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Life Sciences

Recent Trends in Therapeutic Antibodies Development

It's been nearly 50 years since Georges J.F. Köhler and César Milstein pioneered the hybridoma method for producing monoclonal antibodies(mAb).[1] In 1984, they were awarded the Nobel Prize for their work. Just two years later, the first therapeutic mAb – muromonab-CD3 – was approved by the FDA for use as an immunosuppressant to prevent organ rejection in transplant patients.[2,3] It was another decade before the next therapeutic mAb was approved but, today, nearly a fifth of all drugs approved by the US Food and Drug Administration (FDA) are mAbs.[4]

Progress in the field accelerated when James Allison and Tasuko Honjo determined that antibodies could be used to switch off immune checkpoint receptors (CTLA-4 and PD-1) and thus enhance antitumor immunity.[5,6] Based on this research, the first immune checkpoint inhibitors for cancer therapy were approved by the FDA – anti-CTLA-4 (ipilimumab) in 2011 and anti PD-1 (nivolumab) in 2014.[7] In 2018, Allison and Honjo were awarded the Nobel Prize in Physiology or Medicine for their discoveries.[8]

Since then, there has been a rapid expansion in the number of mAbs and antibody–drug conjugates (ADCs) in development. The latter group of therapeutics, ADCs, combine the target specificity of mAbs with a medicinal drug payload, making it possible to directly deliver drugs to their target, both augmenting on-target and reducing off-target effects.[9] This approach is being explored not only in oncology but also for the treatment of infectious diseases. For example, an antibody–antibiotic conjugate has shown promise for targeting intracellular reservoirs of *Staphylococcus aureus* more effectively than vancomycin.[10]

Both mAbs and ADCs have several distinct advantages when compared with small-molecule drugs. Their high degree of specificity reduces off-target binding and drug–drug interactions, both resulting in fewer adverse side effects for patients. mAbs can also be specifically designed against targets unsuitable for small-molecule drugs, such as those with shallow or allosteric binding pockets and protein–protein interactions. This approach opens new avenues for previously untreatable complex diseases and makes them an attractive proposition for drug development, with nearly 200 approved or in regulatory review.[11,12,13] Likewise, the global antibody market is predicted to grow from $247.3 billion in 2023 to $479 billion in 2028, with around 10 therapeutic antibodies being approved in the EU or US each year.[14,15] Despite this progress, it still costs an average of $1.3 billion to bring a new drug to market. Notably, mAbs development costs can vary significantly, with examples such as Dinutuximab costing just $276 million in contrast to the $13.4 billion spent to develop Durvalumab.[16,17]

In this article, we outline trends and challenges in therapeutic antibody development and explore the innovations reducing development timelines and costs.

Antibody modalities

Since the first fully human mAb was developed, advances in antibody engineering have led to the development of an increasingly diverse range of mAb modalities (Figure 1).[18]

Bispecific antibodies (BsAbs)

These are antibodies designed to recognize two different antigens or two different binding epitopes for the same antigen which have the advantage of increased specificity and reduced off-target effects.[18] BsAbs can be either large Ig-like molecules with additional domains or tiny proteins with only two antigen-binding fragments.[18] They are attractive options because of their potential for novel immunogenic activities that cannot be achieved purely by mixing the parental or reference antibodies. They also have the potential to create temporal functionality (when binding events occur in a sequence) or spatial functionality (where linking two binders can be used to recruit immune cells or prevent cells from interacting).[19] For example, a BsAb blocking two inflammatory cytokines simultaneously has been developed to produce a more effective treatment for patients with severe forms of asthma.[20]

Antibody–drug conjugates (ADCs)

