Introduction

Current Status of Biopharmaceuticals: Approved Products and Trends in Approvals

Gary Walsh

Abstract

Biopharmaceuticals represent the fastest growing and, in many ways, the most exciting sector within the pharmaceutical industry. Within this Introduction we first consider what category of product falls within the description of a biopharmaceutical. An overall global snapshot of the current status of the biopharmaceutical sector is then presented, followed by an overview of upstream and downstream processing operations typical of protein-based biopharmaceuticals. General trends in product approvals are next overviewed and this is followed by a summary of the main actual biopharmaceutical products that have gained approval to date (within the EU and/or US). These are considered by product type, the most significant of which are blood-related products, hormones, cytokines, vaccines and monoclonal antibodies. Biopharmaceuticals that have gained approval for veterinary application are then considered, and the Introduction concludes by considering some of the innovations and trends likely to influence the shape of the biopharmaceutical sector in the future.

Abbreviations

AIDS	aquired immunodeficiency syndrome
BHK	baby hamster kidney
BHV	bovine herpes virus
BMP	bone morphogenic protein
CHO	chinese hamster ovary
CSF	colony-stimulating factor
dsRNA	double-stranded RNA
EL	eurifel
EPO	erythropoietin
EU	Europian Union
FSH	follicle-stimulating hormone
G-CSF	granulocyte colony-stimulating factor
GH	growth hormone
GM-CSF	granulocyte macrophage colony-stimulating factor
HAMA	human anti-mouse antibodies
HBsAg	hepatitis B surface antigen

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HER2	herceptin
HIV	human immunodeficiency virus
IB	inclusion body
IFN	interferon
IL	interleukin
LH	luteinizing hormone
mAb	monoclonal antibody
MS	multiple sclerosis
NPV	nuclear polyhedrosis virus
PhRMA	Pharmaceutical Research and Manufacturers of America
PDGF	platelet-derived growth factor
PEG	polyethylene glycol
r	recombinant
rh	recombinant human
RNAi	RNA interference
siRNA	small interfering RNA
TNF	tumor necrosis factor
tPA	tissue plasminogen activator

1 What are Biopharmaceuticals?

What exactly is a biopharmaceutical? The term has now become an accepted one in the pharmaceutical vocabulary, but it can mean different things to different people. A clear, concise definition is absent from pharmaceutical dictionaries, books on the subject, or even in the home pages of regulatory agencies or relevant industry organizations. The term "biopharmaceutical" appears to have originated in the 1980s, when a general consensus evolved that it represented a class of therapeutic product produced by modern biotechnological techniques. These incorporated protein-based products produced by genetic engineering or, in the case of monoclonal antibodies (mAbs), produced by hybridoma technology (see also Part IV, Chapter 16 and Part V, Chapters 1 and 2). During the1990s the concept of nucleic acid-based drugs for use in gene therapy and antisense technology came to the fore (see also Part I, Chapters 6–8 and Part VI, Chapter 6). Such products are also considered to be biopharmaceuticals. On that basis biopharmaceuticals may be defined – or at least described – as proteins or nucleic acid-based pharmaceuticals, used for therapeutic or *in vivo* diagnostic purposes (see also Part III, Chapter 7 and Part V, Chapters 4–7), and produced by means other than direct extraction from a non-engineered biological source. By defining the method of manufacture in negative terminology, proteins obtained by direct extraction from native sources are excluded. The description also encompasses nucleic acid-based products, be they produced by biotechnological means or by direct chemical synthesis – as is the case for most antisense-based products (see also Part II, Chapters 7 and 8 and Part III, Chapter 3). Also, small interfering RNAs (siRNAs) and decoy oligonucleotides are of course considered biopharmaceuticals, regardless of how they were produced (see also Part I, Chapters 9 and 10). However, even that definition is becoming somewhat restrictive as, for example, cell-based products become more prominent (see also Part I, Chapters 11–15).

