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Review

Recombinant Proteins for Industrial versus Pharmaceutical Purposes: A Review of Process and Pricing

John Puetz [1,*] and Florian M. Wurm [2]

[1] Department of Pediatrics, Division of Hematology/Oncology, Saint Louis University School of Medicine, St. Louis, MO 63104, USA

[2] Swiss Federal Institute of Technology, Lausanne (EPFL), 1015 Lausanne, Switzerland

* Correspondence: john.puetz@health.slu.edu; Tel.: +1-314-577-5332

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Abstract: Recombinant proteins have been produced for over 30 years. Applications range from enzymes used in laundry detergents to antigen-detecting antibodies in cancer therapy. Despite similarities in manufacturing, drastic differences in retail pricing between recombinant proteins used for industrial (non-medical) versus pharmaceutical purposes exist. Industrial proteins often have a retail price in the tens of dollars per kilogram while recombinant proteins for medical use may cost billions of dollars per kilogram. This manuscript will briefly review manufacturing techniques and contrast the differences between industrial versus pharmaceutical production. Maximizing manufacturing technologies to reduce cost-of-goods (CoG) is desirable. However, the major reason for the very high pricing of pharma protein products does not reflect CoG, but the financial obligations of clinical trials, research and development, patent constraints, marketing, and return on investment.

Keywords: recombinant proteins; manufactured materials; drug preparation; price

1. Introduction

Humans have been using cultured cells since the dawn of civilization [1,2]. A case can be made that the fermentation of grains by microbes into beer lead to the rise of agriculture and city-states [2]. For centuries, microbes were primarily used to produce human consumables including bread, cheese, alcohol and vinegar. It was not until the 20th century that cultured cells found widespread medicinal and industrial use [3]. Large-scale production was initially based on native plant and animal sources. With the advent of recombinant DNA technology and use of cultured cells, a large variety of proteins became available [3,4]. Today, more than 170 recombinant proteins are used worldwide in medicine [5]. Initially, pharmaceutical recombinant proteins (PRPs) were designed to be as similar as possible to the naturally occurring human protein. More recently, genetic variants of existing proteins and entirely new protein-designs have been created for superior therapeutic values and protein stability. These include gene-fusion products to extend the circulating half-life of coagulation proteins or engineered antibodies for cancer treatment [6–8].

Recombinant proteins for industrial use (IRPs) were developed at the same time as PRPs. Included are enzymes (proteases, lipases, amylases etc.) and structural proteins with wide-ranging applications including the production of food and beverages, conversion of carbohydrates into fuel ethanol or biodiesel, components for clothing and cosmetics, biopolymers, cleaning materials, and waste management [4,9]. IRPs are also genetically engineered for advantageous traits such as improved stability at a different pH, insensitivity towards oxidation, and resistance against heat-induced inactivation, misfolding or aggregation [10–12].

Despite similarities in manufacturing techniques used to produce PRPs and IRPs, drastic differences in retail pricing exist. IRPs often sell for tens of dollars per kilogram (kg) while PRPs can sell for billions of dollars per kg (Table 1) [13–15].

This review will compare PRPs and IRPs and attempt to arrive at an understanding for the dramatic differences in pricing structure.

Table 1. Retail pricing of recombinant proteins. Abbreviations: r—recombinant; PNH—paroxysmal nocturnal hemoglobinuria; HGH—human growth hormone; rFVIIa—recombinant activated factor VII; rFVIII—recombinant factor VIII.

Pharmaceutical Protein			
Product	**Cell Line**	**Application**	**Retail Price per Kg**
Rituximab	Hamster	Lymphoma	$9,500,000.00
Eculizumab	Murine Myeloma	PNH	$23,000,000.00
rHGH	*E. coli*	GH deficiency	$137,000,000.00
rFVIIa	Hamster	Hemophilia with Inhibitor	$2,070,000,000.00
rHepatitis B Surface Antigen	*S. cerevisiae*	vaccine	$5,400,000,000.00
rFVIII	Hamster	Hemophilia	$9,600,000,000.00
Industrial Protein			
Product	**Cell Line**	**Application**	**Retail Price per Kg**
Cellulase	*T. reesei*	Fuel Ethanol	$10.00
rβ-Glucosidase	*E. coli*	Fuel Ethanol	$37.00

Note: due to fluctuating prices, calculations were based on the best available published sources; errors are those of the authors. Calculations used can be found in Appendix A.