As outlined earlier, ADCs comprise a mAb, a cytotoxic payload and an appropriate linker. They are predominantly being explored in oncology, owing to their ability to target the tumor cell surface and then release the cytotoxic payload into tumor cells through receptor-mediated endocytosis.[18] This is more powerful than using mAbs alone, which are often not as lethal against cancer cells as conventional chemotherapy.[2] By combining the high specificity of mAbs with the potent anti-tumor activity of cytotoxic agents, ADCs can improve the therapeutic window of both agents.[22] Since the first ADC therapy approval in 2000, at least 14 ADC therapies have been approved and there are over 100 ADC candidates in development.[23] At the end of 2022, of 138 antibody-based drugs in late development, 19 were bispecifics and 11 were ADCs.[15,24]

Antibody fragments

Several small molecular weight Ab fragments are currently being developed. For example, the fragment antigen binding (Fab) region, the single chain fragment variable (scFv) and the single variable domain on a heavy chain (VHH) region (also known as a nanobody) show intense penetration and lower production costs relative to mAbs.[18]

Camelid nanobodies have the potential to boost crop resistance to viruses, as they can bind efficiently to viral epitopes that are cryptic or inaccessible to conventional antibodies.[25] VHH-based gut therapeutics are also being explored. For example, when fused with fibers on the surface of a probiotic *Escherichia coli* strain, VHHs were expressed in high levels in the gut and found to neutralize enteric bacterial toxins.[26] Nanobodies also present an alternative to mAbs as a scalable treatment option for COVID-19 or future pandemics, where mAb production in mammalian cells is not scalable to meet the global demand.[27]

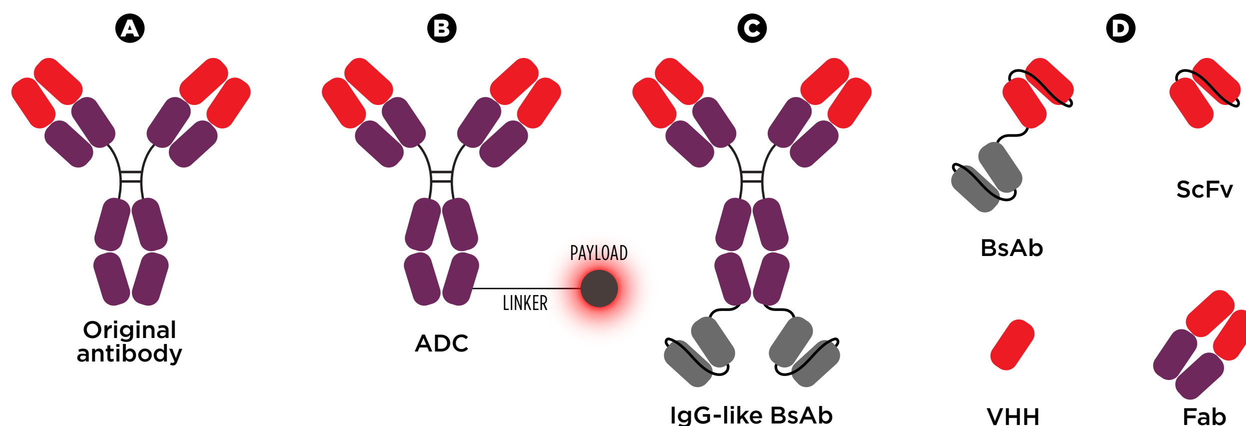

Figure 1. Different mAb modalities. A) An original Ab showing the constant (purple) and variable (red) domains. B) An Ab–drug conjugate, where an mAb is linked covalently to a cytotoxic payload. C) Large IgG-like bispecific Ab with additional domains. D) Ab fragments including (clockwise from left) tiny bispecific antibody containing only two antigen-binding fragments; single chain fragment variable (scFv); fragment antigen binding (Fab) region; single variable domain on a heavy chain (VHH) region (nanobody).

Trends in development

The first therapeutic mAbs were developed in mice (murine mAbs), but these were recognized as foreign material in the human body, resulting in human anti-mouse Abs (HAMA) production.[18] To reduce immunogenicity and enhance the response duration, researchers developed techniques to transform murine mAbs into structures similar to human mAbs (Figure 2). These were called chimeric mAbs. The first chimeric mAb – the anti-P-glycoprotein antibody (abciximab) – was approved for use in 1994 by the FDA for the treatment of platelet aggregation.[18] Chimeric mAbs caused less immunogenic reactions than murine mAbs but, because they retained the mouse variable chain, they still produced some HAMA effects.