2 A Global Snapshot

It is now 22 years since approval of "humulin" (recombinant human insulin), produced in *Escherichia coli* and developed by Genentech in collaboration with Eli Lilly [1]. Lilly received marketing authorization in the US for the product in 1982. This marked the true beginning of the biopharmaceutical industry. Currently some 142 biopharmaceuticals have gained approval for general human use in the EU and/or US (see also Part II, Chapter 4, Part VII,

Chapter 4 and Part VIII, Chapter 1). The major companies marketing one or more approved biopharmaceutical products in these regions are listed in Tables 1–9, as presented later. Additional relevant company and product information is generally available via the company web pages, the details of which are also provided in Tables 1–9. Approximately one in four of all genuinely new drugs currently coming on to the market is a biopharmaceutical and the biopharmaceutical sector is estimated to be worth in excess of $ 30 billion, approximately double its global value in 1999 [2].

Some 250 million people worldwide have been treated to date with biopharmaceuticals. The vast majority are protein based – either recombinant proteins or monoclonal/engineered antibodies [3]. A small number of cell-based products continue to gain marketing approval and one antisense-based product (Vitravene, ISIS Pharmaceuticals) has also been approved for general medical use (see also Part III, Chapter 3). Thus far only a single gene-therapy product has gained approval anywhere. The product, trade name Gendicine, is a human adenovirus engineered to contain the human p53 tumor suppressor gene. It was approved in October 2003 in China, and is indicated for the treatment of head and neck squamous cell carcinoma [4].

The major categories of product indications are as one might expect; mirroring major killers in the "first" world, including various forms of cancer and heart attacks. The single most lucrative product is that of erythropoietin (EPO). Combined sales of the recombinant EPO products "Procrit" (Ortho biotech) and "Epogen" (Amgen) have reportedly surpassed the $ 6.5 billion mark. The biopharmaceutical sector has matured rapidly over the last decade and is set to continue to grow into the foreseeable future – and follow-on biopharmaceuticals are also coming onto the scene (see also Part VIII, Chapter 3).

3 Upstream and Downstream Processing

Protein-based biopharmaceuticals are invariably produced by an initial cell culture/microbial fermentation step (upstream processing), followed by product recovery, purification and formulation into final product format (downstream processing) [5, 6]. In the region of 40% of all protein biopharmaceuticals approved to date are produced by recombinant means in *E. coli*. *E. coli* displays several advantages as a production system. Its molecular genetics are well characterized. It is easy to grow, and grows rapidly and on relatively inexpensive media. Furthermore, high product expression levels are generally achieved. Many of the earlier approved *E. coli*-based products accumulate intracellularly in the form of inclusion bodies. This complicated subsequent downstream processing as it necessitated inclusion body recovery, solubilization and renaturation of the product. However, some *E. coli* product expression systems now used promote export of the desired protein into the periplasmic space in fully folded format, from where it can be conveniently recovered without the necessity for cellular disruption (see also Part IV, Chapters 7 and 12).

Several products are produced using engineered *Saccharomyces cerevisiae*. These include various insulin-based products manufactured by Novo [7] (see also Part IV, Chapter 13), recombinant hepatitis B surface antigen (rHBsAg) produced by SmithKline Beecham as well as a recombinant form of the anticoagulant hirudin [8, 9]. The majority of approved biopharma-

ceuticals are, however, expressed in animal cell lines, mainly Chinese hamster ovary (CHO) (see also Part IV, Chapters 1 and 4), but also baby hamster kidney (BHK) cells [10] (see also Part IV, Chapter 12).