2. The Basics of Manufacturing Recombinant Proteins

Whether manufactured for industrial or pharmaceutical use, recombinantly produced proteins follow the same basic manufacturing process as outlined in Figure 1.

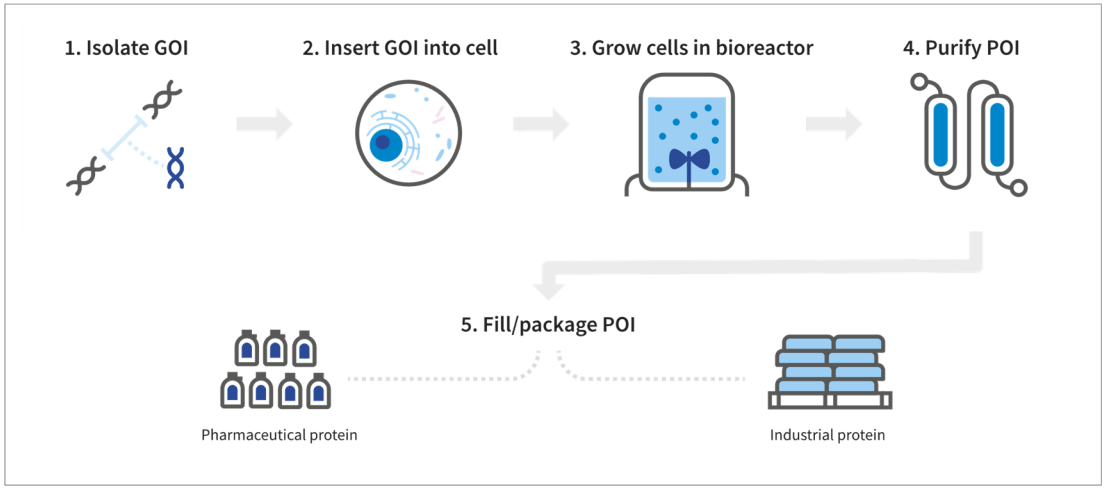

Figure 1. From gene of interest to protein of interest—a simplified scheme.

The first step in manufacturing the protein of interest (POI) is to isolate the corresponding nucleic acid sequence (GOI). In the early phases of the biotech industry, cloning of DNA sequences from mRNA was a crucial step. Today, this is mostly replaced by direct DNA synthesis aided by the availability of genome sequences from a wide variety of species. GOI expression plasmids are then inserted into an appropriate host system to establish a recombinant cell/organism which can be frozen for later use. From vials of frozen cell banks, cultures can be established to initiate a production run. Typically, harvests are executed when cultures have achieved high cell density in batch or fed-batch cultures.

At harvest, the production medium and/or cells that contain the POI are then processed further for purification, packaging and distribution.

Although most recombinant proteins follow this basic manufacturing outline there are vast differences in the processes depending on the POI and its application. These are not only between industrial and pharmaceutical manufacturing processes but also amongst different proteins produced for industrial or for pharmaceutical purposes.

3. Differences in Manufacturing Pharmaceutical and Industrial Recombinant Proteins

3.1. DNA Vector Construction and Gene Transfer to Host Systems

The expression of a POI is initiated by the construction of a suitable nucleic acid vector. To maximize the efficiency of production, the expression vector and GOI are optimized. DNA elements can be added to the vector to promote gene stability and protein secretion. Efficient promotors and enhancers, sequences to optimize copy number of the GOI once it is in the cell, codon optimization, and reporter genes help select for highly productive cells [16–18]. In bacteria, the GOI vector is typically encoded on a plasmid which can be maintained as an episomal DNA element. An example for this is the production of human insulin in *E. coli*, one of the first recombinant proteins made for human therapy [19]. Since plasmids have the tendency to be deleted from bacterial cells, genome-integrated DNA are now preferred. In animal cells, the POI is usually encoded from chromosomally integrated DNA. For GOI transfer into the cell, transfections with calcium phosphate, polyethyleneimine or lipids are used, as are electric shock and microinjection [18].