Further antibody engineering efforts culminated in the first humanized mAbs. Initially, this was achieved by grafting the complementarity-determining region (CDR) of the mouse mAb into a human mAb framework. This approach maintains the specificity of the mAb while removing most of the mouse components. The first humanized mAb, anti-interleukin-2 receptor Ab (daclizumab), was approved by the FDA in 1997.[18] This paved the way for a new class of biologics that could target chronic diseases requiring long-term treatment.

Today, several humanization techniques are used, each iteratively reducing off-target immunogenicity. For example, grafting the specificity-determining region (SDR) – instead of the CDR – further reduces the mouse components of the antigen-binding residues. Another technique involves using human germline genes as the antibody framework rather than IgG genes. Also, chain shuffling enables rapid identification of various human framework combinations that can support the CDRs required for binding and functional activity.[28,29,30]

The success of these humanized mAbs led to the next significant advance in the field: the application of phage display technology to antibody development. For this discovery, Greg Winter and George P. Smith were awarded a share of the 2018 Nobel Prize in Chemistry.[31] Phage

display technology transformed antibody engineering and screening and led to the first approval of a fully human therapeutic mAb, adalimumab (Humira), in 2002 for rheumatoid arthritis.[18,32]

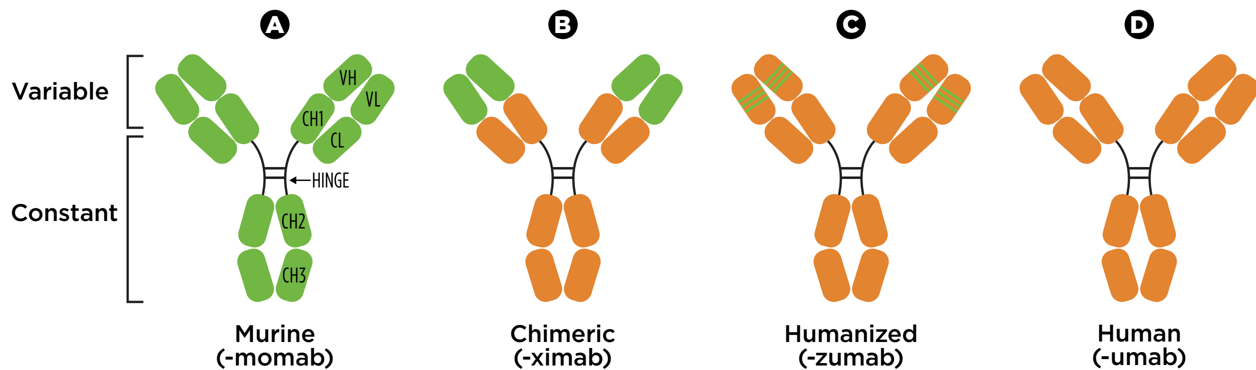

Figure 2. Overview of therapeutic antibody humanization. Abs comprise two large heavy chains and two light chains linked to form a tetrameric structure. Each Ab has a constant domain and a variable domain. The constant domain determines the Ab class (isotype), while the variable domain determines its antigen specificity. A) Murine mAb. B) Chimerical mAb comprising murine variable regions and human constant regions. C) Humanized mAb in which only the hypervariable regions are of mouse origin. D) Human mAb.

From discovery to the market

Bringing an effective new therapeutic to the market is a long and costly process, especially for complex biologic drugs such as mAbs.[16] The longer the development process, the higher the final product's cost and the greater the negative impact on patients who may have no effective treatment options (Figure 3). Each stage in a mAb development pathway brings unique challenges requiring innovative solutions.

