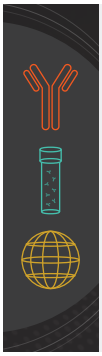

A Blueprint for Robust, Cost-Efficient Biosimilar Purification Using Ion Exchange Resins

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Abstract

Biologic drugs (biologics) are the fastest growing and most expensive sector of the pharmaceutical market, with global sales expected to approach $300 billion in 2021. Unlike chemically synthesized drugs, biologics are generated by living cells. Perhaps the most well-known example of a biologic drug is the monoclonal antibody (mAb). Given their complex structures and the time it takes to develop them, biologics can cost patients up to 22 times more per day than small-molecule drugs (Makurvet 2021). As the number of biologics approved increases each year, the cost of these medications places a heavy burden on healthcare systems. Therefore, streamlined processes for producing cheaper alternatives (namely, biosimilars) are necessary to meet the growing demand for these drugs as treatments for autoimmune conditions and beyond.

Introduction

The Drug Price Competition and Patent Term Restoration Act of 1984 (also known as the Hatch-Waxman Act) promoted the development of generic drugs by establishing the modern process for regulating them. Twenty-five years later, the Biologics Price Competition and Innovation Act of 2009 created a similarly abridged approval pathway for biosimilars (U.S. FDA 2016). The U.S. Food and Drug Administration (FDA) specifically defines a biosimilar as "a biological product that is highly similar to a United States licensed reference biological product" with no discernible differences in potency, quality, purity, and safety (U.S. FDA 2017). The high cost of biologics, which can exceed tens of thousands of dollars per patient annually, underscores the need for biosimilars to reduce healthcare costs and improve patient access to medications.

Since 2006, more than 700 biosimilars have been approved worldwide with global markets estimated to exceed $80 billion over the next 5 years (Harston 2021). This is a massive increase, as the previous 5-year span reached only $14 billion. Moreover, the availability of these cost-effective drugs is predicted to result in a savings of $100 billion through 2024. To date, 29 biosimilars against nine reference biologics have been approved by the FDA (U.S. FDA 2020). More than 42 patents for biologic drugs are set to expire, setting the stage for new biosimilars to enter the market (Table 1). In fact, regulatory authorities outside the U.S., including the European Medical Association (EMA) and the Central Drug Standard Control Organization (CDSCO) in India, began approving biosimilars a decade or more before the FDA. In 2020, the EMA approved eight biosimilar drugs while the FDA approved only two. India has become a global leader in biosimilar approvals with 98 in 2020 (Jeremias 2020). The number of biosimilar approvals worldwide highlights the growing demand for low-cost biosimilars.

Although they are meant to be cost-effective, biosimilars can be costly to produce. Developing a biosimilar involves (1) identifying a suitable expression system (that is, a cell line) for producing the drug; (2) culturing the cell line in large-scale fermentation bioreactors for drug synthesis; (3) harvesting the drug from either the cell mass or cell supernatant; (4) preparing cell samples by either filtration or centrifugation; (5) isolating the protein of interest from host cell proteins and host cell DNA using chromatography resins; (6) characterizing the functionality of the drug; and (7) formulating the final product. When producing biosimilars, pharmaceutical companies should strive to make the process as cost-efficient as possible while ensuring the final biosimilar passes regulatory guidelines, intellectual property restrictions, and tests of functionality. In the case of mAb biosimilars, the vast majority (~80%) of production costs are incurred by post-fermentation processes; indeed, chromatography accounts for over half (~60%) of these costs (Bracewell et al. 2015). Therefore, identifying a robust purification process is key to competing and succeeding in the biosimilar space. Ideally, this process should use a low-cost chromatography resin that maintains high functionality and performance.

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Table 1: Patent expiration dates for biologics in the U.S. and the EU. The expiration of these patents indicates when biosimilars can enter the market for each biologic.

