White Paper: Innovative Technical Approaches are Needed to Reduce the Cost of Antibody Production for Global Use

Executive Summary

Monoclonal antibodies (mAbs) have revolutionized the treatment of many complex diseases over the last two decades, but they are expensive and have primarily benefited patients in middle- and high-income countries. For the rest of the world, the unrealized therapeutic potential of mAbs is enormous, not only for expanded access to current or future therapies, but also for the prevention of infectious diseases like HIV and malaria that kill hundreds of thousands of people every year.

Despite extensive research and development, the cost of manufacturing antibodies typically exceeds $50 per gram of purified antibody or drug substance ($50/g of DS), which is far higher than the target of $10/g of DS required to make them affordable in some of the world's poorest regions. This goal is driven by the projected efficacious dose as well as the price target of $5 per dose, which is comparable to other treatments for infectious diseases, such as malaria chemoprevention that costs $2-$8 per season per child. Despite their clinical effectiveness against malaria and many other diseases, mAbs remain out of reach for low- and middle-income countries, in part because of the high cost of manufacturing. To make mAbs more accessible globally, particularly for infectious diseases and emerging health threats, innovative manufacturing approaches are urgently needed to drive costs below $10/g. Lowering the production costs of mAbs would also enhance access to treatments for chronic diseases, such as cancer and autoimmune disorders, and pave the way for affordable therapies targeting conditions like Alzheimer's disease and arthritis.

These cost-saving innovations could arise from a variety of sources: they could build upon recent advances in manufacturing development for mammalian or other non-mammalian host cells, or they could draw on practices from adjacent industries such as those for blood products, foods, or industrial enzymes. The challenge of cost reduction could be approached either holistically, by applying a solution across the entire process, or very specifically, by improving an individual process element that could then be combined with other innovations for integration into a lower-cost overall process.

The goal of this White Paper is to introduce the current landscape of antibody manufacturing along with its economics, provide examples of recent advances that have contributed to cost reduction, and encourage learnings from adjacent industries with relevant processes. The examples provided are intended to illustrate the broad range of potential approaches and to spark creative ideas that could be implemented to further reduce overall manufacturing costs. Achieving antibody production at $10/g of DS or lower would expand access to these life-saving treatments for the vast populations around the world where they can have the greatest impact, while also favoring the development and commercialization of novel mAbs for diseases that do not have effective treatments.

1. Background and Significance

The "monoclonal antibody revolution" of the past 20 years has seen an extraordinary expansion of the therapeutic applications of antibodies, Fc fusions, and related proteins, for example in the

treatment of cancer, autoimmune diseases, and infectious diseases [1, 2]. Of particular interest to the Gates Foundation is the use of antibodies for the prevention of infectious diseases, including HIV, malaria, and RSV.

Mammalian cell culture, especially using Chinese Hamster Ovary (CHO) cells, remains the dominant platform for monoclonal antibody (mAb) production. CHO systems are highly productive and benefit from well-established regulatory acceptance [3]. However, these systems are capital-intensive and require large manufacturing facilities, expensive raw materials and long development timelines, making it challenging to reduce costs significantly. Moreover, infectious disease mAbs often require larger doses, which result in higher tonnage demands that would strain the current global manufacturing capacity.

The cost to manufacture pharmaceutical-quality antibodies keeps these drugs out of reach for low- and middle-income countries (LMICs), with cost of goods (COG) values typically exceeding $50 per gram of drug substance ($50/g of DS). This cost must drop below $10/g of DS to be accessible for LMICs. For example, to compete with alternative treatments for infectious diseases, mAbs would require a price per dose of approximately $5-10. If the dose is 0.5-1 g, this would translate into a break-even cost of $10/g of DS. This value can be used as a good approximation for mAbs in general, although many factors, including value-based pricing that accounts for efficacy and duration, would be used to define a cost target for a specific mAb.

A cost of $10/g of DS would allow mAbs to become more affordable, in line with other treatments such as PrEP for HIV or chemoprevention for malaria. For example, the administration of sulfadoxine-pyrimethamine plus amodiaquine as a chemoprevention against seasonal malaria costs $8 for 4 doses and corresponds to a range of $2-$8 per child per season [4]. Although this intervention is considered cost-effective, there are challenges in reaching a large population with multiple doses per year. In contrast, recent clinical studies of a mAb to prevent malaria in Mali have shown high efficacy with a single subcutaneous dose that can protect for an entire season [5]. Because 94% of world-wide malaria cases in 2022 occurred in Africa, and the disease killed 600,000 people (mostly children under 5), a single-dose mAb could save countless lives if it was affordable and could be manufactured at the scales needed to support an estimated worldwide demand of thousands of kilograms per year.

The most widely used host cells for mAb culture are CHO cells, and recent advances in cell culture and purification, such as continuous and integrated processing, have so far reduced the footprint and improved the flexible usage of space, but the potential cost reduction has so far been demonstrated only at small proof-of-concept scale. Other innovations have simplified the process or reduced the cost of individual components, but they have not sufficiently lowered the overall production cost. Alternative host cells also show potential for cost reduction in some areas, but additional work is needed.