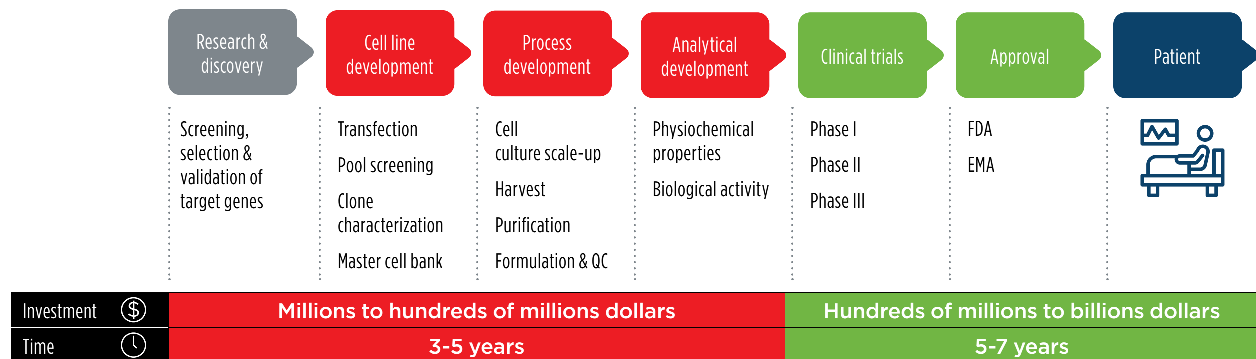

Figure 3. Overview of mAbs development pathway. In research and discovery, Abs are produced and screened for affinity against the desired target. Genetically stable and prolific cell lines are developed for reliable long-term production. Cell culture is scaled up and processes for harvesting, purification, formulation and quality control are developed. The formulated Ab product is tested in clinical trials before it can be approved for clinical use and reach patients.[33]

Research and discovery

Mouse hybridoma

Mouse hybridoma technology is still the most widely used technique for producing murine mAbs. Yet, the HAMA response can cause rapid clearance, reduced efficacy and adverse reactions.[18,34] This can be addressed in several ways, such as CDR or SDR grafting to create humanized Abs and surface amino acid modifications to generate less immunogenic molecules.[18]

Producing humanized mAbs requires custom-developed methods, extensive modification and several iterations. To accelerate this step, computational methods – such as molecular modeling, bioinformatics and machine learning – are now being employed. These methods leverage advances in structural biology and sequencing to predict Ab–antigen interactions and optimize Ab design.[35,36] For example, by using atomistic simulations to select Abs designs by energy and structural integrity it was possible to design humanized mAbs with similar affinities to those of the parental Abs.[37] These techniques support the rational design of synthetic Abs, reducing the need for extensive experimental screening.

Antibody libraries

Abs libraries, such as phage display, yeast display or mammalian display libraries, can be used to rapidly screen for potent, fully human mAbs.[38] In these display libraries, the Ab is fused to the surface coat of cells or phage retaining its ability to fold and bind antigens.[18]

Using this approach, it's possible to construct full-length Abs from screened Fab fragments. Camelid VHH libraries, for example, can retrieve high-affinity binders more efficiently.[18] A limiting step of such an approach is the affinity enrichment stage. Nonetheless, they allow for the generation of diverse Ab libraries facilitating the screening and selection of Abs with desired properties. This leads to more efficient Ab discovery and optimization, with many approved mAbs derived from these technologies.[18] High-throughput screening techniques also allow for the screening of large mAb libraries against specific targets, enabling the quick identification of potential candidates.

Transgenic mice

In 1985, researchers first proposed using transgenic mice to produce Abs.[39] Today, advances in gene editing technology have made human Abs transgenic mice a reality. Advantages to this approach include the removal of humanization steps, the ability to mature Abs *in vivo* and Ab optimization through clonal selection. However, the technology has proven challenging because of the large size of human Ab genes and the need to express other human Ab fragment genes in high amounts in mice. Fully human Ab mice have been developed to address this problem alongside technologies that replicate the human Ab response or express chimeric Abs with human idiotypes.[18]