Although expression in animal cell lines is more technically complex and expensive when compared to *E. coli*-based systems, eukaryotic cell lines, unlike prokaryotic ones, are capable of carrying out post-translational modifications such as glycosylation (see also Part IV, Chapters 2 and 7). Many key biopharmaceuticals are naturally glycosylated. Examples include EPO, many (although not all) interferons (IFNs), blood factor VIII (see also Part II, Chapter 3), and gonadotrophins such as follicle-stimulating hormone (FSH) and luteinizing hormone (LH). In some instances unglycosylated versions of a naturally glycosylated protein retain the therapeutic properties of the native protein and several such products produced in *E. coli* have gained regulatory approval. A prominent example is that of "Filgrastim" – a recombinant granulocyte colony-stimulating factor (G-CSF) produced in *E. coli* and which displays a biological activity similar to the native glycosylated protein [11] (see also Part VIII, Chapter 3). Additional examples include "Betaferon" (Schering, Berlin) and "Neumega", nonglycosylated versions of IFN-β and interleukin (IL)-11, respectively, both of which are produced in *E. coli* [12, 13]. The glycocomponent of many glycoproteins, however, may be necessary for/impact upon the biological activity of a protein, or may influence protein stability or its circulating half-life [14]. In such instances, expression in a eukaryotic system becomes desirable, if not necessary. While expression in lower eukaryotes such as *S. cerevisiae* is possible, glycosylation patterns more similar to a native human protein are obtained if the protein is expressed in an animal cell line. Although glycosylation represents the most common post-translational modification characteristic of such modified biopharmaceuticals, some other forms of post-translational modification can also occur and be relevant to the therapeutic/biological activity of the protein. A prominent example is that of the anticoagulant activated protein C (trade name Xigris), which harbors several γ-carboxylated glutamic acid residues and one β-hydroxylated aspartic acid residue [15]. Both forms of post-translational modification are necessary to underpin full functional anticoagulant activity.

Although the research literature contains numerous examples of high-level recombinant protein production using insect cell lines, this approach has not been used thus far to produce any commercial biopharmaceutical for human use. Insect-based systems are, however, employed in the manufacture of several protein-based veterinary biopharmaceuticals, as described later. Insect cell line culture is usually straightforward and inexpensive, and cell growth is rapid (see also Part IV, Chapter 14). Many insect cell lines are sensitive to infection by baculovirus. Upon infection, up to 50% of all cell protein produced is that of the viral protein polyhedrin. A common recombinant production strategy used therefore entails introducing the gene coding for the protein of interest into an engineered baculovirus, under the influence of the polyhedron promoter [16].

Downstream processing for virtually all protein biopharmaceuticals follows a fairly predictable sequence of events (outlined in Fig. 1) [17]. Following initial product recovery and concentration, multiple chromatographic steps are undertaken (usually between three and six individual fractionation steps). While gel filtration and ion exchange are particularly common, down-