Part of the reason that current mAb costs remain above $50/g of DS is that pharmaceutical process development is constrained in several ways: the urgency of preparing clinical batches to obtain an early indication of the performance of a drug in humans, the need to use established

process platforms that can be transferred to existing manufacturing networks, and the need to address regulatory requirements. As a result, there has been limited focus on the overall COG early in development, and it becomes more challenging to change the materials or the process to reduce costs at later stages of development. Furthermore, because of the established process platforms and strict regulatory requirements, most pharmaceutical companies are not incentivized enough to take risks on innovative (non-platform) manufacturing technologies. In fact, for for-profit companies, $50/g (or even $200/g) of DS is acceptable because COGs represent only a small fraction (<10%) of the product sales price. Some significant contributions to the final price of mAbs include the costs of R&D, marketing and distribution, and profit margins. In contrast, other proteins such as blood products, food proteins, and industrial enzymes can be manufactured at far lower costs, often using technologies not widely implemented for biopharmaceuticals.

Deploying innovative approaches and technologies to further reduce the cost of mAb production would address existing gaps in global health. The availability of effective treatments for cancer, autoimmune disorders, and infectious diseases could be extended to low- and middle-income countries that cannot currently afford them. In addition, novel discoveries and treatments could be developed, commercialized, and made available faster to patients everywhere. And, specifically for endemic or emerging infectious diseases, the spread of the infectious agents could be slowed or stopped, and the world would be better prepared for future pandemics, no matter where they arise.

2. Biomanufacturing Format and Economic Analysis

The manufacturing of mAb DS involves two main steps: upstream processing and downstream processing. During upstream processing, mAb molecules are produced by genetically engineered cells, typically mammalian cells such as CHO cells. These cells are cultured in bioreactors, where they grow and express the mAbs, which are then harvested from the culture fluid. The bioreactors can operate in different modes: batch, fed batch, or perfusion (continuous). Fed batch is the most frequently used mode in industrial mAb production because of its ease of operation and economy of scale for high demand products. Recently, the industry has been shifting towards the use of single-use bioreactors (SUBs) rather than traditional stainless-steel bioreactors. Single-use technologies are often favored because of advantages such as greater space flexibility, reduced cleaning and sterilization needs, lower capital investment, and shorter time to build a facility. In downstream processing, chromatographic and filtration steps are employed to purify the mAb and remove both process- and product-related impurities such as host cell proteins, DNA, and aggregates, thus ensuring product quality and safety. The process typically includes Protein A affinity chromatography, ion exchange, and other polishing steps, followed by ultrafiltration/diafiltration for the final formulation. Recently, efforts have been made to integrate upstream and downstream processes to enable end-to-end continuous manufacturing with the goal of increasing efficiency and reducing costs. However, this approach is still challenging because of the discrete nature and mismatched throughput of some unit operations using available technologies and equipment. As a result, hybrid continuous processes—combining batch and continuous operations in a way that fits with existing facilities and equipment—are being explored as a compromise. These hybrid systems have shown promising results in proof-of-

concept studies, suggesting that they could serve as a bridge toward fully continuous manufacturing in the future.

To investigate the effectiveness of a new innovation in reducing the overall cost of antibody manufacturing, an economic analysis is required in addition to an assessment of its technical feasibility. This is important because the cost of an individual element of the manufacturing process might be reduced with development work, but other unplanned compensatory changes elsewhere in the process may negate that cost reduction. As a result, the focus should remain on the overall goal of $10/g of DS by using a holistic approach to model the cost of the entire process with the integrated improvements rather than relying on the cost savings from an individual improvement alone.

An illustration of a decisional tool for cost of goods (COG) analysis is provided by Mahal, Branton, and Farid [6]. They used this tool to compare the manufacturing COG for a monoclonal antibody produced by CHO cells at different manufacturing scales by four types of processing: stainless-steel batch, single-use batch, single-use continuous end-to-end, and single use hybrid (continuous/batch). Specific assumptions were made for cell culture titers, upstream and downstream process parameters, and materials costs, as well as process flows and schedules for the four options (Table 2 in [6]). Their tool also broke down the data by source of cost and by upstream vs. downstream portions of the process, and they performed a sensitivity analysis to identify the cost-critical parameters for process optimization.

The results showed that, at the smallest demand studied (100 kg/year), single-use facilities (batch or continuous) performed much better in terms of commercial COG than stainless-steel facilities (Fig. 1). As the annual demand increased up to 3000 kg/year, the COGs decreased for all facility types and were comparable for the continuous facilities and the stainless-steel batch facility. Furthermore, the COG does not reach the target of $10/g of DS, and new innovations are needed beyond continuous processing.