Flow cytometry screening

During a robust immune response, the somatic recombination of B cells produces a diverse repertoire of Abs. This process cannot be precisely replicated in transgenic systems. However, the development of flow cytometry screening has made it possible to isolate antigen-specific B cells using B-cell surface markers, from which the desired Abs can then be isolated.[40] This approach was used to produce the currently approved anti-SARS-CoV-2 mAbs deriving from a single B-cell clone.[18]

Cell line development (CLD)

Therapeutic mAb production begins with establishing genetically stable and prolific cell lines required for reliable long-term and large-scale manufacture.[41]

This process starts when a cell line is transfected with the Ab gene(s) of interest and the most productive clones expressing the Ab are selected.[42] Chinese hamster ovary (CHO) cells are predominantly used because they produce correctly folded glycosylated proteins with human-compatible post-translational modifications (PTM), and they are highly productive and adaptable to large-scale culture.[43] Human cell types are also being explored for transient expression of mAbs, including HEK 293 (embryonic kidney cells), HKB-11 (a hybrid between HEK293 and human lymphoma cells) and PERC6 (a human embryonic retinal cell line).[43,44]

Considerable efforts have focused on cell line engineering to improve or modify product quality. By optimizing Ab gene sequences, expression vectors and host cells, it becomes possible to improve Ab quality and yield.[42,45] The host cells' ability to correctly fold and glycosylate Abs is crucial as these PTM significantly impact on Ab function.[42,44,45] For this reason, the production of whole therapeutic mAbs has conventionally required mammalian expression systems. Yet, several lower organism expression systems such as bacteria (*Escherichia coli*), yeasts (*Saccharomyces cerevisiae* and *Pichia pastoris)*, fungus (*Aspergillus niger),* plants (tobacco), algae and insects (silkworm) are increasingly being explored to improve yield, scalability, cost-effectiveness and ease of production.[43] For example, *Escherichia coli* and *Aspergillus niger* cells are commonly used to generate Ab fragments such as Fabs or recombinant fusion mAbs that lack or require simple glycosylation.[42,43]

In vitro cell-free synthesis technology is also being developed using CHO or bacterial cell lysates. This technique holds promise to reduce undesirable biological by-products and enable continuous production (by recycling cell lysates). For the moment, these lower-organism or cell-free approaches are limited by the endogenous machinery of the host or originating cell. However, expression systems are increasingly being engineered for enhanced functions such as glycosylation and improved Ab secretion.[43]

Another area of focus has been the reduction of programmed cell death (apoptosis) to extend long-term viability of cell cultures by overexpressing anti-apoptotic genes.[42] Inducible genes that can switch on overexpression of Ab genes are also being explored to decouple Ab production from cell growth; this could increase the yield from smaller cell cultures.

After transfection, cells are cultivated and screened for robustness and productivity, and the most stable and efficient cell clones are selected. At this stage, a range of quality tests are carried out to analyze Ab integrity (e.g., omics, peptide mapping, SDS-PAGE), aggregation (e.g., analytical ultracentrifugation, Valita Aggregation Pure plate-based assays), glycosylation (e.g., HPLC or capillary electrophoresis-based glycan assays) and charge heterogeneity (e.g., isoelectric focusing).[42] Selection of the final production clone is a critical decision in development. Switching to a different clone during late development represents a major product change and might require comparability studies or even additional human clinical studies.[42]

Producing a stable cell line can be long and costly. Clone selection has been held back because traditional cell culture methods are expensive and time-consuming.[43,46] Automation and robotics provide a faster and highly consistent process and are now being used to increase the efficiency

of clone selection and evaluation. For example, current automated platforms allow for single-cell clone selection, the subsequent expansion of the selected hybridomas in microbioreactors as well as downstream purification and quantitation of Ab yield.

Once the right clone has been identified, several dozen clones are selected to create safety cell banks. From these, the best one is chosen for the master cell bank which will be the source of the working cell lines during production.[47] These working cell lines need to be expanded and maintained, with the continued high viability of these cultures crucial for successful development.