The GOI can also be manipulated to alter the function and expression of the POI for advantageous traits. Techniques include "brute force" mutagenesis, screening of natural mutants, site-directed mutagenesis, domain deletions or additions, applied molecular evolution and others [3,20].

With respect to DNA construction and gene transfer, there are only marginal differences between IRPs and PRPs. However, investigations into human pathophysiology, pharmacokinetics, and pharmacodynamics are necessary for the upfront design of the DNA vector. These investigations are more complex than protein engineering for industrial applications. By these measures, PRPs require more up-front investigation (and cost). Preclinical and clinical research is not only born by pharma, but includes numerous investigators at clinical, academic, and governmental institutions.

3.2. Cell Host Systems

Various host systems can be used to produce recombinant proteins. They include bacteria, single celled (yeasts) and multicellular fungi, mammalian, plant and insect cells [3]. Transgenic plants and animals are also used but will not be discussed in this review. The choice of host system depends on the POI. Bacteria are advantageous for smaller proteins and peptides that do not require complex folding or post-translational modification. Yeasts can produce larger, more complex proteins, while mammalian cell lines are chosen for the largest proteins with complex folding and post-translation modification [3].

With the emergence of recombinant protein production in the 1970's, yields of product were highest from *E. coli* bacteria; however, this is no longer the case. Even the most complex proteins, such as a heterodimeric antibody, can now be produced in Chinese Hamster Ovary (CHO) cells with harvest concentrations exceeding the 10 g/L range, equivalent to what can be achieved in bacteria or yeast [18]. The benefit of animal cell cultures is that the POI can be secreted from the cells at a high rate. This leads to a lower contaminant load in the product stream. Proteins made in *E. coli* frequently need to be refolded from aggregated protein (inclusion bodies) after lysis of the cells [14]. For reasons outlined above, for IRPs, non-*E. coli* or fungal systems are now preferred, and some of them have the capacity to secrete protein as well.

Generally, IRPs are preferentially produced in bacteria and fungi while the majority of PRPs are made in cultivated mammalian cells, principally CHO [3].

3.3. Growth Media

Mammalian cell lines require much more sophisticated growth and production media than other systems. The inadvertent introduction of any foreign organism, pathogenic or not, into the growth media is to be avoided. Medium substrates, i.e., amino acids, lipids, sugars, rare metal ions, etc., must be chosen and assembled into formulations from providers that source everything stringently, assuring sterility and traceability when completed.

In contrast, microbial systems can be grown on much simpler culture media and for IRPs, there are fewer limitations. Sourcing from slaughter-house wastes and left-overs from bulk food-plant agriculture are being used to provide energy-rich substrates for cells [21]. Even solid substrates are being used in "solid substrate fermentations" which avoid applying any liquid-based mixing approaches [21].

Pharmaceutical regulators now prefer that animal cell cultures are grown in chemically defined media. This means avoiding materials from human or animal sources that have risks in promoting growth of pathogens. IRPs do not have these constraints. Media for cultures with animal cells are likely 100–1000-fold more expensive than those for cultures of bacteria or fungi.

3.4. Bioreactors and the Production Process

Bioreactors vary in size from the milliliter scale to tens of thousands of liters [18]. The needs of the market for recombinant proteins can be at multiple tons per year and both industrial and some pharmaceutical proteins are being produced in this range. Adalimumab is one pharmaceutical, amongst several, that are made from CHO cells in bioreactors of 10,000 L or more [22].

Some PRPs, such as vaccines or factor VIII, require only a few micrograms per dose. For these molecules, a few kilograms can fulfil one year of worldwide utilization. Large, complex proteins are difficult to produce, and thus achieve lower product yields in cell culture. This requires the volumes of cell culture and subsequent processing for purification to be large, even though the total quantity of protein is small.

The difference between industrial and pharmaceutical manufacturing is not necessarily the scale of operation. Distinguishing factors are the need for sterility, the quality of ingredients and the final purity of the product. For sterility, process principles, equipment use, raw-materials and water supply are geared to avoid the introduction of non-host organisms or infectious agents, including any virus, microbe or prion.