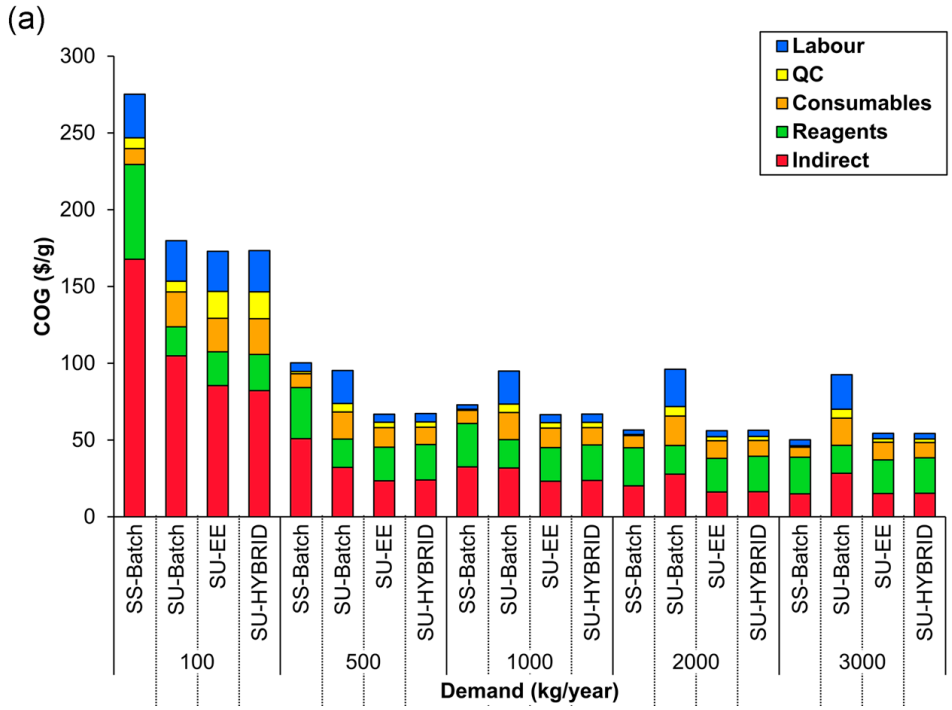

Figure 1. Cost of goods (COG) analysis for CHO-based production of monoclonal antibodies (Fig. 3a from [6], Authors: Mahal H, Branton H, and Farid S., CC BY 4.0, https://creativecommons.org/licenses/by/4.0/). Four types of facilities were assessed: stainless-steel batch (SS-Batch); single-use batch (SU-Batch); single-use continuous end-to-end (SU-EE); and single use hybrid, i.e., continuous for the early part of the downstream process and batch for the later part, (SU-Hybrid).

Several of the cost drivers in bioprocessing may suggest opportunities for further cost reduction. For upstream processing, cell culture medium is very expensive (it could be on the order of $1 M/batch). For downstream processing, chromatography media may represent high up-front expenses (on the order of millions of dollars for Protein A for a 15,000 L batch), but the cost per gram of product can be significantly reduced if the resins can be heavily re-used (over 50 re-uses for Protein A). Virus filtration membranes are single-use and expensive, although some of them may be able to tolerate NaOH at relevant levels and times to permit some re-use. The buffers used for processing can also be expensive.

For facility design and planning, several factors favor single, large, centralized facilities over widely dispersed smaller production facilities. For example, water for injection (WFI) is cheap at large plants with dedicated production of WFI in house; at large scale, steel is cheaper than single-use materials; and analytical costs are relatively small at large batch sizes. Facility operating costs could also be a factor. Depreciation and HVAC may be acceptable on a $/g basis if production from the facility is large. Labor costs may be high but will depend on the region or country where the facility is built.

The industry has made huge progress over the last two decades in reducing the COG from ~$1000/g to ~$50/g. However, even with projected increases in cell culture titer and reasonable estimates of decreased costs of resins and buffers, it is highly unlikely that the overall COG can be reduced to the target of $10/g, and novel approaches in process development and manufacturing are needed for further cost reductions. It will be important to assess new innovations and technologies using the integrated type of decision tool described above [6] to ensure that their impact on the entire process can be captured.

3. Landscape of Antibody Manufacturing and Cost Reduction

a. Recent Advances in Bioprocessing of Mammalian (CHO) Cells

Numerous approaches to improve the economics of biological processes are under active consideration in the industry, and a few examples are provided below for illustration. Integrated continuous processing has received significant attention for its potential to reduce COGs at commercial scale, although its development can be complex. To facilitate the development of these types of processes, the determination of processing parameters could be accelerated through novel optimization methods. To reduce the COGs, continuous precipitation-filtration has been investigated as a cheaper alternative to chromatography, although the resulting increase in the number of steps may limit the overall cost reduction. Finally, an example of flexible modular facility design is provided to illustrate how modeling and facility redesign could be used to accommodate a variety of novel approaches and innovations.