Biologic	Brand Name	Target	U.S. Patent Expiration Date	EU Patent Expiration Date	Biosimilar Antibodies Available from Bio-Rad	Anti-Drug Antibodies Available from Bio-Rad	Protein Type
Adalimumab	Humira	TNF-α	2016	2018	•	•	Antibody
Aflibercept	Eylea	VEGF	2027	2027			Antibody-fusion
Alemtuzumab	Campath/Lemtrada	CD52	2021	2021	•	•	Antibody
Atezolizumab	Tecentriq	PD-L1	2027	2028			Antibody
Avelumab	Bavencio	PD-L1	2035	2032			Antibody
Belatacept	Nulojix	CTLA-4	2021	2023			Antibody-fusion
Belimumab	Benlysta	BAFF	2023	2021			Antibody
Bevacizumab	Avastin	VEGF	2019	2022	•	•	Antibody
Capromab pendetide	ProstaScint	GCPII	2036	N/A			Antibody
Catumaxomab	Removab	CD3 and EpCAM	N/A	2020			Antibody-fusion
Certolizumab pegol	Cimzia	TNF-α	2024	2021		•	Antibody
Cetuximab	Erbitux	EGFR	2016	2014	•	•	Antibody
Daratumumab	Darzalex	CD38	2026	2025			Antibody
Denosumab	Prolia/Xgeva	RANKL	2022	2025		•	Antibody
Durvalumab	Imfinzi	CD274	2029	2028			Antibody
Eculizumab	Soliris	C5	2020	2021	•	•	Antibody
Etanercept	Enbrel	TNFR2	2028	2015		•	Antibody-fusion
Evolocumab	Repatha	PCSK9	2025	2029			Antibody
Golimumab	Simponi	TNF-α	2024	2024		•	Antibody
Infliximab	Remicade	TNF-α	2018	2014	•	•	Antibody
Ipilimumab	Yervoy	CTLA-4	2022	2020		•	Antibody
Mogamulizumab-kpkc	Poteligeo	CCR4	2033	2035			Antibody
Muromonab-CD3	Orthoclone OKT3	CD3R	2021	N/A			Antibody
Natalizumab	Tysabri	Integrin 4	2015	2015	•	•	Antibody
Nivolumab	Opdivo	PD-1	2026	2027		•	Antibody
Ocrelizumab	Ocrevus	CD20	2023	2029			Antibody
Omalizumab	Xolair	IgE	2017	2017	•	•	Antibody
Palivizumab	Synagis	RSV protein F	2015	2015		•	Antibody
Panitumumab	Vectibix	EGFR	2020	2017		•	Antibody
Pembrolizumab	Keytruda	PD-1	2028	2036		•	Antibody
Pertuzumab	Perjeta	HER2	2023	2024			Antibody
Ramucirumab	Cyramza	VEGFR2	2025	2023			Antibody
Ranibizumab	Lucentis	VEGF-α	2022	2020		•	Antibody
Raxibacumab	Abthrax	*Bacillus anthracis* protective antigen	2026	2024			Antibody
Rituximab	Rituxan	CD20	2018	2013	•	•	Antibody
Secukinumab	Cosentyx	IL17A	2020	N/A			Antibody
Siltuximab	Sylvant	IL6	2027	2034			Antibody
Tocilizumab	Actemra	IL6R	2015	2017	•	•	Antibody
Tositumomab	Bexxar	CD20	2029	Withdrawn			Antibody
Trastuzumab	Herceptin	Erb-B2	2019	2014	•	•	Antibody
Ustekinumab	Stelara	IL12 and IL23	2024	2023		•	Antibody
Vedolizumab	Entyvio	Integrin α4β7	2026	2024		•	Antibody

Process Development

Purification of mAb-based biosimilars starts with a capture purification step, which typically uses a Protein A resin. This resin binds mAbs with high affinity and specificity, enabling high purity after a single purification step. In addition, Protein A resins are relatively unaffected by buffer conditions at pH >6. Still, there are several disadvantages associated with using Protein A resins in a mAb purification process. Despite their purification capabilities, Protein A resins cannot differentiate between functional and aggregated mAbs and can even increase mAb aggregation during purification (Jin et al. 2019). Moreover, while mAb biosimilars bind Protein A resins under neutral or basic conditions, elution requires substantially more acidic conditions (pH 3.0–3.6). This low pH can disrupt the structural integrity of mAbs and cause them to aggregate or precipitate. These conditions can also cause the Protein A ligand to leach from the resin into the purified sample, contaminating the purification workflow and limiting the resin's reusability by reducing its binding capacity over time.