3.5. Downstream Processing and Formulation

With cultured animal cells dominating the production of PRPs, the POI is frequently secreted into the liquid of the medium. Thus, the harvest would involve the separation of cells from the liquid as a first step, followed by at least three, but frequently four or more different purification steps [23]. Endotoxins and viruses must also be removed from PRPs [23]. None of these steps recover the secreted product at a 100% rate, and typically, a 70% step success rate is considered good. Thus, following purification, less than 50% of the product initially synthesized by cells will end up in a medicine vial. Purification steps involve chromatographic processes to achieve the 99% or higher purity required for PRPs [23]. Large volumes of the highest quality water, on average at least 10 times the volumes as the production vessel, are used [24].

IRPs also require downstream processing and purification, and share many of the same methodologies used for PRPs. However, PRPs require far higher purity due to the need to remove potentially immunogenic and bioactive cellular debris, protein contaminants, endotoxins, and pathogens.

Significant delays between manufacturing and eventual patient use occur with PRPs. This is not the case with IRPs as the product can be shipped directly from manufacturer to consumer with perhaps a short stay at a distribution center. Whereas PRPs are never shipped directly from manufacturer to

consumer. The need for stability adds complexity to PRPs. The shelf-life of PRPs should be at least one year or longer. The study and optimization of drug formulations is a complex science and takes significant time and resources [25].

3.6. Quality Control

Perhaps the greatest difference in the manufacturing process between IRPs and PRPs is in quality control. Every step in the production of PRPs is tightly regulated. An initiative to further improve qualities and reproducibility of PRPs has been promoted and supported by regulatory agencies. "Quality by Design" (QbD) tries to understand every step and every single ingredient entering the production towards PRPs [26,27].

Analytical tests are applied during manufacturing and records must be kept and compared with historical data. This necessitates hundreds of quality assurance steps and batch production records with tens of thousands of data entries. It has been estimated that a two-week production run of a PRP can require up to 6 months of testing, documentation, and quality review [28].

Since the PRP itself may be a very complex molecule or molecular assembly, it cannot be structurally defined by a single chemical formula. For example, antibodies are built as heterodimers of heavy and light-chain polypeptides which also carry glycosylations. The added carbohydrate moieties differ (ever so slightly) from one molecule to the other. The product is better described as a family of molecules which have (hopefully) all identical polypeptide chains. Thus, the product ending up in a drug vial is the result of all steps involved in the manufacturing process: The process defines the product. Rules and steps on how to implement QbD approaches are defined and described in regulatory documents such as those released by International Conferences on Harmonization (ICH) bringing together Regulatory Agencies from around the world [29].

Manufacturers of IRPs also aim to produce a high-quality product; however, they do not face the same degree of regulatory requirements and oversight that pharma faces.

3.7. Manufacturing Summary

A summary of the differences in manufacturing between PRPs and IRPs reveals that PRPs tend to be manufactured in mammalian cells grown in stringently produced growth media following strict quality control and manufacturing guidelines and are processed to the highest purity. The cost of producing a PRP is far higher than an IRP, but still does not explain the drastic difference in retail pricing. However, PRPs have additional requirements that contribute to their retail price.

4. Clinical Trials, R&D, and Patents

All pharmaceuticals must undergo clinical trials before release to the market. This is obviously not required for IRPs. Even prior to entering clinical trials, extensive research is done with the PRPs using in-vitro and in-vivo studies. It can take years to develop a drug. It is a reasonably fair assumption that only 1 in 5 to 10 molecules that have undergone studies in animals will enter the first phase of clinical assessment. There are high risks to failure. In Phase 1 studies, things can go terribly wrong: A new antibody drug, TGN1412, was injected into six healthy young men at doses 500 times smaller than what had been found to be safe in animal studies. Entirely unexpected, life-threatening conditions developed in each subject [30]. The company behind the product declared bankruptcy following this event.

The costs of a single clinical trial can vary from $5 million for small, non-controlled orphan drug trials to $350 million for large controlled studies. The median estimate is $19 million [31]. Many drugs require several clinical trials prior to gaining authorization for human use. Determining the overall cost for bringing a drug to market has been fraught by different methodologies, data sources, and classification of drugs studied [32]. As a result, there is at least a 9-fold difference in cost estimates [32]. Often cited is the Tufts Center for the Study of Drug Development. Their most recent publication estimated the cost of bringing a single drug to market at $1.4 billion [33]. The Tufts study used a

confidential survey as a data source and has been criticized for its lack of transparency. A more recent estimate using securities and exchange filings placed the median cost at $648 million [34].