i. Integrated and Continuous Bioprocessing

Integrated and continuous bioprocessing (ICB) is being implemented as a flexible and rapid approach to scale up the production of therapeutic proteins from mammalian cells. Coffman et al. [7] describe a common ICB framework that represents the range of processes being implemented in numerous companies. It consists of a perfusion bioreactor linked to an integrated downstream process with the following steps: dual-column chromatography for capture and polishing, virus inactivation and filtration, and ultrafiltration/diafiltration (Fig. 1 in [7]). A staged approach can be used to scale up the plant (Fig. 3 in [7]) because the downstream portion of the process can remain essentially the same while the number of bioreactors is increased. For example, Stage 1 uses a 500 L bioreactor and could produce up to 30 kg per batch, which could support clinical studies and corresponds to about 0.5 tons per year. Stage 2 uses four 500 L bioreactors and could produce up to 2 tons per year. Stage 3 would require larger (2000 L) bioreactors and could produce up to 8 tons per year. The plant sizes are constrained by (1) the solution and media preparation volumes and (2) the flow rate of the pumps on the capture step. The details of the ICB design are provided in a companion paper by Coffman [8].

ii. Rapid Optimization of Integrated Purification to Reduce Development Costs

Because of the complexity of an integrated and continuous downstream process, its optimization is more challenging and takes longer than for a typical batch process. To reduce this development time and associated costs, Crowell et al. [9] propose a method to design and optimize an integrated continuous multi-column process that is considered as a single unit operation. Notably, they were able to design a “straight-through” process that does not require the use of hold tanks or buffer exchanges, such that it could be more easily intensified

during future scale increases. For the optimization, they used an *in silico* tool to select the chromatography resins, conducted conventional, single-column screening to determine the operating ranges, and employed a statistical model using design of experiments (DOE) on the integrated multi-column unit operation to establish the optimum conditions. Using those results, they demonstrated high yields for both an antibody (88%) and a cytokine (86%) as well as low impurity levels (host cell protein, DNA, aggregates) for both products. The authors indicate that this approach could also be used to simplify and accelerate optimization of other downstream unit operations.

iii. Continuous Precipitation-Filtration Process for mAb Capture

To reduce the cost associated with the commonly used Protein A capture chromatography resins, Minervini et al. [10] explored the use of a continuous precipitation-filtration process for initial capture of a mAb from clarified cell culture fluid. To precipitate the mAb, they used $ZnCl_2$ as a cross-linking agent and polyethylene glycol (PEG-3350) as a volume exclusion agent in a tubular precipitation reactor. The dewatering and washing steps were accomplished by tangential flow filtration at two scales: (1) a lab-scale two-stage countercurrent washing system, and (2) a small commercial countercurrent hollow fiber membrane system that consisted of a dewatering module followed by four stages of countercurrent washing (Fig. 1 and 2 in [10]). The authors also advanced process monitoring technology by using an in-line probe to assess particle size distribution at the outlet of the precipitation reactor. This type of real-time feedback on the particle distribution from the reactor can allow more consistent operation of the subsequent dewatering and washing steps.

They demonstrated good antibody yields (87% for lab-scale two-stage; 92-93% for commercial 4-stage) and showed that continuous precipitation-filtration is feasible for antibody capture and can be scaled. However, additional optimization is needed to further reduce DNA levels, assess prolonged use of filters, and reduce the volume of water and other reagents (e.g., salts, PEG).

The economics of end-to-end continuous antibody manufacture have been evaluated with column-free capture alternatives (aqueous two-phase extraction or precipitation) and compared to traditional capture column chromatography with Protein A [11]. The results showed that the costs of precipitation were similar or higher than those of Protein A chromatography, and that further optimization is needed to improve volumetric productivity and yields and reach the COG target.

iv. Flexible Facilities (to accommodate new processes)

Many biopharmaceutical manufacturing facilities now in use were constructed with stainless steel equipment to manufacture a single product. They typically required large initial capital outlays and long lead times, and they are operated at about 70% of their fixed maximum capacity. As a result, it is challenging for manufacturers to respond to large fluctuations in demand such as those seen for antibodies against SARS-CoV2 during the pandemic. Garcia and Gefroh [12] introduce a promising first-generation flexible modular facility that uses ICB and single-use materials to provide small processing spaces, higher throughputs, and lower costs, especially early in the product lifecycle because the modular design allows straightforward scale-up. This example is provided to illustrate the type of flexible facility

design that could accommodate novel approaches to bioprocessing for further cost reduction, even though – in the long run – it might not be as cost-effective as large stainless-steel facilities if demand is high.