Assessment of viability using Trypan blue staining is the current standard of practice during CLD. Automated analyzers combining Trypan blue staining and image analysis work to speed up this process.[42] However, with the advent of more complex culture vessels and greater understanding of the impact of cellular metabolism on culture health and productivity, many labs now also implement metabolic monitoring of cultures. This includes on-line monitoring of oxygen uptake or off-line assessment of mitochondrial activity using fluorescence-activated cell sorting (FACS).[42]

It is also becoming more common for companies to adopt process analytical technology (PAT) for mAb development and manufacture.[42,48] PAT is an integrated set of advanced and automated methods that analyze the composition and biophysical properties of cell culture fluids and biotherapeutic molecules, using in-line or near-line sensors and probes.[48] In upstream mAb development, PAT sensors can be used to monitor media conditions, cell processes and extrinsic factors that influence the mAb quantity and quality, supporting cell line optimization and clone selection.[48]

The traditional method for scaling up cell-based products is to use large bioreactors to produce large harvest volumes. These processes can be fed-batch or continuous, but either system must be carefully optimized for operation parameters including temperature shifts, gas exchange, shear stress and feeding strategy. A new trend is the use of single-use bioreactors which have lower capital and investment costs and eliminate the need for cleaning or sterilization, thus reducing product contamination rates.[49]

Process development

In downstream process development, the focus shifts to Ab purification, yield and productivity.[49] These downstream processes are time-consuming and costly. Hence, manufacturers are investing considerable time and effort in optimizing and simplifying processes to improve productivity. This includes the development of novel technologies and the establishment of Quality by Design (QbD) approaches.[50]

The Ab purification process needs to reliably and predictably produce products suitable for human use – this means eliminating impurities, host cell proteins and aggregates.[49,51] The typical Ab purification process begins with centrifugation or filtration to remove cells and cell debris, followed by protein A chromatography.[52] This yields a highly purified product in a single step, but has the disadvantage of being 50% more expensive than other chromatographic media.[52,53] In addition, Protein A resins cannot distinguish between functional and aggregated antibodies, allowing aggregated Abs into the next production stage.[53] Minimizing the formation of soluble aggregate is crucial during mAb development, as aggregate can reduce drug efficacy and increase undesirable immunogenicity.[54] Thus, manufacturers focus on other methods for use alongside or as an alternative to chromatography. These methods include membrane-based

separation, two-phase separation by size-exclusion chromatography and sedimentation velocity analytical ultracentrifugation (SV-AUC). SV-AUC is the method of choice for characterizing and quantifying aggregates as it can be carried out with several solution compositions and formulations.[54]

Maximizing Ab recovery while maintaining product quality is another key challenge during process development. During the removal of aggregates and host cell proteins, it is inevitable that some product is lost. Here, the selection of appropriate filters during the initial harvesting step, the choice of chromatography media and the addition of excipients to buffers can profoundly impact yield.[51]

Analytical development

During mAb development, thorough characterization of the product must be performed according to regulatory guidelines.[55] This functional and structural characterization of mAbs is essential to ensure the product affinity, specificity and stability.[56] This involves Ab quantification, assessing for impurities, as well as characterization of structural, physicochemical, immunological and biological properties.[55]

As part of the QbD principles adopted by regulatory agencies, manufacturers are required to demonstrate critical quality attributes (CQAs) for every mAb product.[46] A CQA is defined as "a physical, chemical, biological or microbiological property or characteristic that should be within an appropriate limit, range or distribution to ensure the desired product quality".[57] These are the crucial characteristics required to ensure quality from a patient's perspective.[46,58] CQAs can be obligatory (mandated by regulatory authorities, e.g., antibody titer, presence of adventitious agents such as viruses), product-specific (structural or physicochemical attributes that impact product function, e.g., size, aggregation) and process-related impurities (e.g., host-cell protein).[56]

There are several analytical tools available to measure mAbs attributes.[55] Common analytical tools include mass spectrometry for structural testing, alongside infrared spectroscopy and nuclear magnetic resonance spectroscopy for assessing higher-order structure. Size exclusion chromatography (SEC) and/or SV-AUC are used for Abs aggregation quantification.[55]

While SEC has low material requirements and allows for high throughput, sensitivity and precision, it is limited by a need for two-phase separation, which means the sample must be diluted. This can dissociate existing aggregates or promote the formation of new ones. For this reason, SEC is usually carried out alongside SV-AUC for aggregate characterization.[54] For Ab titer quantification, colorimetric assays, HPLC or ion chromatography are commonly used.[55] Novel plate-based methods, such as the Valita Aggregation Pure assay, allow for antibody aggregation analysis within 15 minutes.