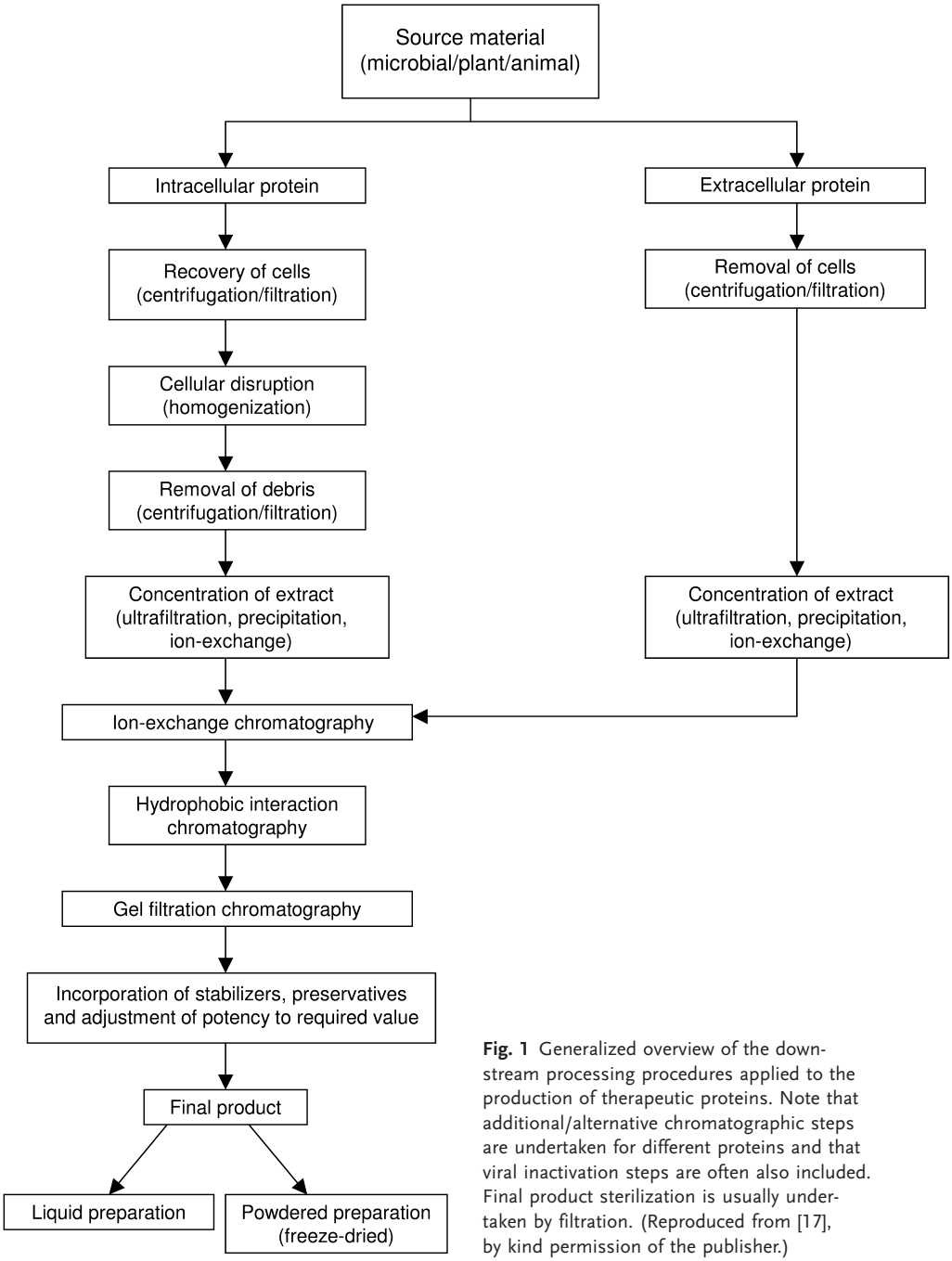

Fig. 1 Generalized overview of the downstream processing procedures applied to the production of therapeutic proteins. Note that additional/alternative chromatographic steps are undertaken for different proteins and that viral inactivation steps are often also included. Final product sterilization is usually undertaken by filtration. (Reproduced from [17], by kind permission of the publisher.)

stream processing of several products also entail the use of the more bioselective technique of affinity chromatography. Examples include the incorporation of an immunoaffinity step in the purification of recombinant factor VIII (see also Part II, Chapter 3) and the use of Protein A affinity columns in the purification of some antibody-based products. Several other downstream processing procedures employ at least one pseudoaffinity step, e.g., the purification of the veterinary product Vibragen ω (discussed later), which involves the use of both dye affinity and immobilized metal affinity chromatography. Preparative high-performance liquid chromatography systems have also been included in the downstream processing of some products, including some recombinant insulins and "Leukine", a recombinant granulocyte macrophage colony-stimulating factor (GM-CSF) sold by Schering.