In addition to the examples discussed above, other advances in bioprocess development include improved process analytical technology (PAT) for integrated testing, new approaches to protein partitioning and precipitation, and novel separations materials, affinity tools, and membranes. Furthermore, engineering biology and AI-driven tools have significant potential to improve both cell line or strain engineering and the design of novel bioprocess materials, such as growth media, purification resins, and filters. Though not yet fully utilized, these advancements could improve upstream and downstream productivity and purity and reduce costs.

b. Alternative Host Cells

Because of the high cost of mAb production from CHO cells, non-mammalian hosts such as yeasts, filamentous fungi, and plants are being pursued as alternatives, in particular for equitable access to these medicines [13]. Potential advantages include faster growth rates, more robust and cheaper fermentations, fewer secreted host cell proteins to be removed, and reduced susceptibility to adventitious agents. These could lead to faster development times and cheaper products that would be more accessible worldwide. However, a common challenge with alternative host cells is achieving a glycosylation pattern that does not adversely impact the pharmacokinetics or potency of the antibody in humans. And additional work is needed to realize meaningful cost reductions, such as by developing open-source strains and establishing integrated continuous platform processes for antibody purification. Examples of mAb production with three types of organisms are discussed below to illustrate some of the recent advances in the field.

i. Filamentous Fungi

Kaiser et al. [14] used a genetically engineered filamentous fungus, *Thermothelomyces heterothallica* ("C1") to produce a well-characterized human monoclonal antibody (HuMab 87G7) against SARS-CoV2. The fermentation used a 4–7-day fed-batch process with inexpensive media components, and the antibody was purified with Protein A chromatography.

The C1-produced antibody and the mammalian-produced antibody showed comparable *in vitro* virus binding and neutralization. However, the glycosylation patterns were different, and further study is needed to assess the potential long-term clinical effects of these differences. *In vivo*, the C1-produced antibody provided protection against multiple variants in hamsters and against the Delta variant in non-human primates. This study shows that it is possible to produce human mAbs in the C1 host for the prevention and treatment of respiratory virus infections, and it seems likely that the short fermentation process and low-cost media can provide an advantage over mammalian cell cultures, especially when dealing with a rapidly mutating virus like SARS-CoV2 during a pandemic.

The current use of C1 to produce mAbs is an example of an innovation inspired by a different industry. This organism was originally used to manufacture industrial enzymes and other proteins, and it was later genetically engineered for successful biotechnology applications.

ii. Yeast (Pichia)

The yeast *Pichia pastoris* (also known as *Komagatella phaffi*) has been widely used for the industrial production of proteins, and it is considered as "generally regarded as safe" (GRAS) by the FDA. Some of its advantages are that it grows to very high cell densities, uses methanol as a cheap growth substrate, and secretes most recombinant proteins into the culture supernatant, which facilitates their purification. However, the wild-type yeast produces glycoproteins with high levels of added mannose. Because the surface carbohydrates are critical to the function of most antibodies, significant glycoengineering has been performed to avoid this mannosylation and to add the enzymes that would produce antibodies with human glycosylation patterns. Nylen and Chen [15] provide a detailed stepwise protocol to prepare full-length monoclonal antibodies from a glycoengineered humanized *P. pastoris* expression system. In general, antibodies from both CHO cells and *P. pastoris* have a heterogeneous glycosylation pattern. Liu et al. [16] developed a way to prepare the mAb trastuzumab with homogeneous glycosylation by first expressing it in *P. pastoris*, purifying it from the culture supernatant, and then remodeling its glycosylation pattern *in vitro* using a series of endoglycosidases. The resulting homogenously glycosylated antibody showed strong binding affinity to FcγRIIIA , which is essential for its therapeutic activity.

So far, one full-length antibody produced in *Pichia* has been approved by the FDA: Eptinezumab for the prevention of migraines. Further work is needed to realize the potential of *Pichia* as a cost-effective platform for the production of antibodies by increasing fermentation titers and better characterizing the genes to engineer desired product characteristics with high efficiency.

iii. Plants

Glycoengineered plants have also been used to produce mAbs with mammalian glycoforms, and they are low-cost, readily scalable, and not vulnerable to human pathogens [17]. Multiple plant-based mAbs are in pre-clinical and early clinical stages against viruses like Ebola, HIV, West Nile, dengue, and Zika (Table 1 in [17]). However, a significant challenge for downstream processing is that mAbs produced in plant cells are not secreted but are retained within the cell wall, and the recovery and purification steps are much more complex as a result. Work is ongoing into methods like aqueous two-phase separation as an early step to purify and concentrate the mAbs and the addition of enzymes to disrupt the cell wall and facilitate recovery. In addition, mAb expression levels need to be increased to levels comparable to those in mammalian cells to compete at a commercial scale.

iv. Cell-Free Protein Synthesis

Emerging platforms such as cell-free synthesis (CFS) offer potential cost savings by bypassing the need for complex cell line development and reducing production times from weeks to hours. Although not yet economically viable compared to CHO systems, further advancements in process yield and efficiency could position CFS as an attractive route to reducing mAb COGs values [18] while simplifying infrastructure demands in LMIC regions for enhanced accessibility.

c. Adjacent Industries

Protein production is important, not only for pharmaceuticals, but also for several other industries such as food (including cultured meat), industrial enzymes, and blood fractionation. Some of

these have low manufacturing costs or have significantly reduced those costs over time. In addition, similar processing steps such as membrane filtration are used in some medical applications. Process development for mAbs may be able to draw on practices from these adjacent industries, which share commonalities in processing technologies that could inspire routes to achieving cost reductions.