Clinical trials

Clinical development of therapeutic mAbs follows the same three phases of development as other therapeutics. In phase 1, an initial batch of the mAb will be evaluated for safety and dose optimization. In phase 2, the mAb is evaluated in a larger group of patients for both safety and efficacy. If the results of phase 2 trials are promising, the mAb will be evaluated in larger phase 3 trials to compare it with existing treatments.

Process and product development efforts have reduced phase 1 timelines to around 10–12 months, but the overall clinical development process can take 5–7 years.[46] There are currently over 130 therapeutic mAbs undergoing evaluation in pivotal late-stage clinical studies (phase 2/3).[12] Taking the latest approval rates into account, one in five of these therapeutics will be approved.[46]

Regulatory advances

The journey to therapeutic mAb approval has conventionally been a long, complex road, but regulatory agencies are now working together with the pharmaceutical industry to create more efficient pathways. Initiatives such as the FDA's expedited review programs aim to accelerate the availability of innovative new treatments to patients. In addition, conditional or emergency use authorizations are in place. For example, the approval of the mAbs targeting SARS-CoV-2 represent the fastest ever therapeutic mAb development to date.[59]

As the clinical experience with mAbs modalities expands, regulators are increasingly looking to streamline development by using approved "benchmark" molecules to assess the risk of novel mAbs. For example, the FDA and European Medicines Agency use data on anti-drug antibodies for approved benchmark drugs to estimate the immunogenicity risk of new drug candidates.[59] Additionally, the FDA's recent guidance on the Platform Technology Designation Program allows companies to use platform technologies to manufacture more than one drug or biological product through a standardized production or manufacturing process.[60] The goal of this program is to create efficiencies in the development, manufacturing, and review processes for drug products.

Harnessing collective knowledge, resources and expertise on mAb development, paired with more rapid approval processes, will facilitate the translation of research findings into clinical applications.

Conclusion and future perspectives

The therapeutic mAb development pathway is a long, challenging and costly process, with many development programs stopped at a late stage due to poor Ab quality, production issues or failure at the clinical development stage. Each step in the journey, from Ab discovery through to large-scale production, requires a labor-intensive optimization process, especially during CLD. Likewise, current ADCs are challenged by a combination of uncontrolled payload release, which limits the ability to achieve sufficient potency in cells, and the risk of potentially toxic side effects. These challenges highlight the need to optimize development methodologies shortening the time to market and ensuring these promising treatments reach patients faster. Collaboration between academia, industry, research institutions and regulators is crucial for advancing the discovery and development of mAbs.

There are four pillars to a more optimized approach to antibody production. First, automation of key manual steps in the process (e.g., antibody screening, clone selection and real-time continuous monitoring of cell culture) could expedite the process. Second, advanced analytical tools for checking cell viability combined with in-line or near-line sensors and probes can provide tighter control of Ab production and quality, reducing chances of contamination throughout the process. Third, using platform processes and integrating multimodal analytical tools across the lab can help speed up the crucial step of characterizing mAb CQAs. Fourth, automated real-time data capture and sophisticated data management tools can provide insights into process optimization to facilitate faster setup and scale-up of antibody production processes.

Despite these technological advances not all mAb-based drugs perform well in clinical settings, likewise the costs associated with their development can be prohibitive. This highlights the need for continued innovation and efficiency in the mAb development process, with the goal of balancing clinical performance with cost-effectiveness to ensure the high quality products reach patients sooner.

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2024-GBL-EN-105538-v1