Inclusion of a viral inactivation step is generally also characteristic of downstream processing procedures, especially if the product is derived from an animal cell line [18]. Chromatographic steps are themselves usually quite effective in removing viruses from product streams (e.g., gel filtration will separate most viruses quite effectively from much smaller therapeutic proteins), and, of course, the ability of the downstream processing procedure to remove typical animal cell viruses from the product will have been tested and validated during the purification design stage (see also Part I, Chapter 6 and Part VII, Chapter 1). Amongst the various "safety net" viral removal approaches often included in downstream processing procedures are multiple repeat filtration through a 0.1 μm filter, heat/UV treatment or treatment with chemical inactivation agents such as β-propiolactone (used for some veterinary products at least).

Final product may be formulated in liquid or freeze-dried form, and virtually all biopharmaceutical products are sterilized by filtration followed by aseptic processing. The most commonly employed excipients include human serum albumin, polysorbate 20 or 80, mannitol, sucrose or maltose, amino acids (usually glycine, arginine or histidine) and a buffer (often citrate, acetate or phosphate based).

4 Trends in Approvals

4.1 Protein Engineered Products

The bulk of first-generation (early approved) biopharmaceuticals were unaltered mAbs or simple replacement proteins such insulin, blood factor VIII and IFNs. An increasing number of modern biopharmaceuticals, however, have been engineered in order to tailor their therapeutic properties. The most common form of protein engineering involves the alteration of amino acid sequence in order to achieve one or more of the following goals:

- Alteration of biological half-life of the protein.
- Reduction or elimination of issues of product immunogenicity.
- Generation of either fast- or slow-acting product (e.g., variants of insulin).
- Generation of novel, hybrid protein therapeutics.

Second-generation tissue plasminogen activator (tPA) products represent the most prominent example of a biopharmaceutical engineered in order to alter biological half-life [19]. Unmodified tPA, although an effective thrombolytic agent, displays a half-life of some 3 min after i.v. administration.

From a practical standpoint this necessitated product administration by continuous infusion over a 90-min period. Domain-deleted engineered variants (trade names Ecokinase and Retavase), however, display half-lives in the region of 15–20 min, facilitating product administration by a single i.v. injection.

First-generation mAbs approved for medical use were invariably unmodified murine monoclonals produced by classical hybridoma technology [20]. These suffered from a number of clinical disadvantages, not least the fact that they were highly immunogenic when administered to man. Administration elicited the production of human anti-mouse antibodies (the HAMA response) that limited efficacy, particularly upon repeat administration. Murine monoclonals also displayed relatively short half-lives (typically 30–40 h) when administered to man and they proved to be poor triggers of human immune effector functions, such as the activation of complement. The majority of antibody-based biopharmaceuticals gaining marketing approval in recent years are engineered in order to reduce or effectively eliminate such problems [21, 22]. Chimeric antibodies are murine–human hybrid antibodies produced by splicing the gene sequences coding for the mouse-derived antibody variable regions (which contain the antigen binding site) to nucleotide sequences coding for the constant regions of a human antibody (see also Part IV, Chapter 16 and Part V, Chapters 1 and 2). Such chimeric products, when compared to first generation murine mAbs, display significantly extended half-lives (of up to 250 h), are capable of activating human immune effector functions and are significantly less immunogenic. Humanized antibodies are more extensively engineered, effectively produced by grafting (at the DNA level) the actual murine antibody antigen binding regions (the complementarity-determining regions) into a human antibody sequence. Such products display half-lives essentially identical to fully native antibodies and are significantly less immunogenic in man, even when compared to chimeric antibodies.

Engineered insulin analogs represent the most prominent group of second-generation biopharmaceuticals modified in order to generate either short- or long-acting forms of the native therapeutic protein (see also Part IV, Chapter 13 and Part VI, Chapter 4). Both long- and short-acting insulin, and the engineering principles underpinning their generation are considered subsequently in this Introduction. A number of novel hybrid proteins have also been generated by protein engineering and have gained medical approval for various conditions. Examples include "Enbrel" [a tumor necrosis factor (TNF) receptor fragment linked to an antibody fragment, indicated in the treatment of rheumatoid arthritis] and "Ontak" (an IL-2–diphtheria toxin fusion protein used to treat cutaneous T cell lymphoma).