Three examples are provided below to illustrate some of the similarities in processing: the production of human serum albumin from blood products, the removal of waste products and extra fluid from the blood during hemodialysis, and the production of whey proteins from milk.

i. Blood Fractionation for Production of Human Serum Albumin

Human Serum Albumin (HSA) is the most common circulating protein present in blood, and it has long been used clinically to treat a wide variety of conditions, including the restoration of blood volume in emergencies. Its production typically occurs at very large scale from pools of human plasma that contain high concentrations of HSA. The original process developed in the 1940s and 1950s involved fractionation through stepwise increases in ethanol concentration to remove specific blood components at each step. The process has been modified over time to include ultrafiltration/diafiltration or chromatography steps to increase product purity and safety while remaining cost-effective, and different manufacturers may use different processes to meet pharmacopeial requirements [19]. In addition, a variety of alternative technologies and approaches, such as multiple precipitation and chromatography techniques, have been assessed at smaller scales [20]. Although some show promise, the authors indicate that their low efficiency may limit their use to lab-scale purifications.

Note that the requirement for HSA purity in the United States Pharmacopeia is ≥ 96%, which is less stringent than for mammalian-derived mAbs, and it is possible to achieve this level of purity more cheaply without chromatography. In contrast, a typical purity requirement for mAbs is ≥ 98%, and any impurities present at a level of 1% or greater need to be well-characterized. In addition, all product variants must be thoroughly characterized for mAbs but not for HSA, and there are no specifications for host cell proteins for HSA because there is no cellular host. Although the standards are more stringent for mAbs, some of the approaches and processes unique to blood product manufacturing could spark ideas for mAb cost reduction.

ii. Hemodialysis

Hemodialysis is a life-saving treatment for patients with acute or chronic kidney failure. It uses membrane filtration to remove waste and water from the blood, adjust blood pressure, and control salt concentrations [21]. Because patients typically receive hemodialysis several times a week and use large volumes of highly purified water and dialysate each time, both the purity of the water used to prepare the dialysis solution and the quality of the hemodialysis filter itself are critical components of the safety of the procedure.

The water used in dialysis facilities is purified from the local drinking water using a series of filtration and ultrafiltration steps to remove chemical and microbiological contaminants. It is

then used to prepare the dialysis fluid that contains the buffers and components needed to exchange with a patient's blood [22]. The quality of the dialysis fluid is specified by standards from multiple groups: the Association for the Advancement of Medical Instrumentation (AAMI), the Center for Medicare and Medicaid Services (CMS), ISO 23005-5, and some pharmacopeial monographs. Some filter manufacturer websites indicate that they can purify water to "ultrapure" standards.

Developments have also continued for the hemodialysis systems and filters. The design and performance of single-use hollow fiber membranes has been improved, for example to increase efficiency of waste product removal or to tightly control pore sizes to bring the performance closer to that of the kidney and improve patient outcomes. Another advantage of these countercurrent dialysis units is the single-pass, low-shear operation that can handle the high viscosity of blood. Because they are used so frequently in hemodialysis equipment, the pre-sterilized cartridges are designed for straightforward set-up and process control, and they are manufactured in very large numbers that substantially reduce the cost.

It is possible that some of the technologies and approaches used to enhance the performance and safety of the hemodialysis filters could be applied to mAb purification. For example, hollow fiber countercurrent dialysis has now been demonstrated for continuous buffer exchange of a concentrated IgG solution [23], and it could potentially replace batch diafiltration for buffer exchange and formulation. The authors used commercially available hollow fiber dialyzers to process over 0.5 kg of IgG per day in a scalable, low-cost process with buffer requirements several fold lower than in standard batch diafiltration. They suggest that continuous countercurrent dialysis for buffer exchange and formulation could substantially reduce the cost of this step. Furthermore, the framework for integrated and continuous biomanufacturing described in Section 3.a.i. could accommodate this use of hollow fiber dialyzers and could potentially provide an eight-fold reduction in buffer volume [8]. However, the typical high molecular cut-off on the order of 50,000 Da used for hemodialysis may limit their use to buffer exchange.

iii. Whey Protein

An example from the food industry is the manufacturing of whey protein from milk, often as a byproduct of cheesemaking. A variety of whey products prepared with different levels of processing are added to food, nutritional supplements, and infant formulas as a source of protein or as an emulsifier.