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Finally, Protein A resins can be two to three times more expensive than chemical resins depending on the manufacturer. For these reasons, replacing Protein A resins in the purification workflow should be prioritized as a means of reducing the production costs of biosimiliars. An ion exchange resin like the Bio-Rad Nuvia S Strong Cation Exchange Resin is a prime substitute, as it offers high binding capacity and robust chemical stability at a lower price point.

A greater than fourfold reduction in chromatographic costs can be achieved by replacing Protein A resins with the Nuvia S Strong Cation Exchange Resin (Posch et al. 2020; Drevland et al. 2018). Although it is not quite as selective as affinity resins, Nuvia S Resin is more stable and reusable, making it more robust than protein-based resins. Nuvia S Resin also has a greater dynamic binding capacity (nearly threefold higher) than standard Protein A resins. To illustrate the capabilities and economic benefits of replacing Protein A resins with Nuvia S Resin, we developed a three-step purification process that can be applied to the production of future mAb biosimilars as their reference drug patents expire (Figure 1; Posch et al. 2020, Drevland et al. 2018). The mAbs used in these studies — adalimumab (Drevland et al. 2018) and rituximab (Posch et al. 2020) — were produced by Chinese hamster ovary (CHO) and human embryonic kidney (HEK) 293 cells, respectively. During the capture step for each biosimilar, Protein A resins efficiently removed ~99% of the host cell proteins, whereas the Nuvia S Resin removed 94–96% of these proteins. Additional experimental details can be found in bulletins 7130 (Drevland et al. 2018) and 7382 (Posch et al. 2020).

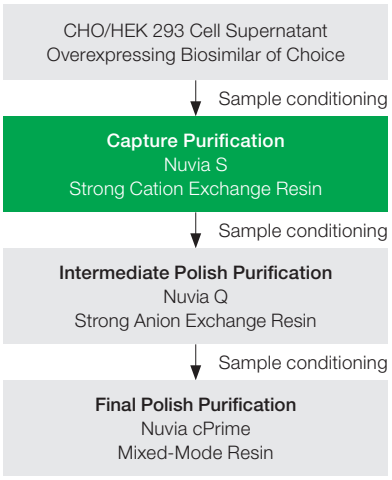

Fig. 1. An ion exchange–based biosimilar purification workflow. Biosimilars can be purified from overexpressing cell lines using ion exchange resins. After the initial capture purification step using Nuvia S Resin, additional polishing steps are applied to remove host cell proteins and DNA. This example workflow also uses Nuvia Q Strong Anion Exchange Resin in flow-through mode to remove contaminating proteins and DNA. The final polishing step eliminates the remaining contaminating proteins using Nuvia cPrime Mixed-Mode Resin. CHO, Chinese hamster ovary; HEK 293, human embryonic kidney 293.

Figure 2 illustrates the economics of purification processes using Protein A resin versus Nuvia S Resin. Purifying 1 g of a mAb using a Protein A resin costs about $1,300 for the buffers and resin. Alternatively, purifying the same amount of mAb using Nuvia S Resin costs approximately $50. The price difference between the two resins reflects the higher dynamic binding capacity of Nuvia S Resin as well as its reusability. Yield also has to be considered in these calculations. Fortunately, yields using ion exchange resins can reach levels (85%) that are only modestly lower than those obtained using Protein A resins (90%) (Posch et al. 2020). Although both capture steps require subsequent polishing steps, the ion exchange approach may require a second polishing step to ultimately achieve the purity required of a biologic. Nevertheless, even with an addition of a third resin, the ion exchange purification process is far more cost-efficient than the Protein A–based process.