4.2 Engineering via Post-translational Modification

Although the majority of engineered biopharmaceuticals have been altered specifically in terms of their amino acid sequence, several products have now come on stream that are engineered post-synthesis. The changes introduced normally entail the covalent attachment of a chemical group to the protein's polypeptide backbone or the alteration of a specific pre-existing post-translational modification, i.e., the glycocomponent of glycoproteins (see also Part IV, Chapters 2 and 7).

Thus far, engineering by attachment of a chemical group has centered around PEGylation and, to a lesser extent, attachment of fatty acid groups. PEGylation entails the covalent attachment of one or more molecules of polyethylene glycol (PEG) to the polypeptide backbone [23]. PEGylation is technically straightforward to achieve and chemically activated PEG molecules for conjugation can be generated *in-house* or purchased commercially. PEGylation generally increases the plasma half-life of a protein, by decreasing the rate of systemic clearance. This decreases the frequency of administration required, with consequent economic savings and improved patient experience, often along with reduced treatment side-effects.

Native IFNs have relatively short plasma half-lives, typically of the order of 4 h. PEGylation can increase this value up to 24 h (see also Part VI, Chapter 2). Intron A, for example, is a recombinant human IFN-*α*2b produced by Schering Plough. It is approved for the treatment of various cancers including leukemia, as well as for some viral infections such as hepatitis B and C. Generally, administration schedules entail product injection 3 times a week. PEGylated Intron A, however, need only be administered once weekly to achieve the same effect [24].

Levemir is a recently approved insulin analog (Table 3) whose principal engineering feature related to the covalent attachment of a fatty acid side-chain, as described later.

Thus far, at least two approved therapeutic proteins are engineered by modification of their glycocomponent. Nespo (Aranesp in the US) is a recombinant human EPO molecule expressed in a CHO cell line. Native EPO harbors three *N*-linked carbohydrate side-chains, whereas the engineered recombinant product displays five such carbohydrate side-chains. The increased carbohydrate content extends the product's serum half-life significantly, again facilitating once weekly administration [25].

Cerezyme is the trade name given to recombinant human glucocerebrosidase, a lysosomal enzyme central to the metabolism of glucocerebrosides (glycolipids found naturally in the body). Lack of glucocerebrosidase activity triggers Gaucher's disease, a genetic condition characterized by accumulation of glucocerebrosides, particularly in tissue-based macrophages. An obvious therapeutic strategy in treating Gaucher's disease would be the direct administration of the missing enzyme. However, injected glucocerebrosidase is quickly removed from the bloodstream by the liver. Cerezyme is produced in an engineered CHO cell line. However, downstream processing includes an enzyme-based processing step using an exoglucosidase enzyme. The exoglucosidase removes the sialic acid sugar caps of the oligosaccharide side-chains. This exposes side-chain mannose residues, which in turn promotes macrophage-specific enzyme uptake, mediated by mannose-specific receptors present on the macrophage cell surface. In this way the sugar engineering promotes targeted delivery of the biopharmaceutical to the cell type most affected [26] (see also Part VI, Chapters 5 and 3, and Part VI, Chapter 1).

5 Declining Number of Approvals

A marked decrease in the number of new biopharmaceuticals gaining marketing approval has become evident over the last 2–3 years, both in Europe and the US (see also Part II, Chapter 4, Part VII, Chapter 4 and Part VIII, Chapter 1). Fig. 2 presents