During milk processing, the liquid whey is separated from the cheese curds, filtered to remove insoluble compounds, and microfiltered to reduce the fats, carbohydrates (mainly lactose), and water, and produce whey protein concentrate (WPC) at a range of protein levels (25-80%). This can be further ultrafiltered to remove additional fat and lactose and produce the more expensive whey protein isolate (WPI, ≥ 90% protein) [24]. A variety of membranes and technologies can be implemented to improve process efficiency and yield as well as recover additional high-value fractions [25].

Whey protein is included to illustrate the purification processes designed to produce a variety of consumer products at different purity levels and at costs that are typically much lower than

those of mAbs. These and other approaches from the food industry could be considered among potential sources of innovations.

4. Sources of Potential Technical Opportunities

Extensive development work across both industry and academia over many years has provided innovations and advances that led to increases in yield, scale, and purity as well as decreases in cost for the production of mAbs for therapeutic use. However, these improvements have not been sufficient to achieve the goal of $10/g of DS that would allow these products to be used more broadly around the world, and new innovations are needed.

This White Paper has described multiple recent innovations for the production of monoclonal antibodies in mammalian cells (e.g., integrated continuous bioprocessing, precipitation, process optimization strategies) and the use of alternative hosts such as filamentous fungi, yeast, and plants. Although each of these approaches has yielded benefits, there are also limits to their implementation or to the magnitude of cost reduction that they can provide, and the search for solutions needs to be broadened to include best practices, new technologies, and innovations from other relevant industries, such as those for food, blood products, and industrial enzymes.

Although pharmaceutical-grade antibodies are different from other industrial products because of their extensive quality control and the stringent regulation of their processes and facilities, it should be possible to adapt a variety of innovative technologies or approaches from a broad range of sources and incorporate them into future antibody production processes and facilities. In fact, achieving the goal of $10/g of DS may well require the combination of multiple individual innovative solutions.

5. Conclusions

The goal of this White Paper is to spark creative thinking for novel solutions across multiple disciplines, industries, and areas of expertise to further reduce the cost of antibody manufacturing to $10/g of DS. Potential solutions need to be developed and assessed both for their technical feasibility and for the overall cost reduction that could be achieved from their implementation. Both large and small innovations are needed: they could either reduce the cost significantly on their own, or – more likely – they could address a key contributor to the cost. Multiple innovations from a variety of sources could then be combined to give rise to an integrated process that meets the target of $10/g of DS and allows the broader worldwide use of mAbs to improve human health.

6. References

1. de Jong, H., Grobusch, M. (2024) Monoclonal antibody applications in travel medicine. *Trop Dis Travel Med Vaccines* **10**, 2. https://tdtmvjournal.biomedcentral.com/articles/10.1186/s40794-023-00212-x

2. Lu, R. et al. (2020) Development of therapeutic antibodies for the treatment of diseases. *J Biomed Sci* **27**, 1. https://jbiomedsci.biomedcentral.com/articles/10.1186/s12929-019-0592-z

3. Alldread R. et al. (2014) Large Scale Suspension Culture of Mammalian Cells, in *Industrial Scale Suspension Culture of Living Cells* (eds H.-P. Meyer and D. R. Schmidhalter), Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany. doi: 10.1002/9783527683321.ch12 https://onlinelibrary.wiley.com/doi/10.1002/9783527683321.ch12 (Summary only)

4. Gilmartin C. et al. (2021) Seasonal malaria chemoprevention in the Sahel subregion of Africa: a cost-effectiveness and cost-savings analysis. *The Lancet Global Health*, 9(2) e199-e208. https://www.sciencedirect.com/science/article/pii/S2214109X20304757

5. IAVI report (2024) Will monoclonal antibodies be a new weapon in the fight against malaria? https://www.iavi.org/iavi-report/monoclonal-antibodies-for-malaria-prevention-iavireport/

6. Mahal H, Branton H, and Farid S. (2021) End-to-end continuous bioprocessing: Impact on facility design, cost of goods, and cost of development for monoclonal antibodies. *Biotechnol Bioeng* 118: 3468-3485. https://analyticalsciencejournals.onlinelibrary.wiley.com/doi/10.1002/bit.27774

7. Coffman J, Brower M. et al. (2021) A common framework for integrated and continuous biomanufacturing. *Biotechnol Bioeng* 118: 1735-1749. https://analyticalsciencejournals.onlinelibrary.wiley.com/doi/10.1002/bit.27690

8. Coffman J, Bibbo K et al. (2021) The design basis for the integrated and continuous biomanufacturing framework. *Biotechnol Bioeng*, 118: 3323–3333. https://analyticalsciencejournals.onlinelibrary.wiley.com/doi/10.1002/bit.27697

9. Crowell L. et al. (2021) Rapid optimization of processes for the integrated purification of biopharmaceuticals. *Biotechnol Bioeng*, 118: 3435-3446. https://analyticalsciencejournals.onlinelibrary.wiley.com/doi/10.1002/bit.27767