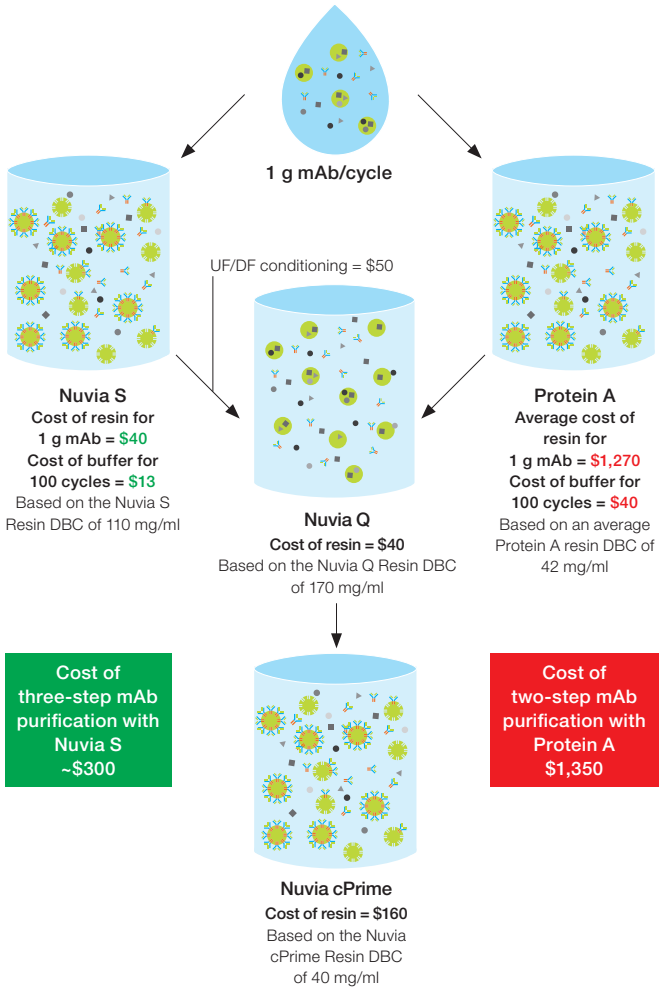

Fig. 2. Process economics comparison of purification workflows using either Nuvia S Resin or Protein A resin in the capture step. Cost calculations reflect the amount of resin required to purify 1 g of mAb per cycle for 100 cycles. The cost of the Nuvia S Resin is based on the price of a 25-ml bottle of resin (U.S. list price: $115) with a dynamic binding capacity of 110 mg/ml (as measured for adalimumab). The cost of the Protein A resin was calculated as the average price of a 25-ml bottle of resin, determined from three commercially available Protein A resins (U.S. average list price: $1,334): MabSelect PrismA (Cytiva), Praesto Jetted A50 (Purolite Corporation), and POROS MabCapture A (Thermo Fisher Scientific Inc.). The average dynamic binding capacity of these Protein A resins is 42 mg/ml. Costs are calculated based on 2021 pricing. UF, ultrafiltration; DF, diafiltration; DBC, dynamic binding capacity.

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Confirming Functionality

In order to obtain regulatory approval, biosimilars must be demonstrably similar to their reference biologics in terms of safety, efficacy, and immunogenicity (Sharma et al. 2020). This can be done using multiple assays, including pharmacokinetic (PK), anti-drug antibody (ADA), and functional assays. As part of the totality of evidence required by regulatory agencies, the results of these assays must be robust. Therefore, the reagents used must meet high performance standards. To provide high-quality reagents for confirming the functionality of biosimilars, Bio-Rad has designed and validated recombinant monoclonal ADAs and research-use biosimilar antibodies for several biologics, as summarized in Table 1.

Bio-Rad offers three types of ADAs that are useful for confirming the functionality of biosimilars. These ADAs target different sites on a biosimilar drug (see Figures 3 and 4), enabling detection of free drug (Type 1), total drug (Type 2), or bound drug (Type 3) (Harth and Frisch 2021). Bio-Rad Anti-Biotherapeutic Antibody Reagents for ADA and PK assay development were selected from Human Combinatorial Antibody Libraries (HuCAL®) via in vitro phage display. Guided selection strategies enable the generation of fully human, highly specific inhibitory and noninhibitory anti-idiotypic antibodies and specialized drug target–complex binders. These different types of antibodies enable development of PK assays to detect free or total drug, or drug bound to its target. Additionally, the inhibitory antibodies can be used as reference standards in an ADA assay, replacing the need to source antibodies raised in animals.