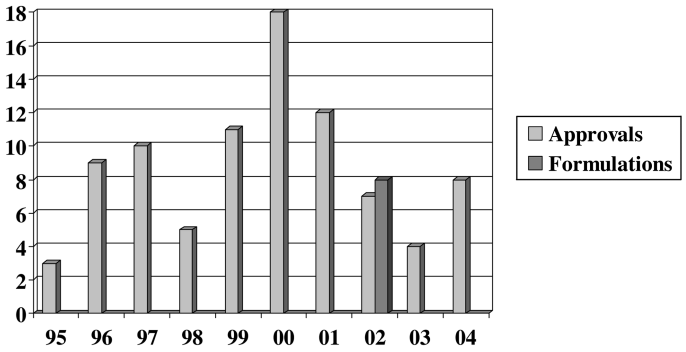

Fig. 2 Biopharmaceutical numbers approved for human use per year since the introduction of the centralized approvals system in 1995.

numbers of biopharmaceuticals approved within the EU per year since the introduction of the centralized European applications procedure in 1995 (see also Part VII, Chapter 5). The first product approved under that new centralized evaluation system was Gonal F, Serono's follicle-stimulating hormone product. It was one of three biotech products approved that year. Approval numbers peaked in 2000, when 18 new biopharmaceutical products gained marketing approval. In 2001 the European figure was 12. Although 14 distinct products were approved in 2002, eight of these were various formulations of the same active ingredient (recombinant insulin produced by Novo in an engineered strain of *S. cerevisiae*) (see also Part IV, Chapter 13). Therefore, in reality only seven genuinely different biopharmaceuticals were approved that year. The downward trend continued in 2003, with the approval of just four biopharmaceuticals, although eight products were approved within the EU in 2004. The reason this "decline" in number of approvals over the last few years is not immediately apparent, but the large numbers currently under clinical evaluation likely renders this decline a short-term phenomenon. New approaches for the accelerated development of biopharmaceuticals (see also Part III, Chapter 2 and Part III, Chapter 1) will most likely also support a healthy increasing trend in approval.

6
Products Approved for Human Use

Here, an overview of biopharmaceutical products thus far approved (within the EU and US at least) is presented. The products have been grouped into nine categories: recombinant blood factors, recombinant thrombolytics, recombinant insulins, additional recombinant hormones, recombinant hematopoietic growth factors, recombinant IFNs and ILs, recombinant vaccines, monoclonal and engineered antibodies, and additional biopharmaceuticals (e.g., cell therapy, gene therapy, siRNA).

6.1
Recombinant Blood Factors

A total of seven recombinant blood factors have gained marketing approval, mainly throughout the 1990s (Table 1). All aim to treat either hemophilia A or B and all are

Table 1 Recombinant blood factors approved to date

Product	Company	Therapeutic indication	Approved
Bioclate (rhFactor VIII produced in CHO cells)	Centeon	hemophilia A	1993 (US)
Benefix (rhFactor IX produced in CHO cells)	Genetics Institute	hemophilia B	1997 (US, EU)
Kogenate (rhFactor VIII produced in BHK cells; also sold as **Helixate** by Centeon via a license agreement)	Bayer	hemophilia A	1993 (US), 2000 (EU)
Helixate NexGen (octocog *α*; rhFactor VIII produced in BHK cells	Bayer	hemophilia A	2000 (EU)
NovoSeven (rhFactor VIIa produced in BHK cells)	Novo-Nordisk	some forms of hemophilia	1995 (EU); 1999 (US)
Recombinate (rhFactor VIII produced in an animal cell line)	Baxter Healthcare/ Genetics Institute	hemophilia A	1992 (US)
Advate (octocog-*α*, rhFactor VIII produced in CHO cell line; the product is similar to *Recombinate* except it is expressed in culture media free from animal-derived proteins and formulated without plasma-derived human albumin)	Baxter	hemophilia A	2004 (EU)
ReFacto (Moroctocog-*α*, i.e., B-domain-deleted rhFactor VIII produced in CHO cells)	Genetics Institute	hemophilia A	1999 (EU), 2000 (US)

produced in engineered animal cell lines in order to facilitate product glycosylation.