10. Minervini M. et al. (2023) Continuous precipitation-filtration process for initial capture of a mAb product using a four-stage countercurrent hollow fiber membrane washing step. *Biotechnol Bioeng* 2023; 1-11. https://analyticalsciencejournals.onlinelibrary.wiley.com/doi/full/10.1002/bit.28525

11. Neves C., Coffman J., and Farid, S. (2024) Evaluating end-to-end continuous antibody manufacture with column-free capture alternatives from economic, environmental, and robustness perspectives. *Biotechnology Progress*. doi:10.1002/btpr.3427 https://discovery.ucl.ac.uk/id/eprint/10186520/1/Evaluating%20end%20to%20end%20continuous%20antibody%20manufacture.pdf

12. Garcia F and Gefroh E. (2023) Reducing biopharmaceutical manufacturing costs through continuous processing in a flexible J.POD facility. *Drug Discovery Today* 28 (7) July 2023. https://www.sciencedirect.com/science/article/pii/S1359644623001356?via%3Dihub

13. Brady J and Love C. (2021) Alternative hosts as the missing link for equitable therapeutic protein production. *Nature Biotechnology* 39: 401-407. https://www.nature.com/articles/s41587-021-00884-w

14. Kaiser F et al. (2024) Filamentous fungus-produced human monoclonal antibody provides protection against SARS-CoV-2 in hamster and non-human primate models. *Nature Communications* 15:2319. https://www.nature.com/articles/s41467-024-46443-0

15. Nylen A. and Chen M-T. (2018) Production of Full-Length Antibody by Pichia pastoris. *Methods in Molecular Biology* (Clifton, N.J.), 01 Jan 2018, 1674:37-48. DOI 10.1007/978-1-4939-7312-5_3. https://link.springer.com/protocol/10.1007/978-1-4939-7312-5_3

16. Liu C-P. et al. (2018) Glycoengineering of antibody (Herceptin) through yeast expression and in vitro enzymatic glycosylation. *PNAS* 115(4) 720–725. https://www.pnas.org/doi/full/10.1073/pnas.1718172115

17. Chen Q. (2022) Development of plant-made monoclonal antibodies against viral infections. *Curr Opin Virol.* 2022 52:148-160. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8844144/

18. Stamatis, C. and Farid, S. (2021) Process economics evaluation of cell-free synthesis for the commercial manufacture of antibody drug conjugates. *Biotechnol J*, e2000238. doi:10.1002/biot.202000238. https://discovery.ucl.ac.uk/id/eprint/10116483/1/Stamatis%20Farid%20Process%20Eco%20CFS%20BiotJ20%20AcceptedArticle.pdf

19. Matejtschuk P. et al. (2000) Production of human albumin solution: a continually developing colloid. *Br J. Anaesthesia* 85(6) 887-895. https://www.bjanaesthesia.org/article/S0007-0912(17)36794-6/fulltext

20. Raoufinia R. et al. (2016) Overview of Albumin and Its Purification Methods. *Adv Pharm Bull*. 6(4), 495-507. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5241407/

21. Hemodialysis. NIDDK/NIH website: https://www.niddk.nih.gov/health-information/kidney-disease/kidney-failure/hemodialysis.

22. Coulliette A. and Arduino M. (2013) Hemodialysis and Water Quality. *Semin Dial.* 26(4): 427–438. doi:10.1111/sdi.12113. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4596525/pdf/nihms-726710.pdf

23. Yehl C. et al. (2019) Hollow fiber countercurrent dialysis for continuous buffer exchange of high-value biotherapeutics. *Biotechnol. Prog*., 35: e2763, 2019 https://aiche.onlinelibrary.wiley.com/doi/10.1002/btpr.2763

24. Burrington, K. (2013) Technical Report: Milk fractionation technology and emerging milk protein opportunities. Published by the Dairy Research Institute and the US Dairy Export Council. https://www.usdairy.com/getmedia/358d8b94-49c6-4630-8837-b1651c7416d8/technicalreportmilkfractionationtechnologypdf.pdf.pdf.aspx

25. Reig M. et al. (2021) Use of Membrane Technologies in Dairy Industry: An Overview. *Foods* 10(11): 2768-2803. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8620702/

7. List of Abbreviations

AAMI Association for the Advancement of Medical Instrumentation

CFS Cell-free synthesis
CHO Chinese hamster ovary cells (commonly used for mAb production)
CMC Chemistry, manufacturing, and controls
CMS Center for Medicare and Medicaid Services
COG Cost of goods

DOE Design of experiments
DS Drug substance

GRAS Generally regarded as safe

HIV Human Immunodeficiency virus
HSA Human serum albumin

ICB Integrated and continuous bioprocessing
ISO International Organization for Standardization

LMIC Low- and middle-income countries
mAb Monoclonal antibody

PAT Process analytical technology
PEG Polyethylene glycol
PPQ Process performance qualification

RSV Respiratory syncytial virus

WPC Whey protein concentrate
WFI Water for injection
WPI Whey protein isolate