When developing the purification process for adalimumab and rituximab biosimilars, anti-idiotypic antibodies from Bio-Rad were used to demonstrate similarity with the reference protein in PK bridging studies. This step is required by all regulatory agencies in the development of a biosimilar (Sharma et al. 2020). In the case of rituximab, an immobilized Type 1 anti-idiotypic antibody was used to capture the biosimilar, after which a Type 2 anti-idiotypic antibody was used to detect total biosimilar levels (Posch et al. 2020). In the development of the adalimumab purification process, immobilized drug target (TNF-α) was used to capture the biosimilar, which was then detected with an anti-idiotypic antibody (Drevland et al. 2018). Flow cytometry was also used in this case to demonstrate functional equivalence in vitro. In both of these studies, the purified mAb behaved the same as the reference drug product, indicating the purification process did not alter functionality or effectiveness of these biosimilar antibodies.

In addition to the off-the-shelf anti-biotherapeutic antibodies, Bio-Rad also offers custom anti-idiotypic antibodies generated using HuCAL technology. Refer to bulletins 37068 and 46147 for more details.

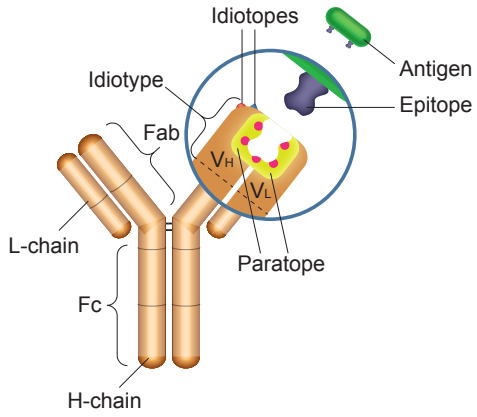

Fig. 3. Schematic of an antibody variable region illustrating epitopes, idiotopes, and paratopes. A paratope refers to a binding site for an antigenic epitope. An antibody idiotope refers to the portion of the variable regions that binds to the paratope of another antibody. Fab, antigen-binding fragment; L-chain, light chain; H-chain, heavy chain; V_H, heavy chain variable domain; V_L, light chain variable domain.

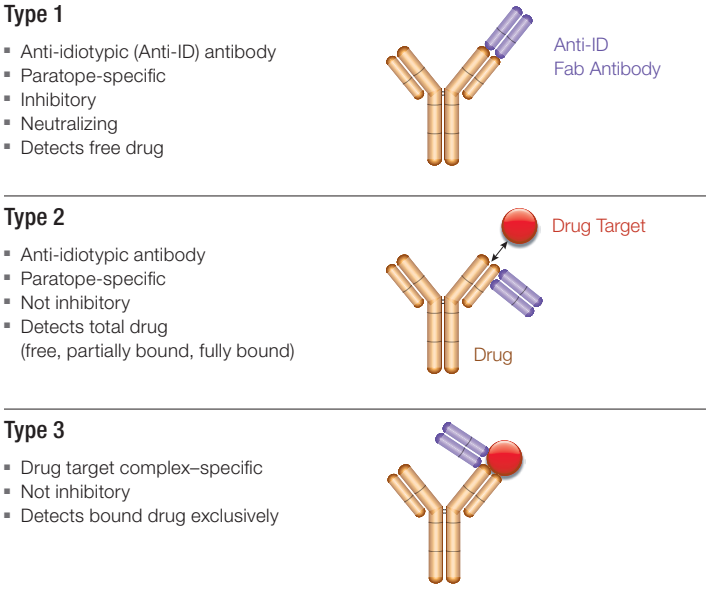

Fig. 4. Three different types of anti-biotherapeutic antibodies. Type 1, inhibitory anti-idiotypic antibodies for measuring free (unbound) drug; type 2, non-inhibitory antibodies for measuring total drug; type 3, drug target complex–specific antibodies for measuring bound drug exclusively.

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Conclusion

The U.S. and EU patents of more than 20 biologic drugs are set to expire within the next two decades, paving the way for the development and approval of more biosimilars. Remaining competitive in this expanding market will require developers to lower production costs while maintaining the purity and efficacy required to meet regulatory standards for biosimilars. To assist with the development of biosimilars, we have provided a blueprint for improving process economics by replacing Protein A resins with Nuvia S Strong Cation Exchange Resin in the capture step of the purification workflow. Nuvia S Resin, along with the catalog of chromatography resins available from Bio-Rad, can provide a more cost-effective purification process for biosimilar development. Additionally, Bio-Rad offers a large range of anti-biotherapeutic antibodies that can be used to help confirm the functionality of biosimilars after purification.

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