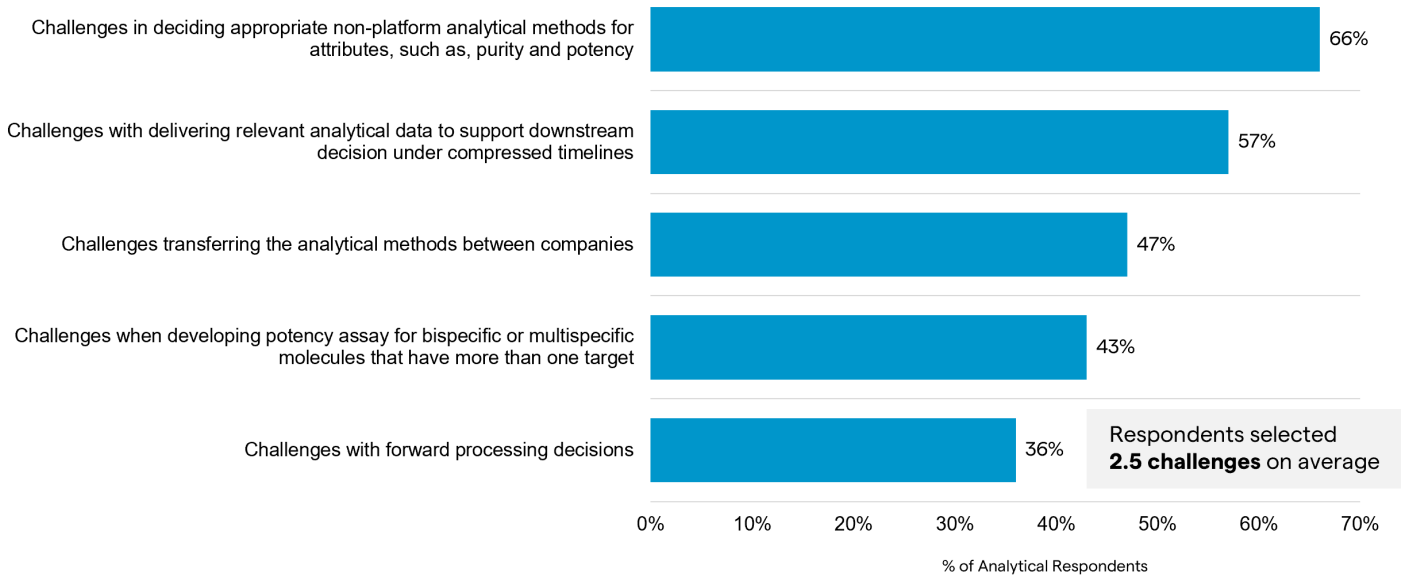

Figure 4: *Challenges experienced by survey respondents during analytical development*

proteins can also create added challenges with purity.

Challenges with delivering relevant analytical data to support downstream decisions under compressed timelines were also widely encountered, with 57% confirming that they have experienced this issue. Just under half of the analytical development respondents (47%) reported Challenges transferring the analytical methods between companies, and 43% relayed Challenges when developing potency assays for bispecific or multispecific molecules that have more than one target. The issue encountered by the smallest proportion of analytical development respondents was Challenges with forward processing decisions (36%).

The incompatibility of these molecules with some platform approaches for mAbs calls on a more flexible and agile approach to early developability and method development. Therefore, Lonza has developed a toolbox approach for product-specific analytical method development, which can be employed up front using material from cell pools to de-risk process development and manufacturing. This toolbox approach also allows its experts to screen for titer and product assembly at an early stage. This ability to gather critical data about a molecule at an early stage of development can help ensure appropriate analytical methods are deployed at the right time, which allows a development team to avoid many of the issues outlined by survey respondents.

CONCLUSION

Overall, the challenges of respondents throughout the survey corresponded with Lonza's expectations, as 40% of the large molecules its teams support fall in this array of modalities from preclinical to commercial-stage development. This is achieved by leveraging extensive expertise from 40 years of experience in developing and manufacturing biologics and a breadth of innovative technologies for mammalian and microbial-derived molecules. These innovative platforms, combined with Lonza's advanced technologies and analytical toolbox, provide its experts with the tools to help address the challenges of new molecular format development and manufacturing in today's changing industry.