IRPs are also thoroughly tested before they are released to the market. While IRPs do not require clinical trials, the amount of effort and investigation necessary for protein engineering should not be underestimated.

Pharmaceutical companies expend about 17% of their revenue on research and development (R&D) [35]. This contrasts with the typical S&P 500 company which expends about 2%. Pharmaceutical companies also devote a higher proportion of their revenues to dividends and stock buy backs—18% versus 8% [35]. Peer reviewed published financial data for IRPs was not readily available.

PRPs and IRPs can both be protected by patents. Protection by patents is limited in time: patent applications are submitted years before the potential drug can be marketed. Protection from a patent is only provided for a maximum of 20 years, and very importantly, the clock starts when the patent is filed, i.e., years before it is granted. In addition, the development of a PRP and the research phases, including the clinical approval processes can take 10 years or more. Thus, pharma companies have a much shorter time to recoup their investment compared to general industrial companies.

5. Marketing and Liability

Both company categories market their products. In general, industry markets to a small group of highly selective customers, while pharma markets to health care providers, commercial pharmacies, hospitals, and directly to consumers. The marketing of PRPs is heavily regulated and varies from country to country.

Estimates of marketing costs for pharmaceuticals vary widely. A recent publication estimated annual marketing expenditures for all pharmaceutical companies in the US in 2019 dollars ($-2019) at 31.5 billion while another study published over a decade ago estimated the costs at 76 billion $-2019 [36,37]. It has been claimed that pharmaceutical companies spend twice as much on marketing as R&D [38]. The annual survey of pharmaceutical members listed annual R&D spending in $-2019 at 74 billion dollars [39]. To the degree this self-reported data is accurate, it suggests that marketing expenditures may be equal to or half that of R&D. A Canadian study also showed that R&D spending is 1.45 to 2.8 times that of promotional spending [40]. It is unclear if pharmaceutical companies promote recombinant products differently than other products, but this is unlikely.

Since the global revenue for all IRPs is less than $10 billion per year, pharma clearly spends far more on marketing then companies making IRPs [41].

Both PRP and IRP manufacturers face liability costs. Liability expenses for pharmaceutical companies are about 2% of revenue [42]. Documentation of the liability costs for industrial enzyme manufacturers could not be located but it is anticipated that the liability for a PRP is much higher than for IRP.

6. Conclusions

There are vast differences in the retail pricing of recombinant proteins manufactured for industrial versus pharmaceutical applications. Manufacturing practices vary, especially quality control, but these do not account for the differences in price of 8–9 orders of magnitude. Clinical trials, R&D, patent constraints, marketing, and return on investment to shareholders are the dominant drivers of the cost difference. Revenue generated from pharmaceutical sales has financed decades of basic and applied research. Revenue has also been returned to investors. Estimates of R&D and marketing costs vary greatly. Additional transparency by the pharmaceutical companies is required to more accurately gauge R&D and marketing costs and to determine if the high cost of PRPs is justified.

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Appendix A

Calculations were derived from the United States Centers for Medicare and Medicaid Services (CMS) Average Sales Price (ASP) and the pharmaceutical package insert [14].

rFVIII: 7.5×10^3 IU/mg $\times 10^6$ mg/kg = 7.5×10^9 IU/kg $\times$ \$1.28/IU = \$$9.6 \times 10^9$/kg
rFVII: \$2.07/mcg $\times 10^9$ mcg/kg = \$$2.07 \times 10^9$/kg
Rituximab: \$95/mL $\times$ 1 mL/10 mg $\times 10^6$ mg/kg = \$$9.5 \times 10^6$/kg
rHGH: \$55/0.4 mg $\times 10^6$ mg/kg = \$$1.37 \times 10^8$/kg
eculizumab: \$230/mL $\times$ 1 mL/10 mg $\times 10^6$ mg/kg = \$$2.3 \times 10^7$/kg
rHepatitis B surface antigen: \$27/5 mcg $\times 10^9$ mcg/kg = \$ 5.4×10^9/kg

The price of cellulase and glucosidase were determined from the cited references for Table 1 [13,14].

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