Blood factor VIII-based products are indicated for the treatment and prophylaxis of patients with hemophilia A (see also Part II, Chapters 1–3). This is a genetic disease characterized by the total lack or presence only at low levels of blood clotting factor VIII. Lack of adequate levels of this clotting factor results in prolonged bleeding episodes, occurring spontaneously or after trauma/surgery.

Recombinant blood factor products have proven to be as effective as the plasma-derived product, without suffering the disadvantage of the potential risk of transmission of human blood-borne pathogens (see also Part II, Chapter 3).

Blood factor IX-based products are indicated for the control and prevention of bleeding episodes in patients with hemophilia B. Hemophilia B again is a hereditary disorder caused by a deficiency in circulating levels of coagulation factor IX, resulting in impaired blood clotting ability (see also Part III, Chapter 6).

NovoSeven is an unusual product in that it is (a recombinant form of) human coagulation factor VII (FVII). The product is converted in an autocatalytic fashion into the active two-chain form (FVIIa) during its chromatographic purification. NovoSe-

ven is employed to stimulate the coagulation process in hemophilic patients with inhibitors to factor VIII and IX. It achieves its therapeutic effect by inducing the activation of factor X to factor Xa, which converts prothrombin into thrombin (see also Part II, Chapters 1 and Part III, Chapters 6). This, in turn, triggers the final clotting step, where fibrinogen is converted into fibrin to form the hemostatic plug. The whole clot-triggering process is therefore achieved by bypassing the action of factor VIII and IX. This activation occurs only in the presence of tissue factor (a membrane protein not present in plasma), calcium and phospholipids, so that coagulation is stimulated only when an injury has occurred to a vessel, with resulting local hemostasis. This complex process is nicely shown in a video animation on the supplementary CD-ROM.

6.2 Recombinant Thrombolytics

Six tPA-based thrombolytic products have gained approval thus far (Table 2). Native tPA is a 527-amino-acid glycosylated serine protease synthesized predominantly in vascular endothelial cells, from where it enters the bloodstream. It is the major activator of the natural thrombolytic process and therefore has obvious application in the accelerated removal of blood clots that form under inappropriate conditions. A recombinant form of native human tPA was first marketed by Genentech in 1987. Most subsequent tPA-based products are engineered in some way, in order to extend their plasma half-lives, as previously mentioned.

Table 2 Recombinant tPA-based products thus far approved

Product	Company	Therapeutic indication	Approved
Activase (Alteplase, rhtPA produced in CHO cells)	Genentech	acute myocardial infarction	1987 (US)
Ecokinase (Reteplase, rtPA; differs from human tPA in that three of its five domains have been deleted; produced in *E. coli*)	Galenus Mannheim	acute myocardial infarction	1996 (EU)
Retavase (Reteplase, rtPA; see *Ecokinase*)	Boehringer Mannheim/ Centocor	acute myocardial infarction	1996 (US)
Rapilysin (Reteplase, rtPA; see *Ecokinase*)	Boehringer Mannheim	acute myocardial infarction	1996 (EU)
Tenecteplase (also marketed as **Metalyse**; TNK-tPA, modified rtPA produced in CHO cells)	Boehringer Ingelheim	myocardial infarction	2001 (EU)
TNKase (Tenecteplase; modified rtPA produced in CHO cells; see *Tenecteplase* entry above)	Genentech	myocardial infarction	2000 (US)

6.3 Recombinant Insulins

In many ways insulin remains the prototypic biopharmaceutical. Used to treat diabetes mellitus, early commercial preparations were extracted directly from the pancreatic tissue of slaughterhouse animals. The WHO estimates that some 170 million people suffer from diabetes, a figure that is likely to double by 2030. Although only a minority of these sufferers actually require daily insulin injection, the current world market for insulin is valued at in excess of $ 4.5 billion, a figure that is likely to reach $ 8 billion before the end of the decade (see also Part IV, Chapter 13 and Part VI, Chapter 4). Commercially feasible methods of enzymatically converting porcine insulin into a product identical to human insulin were developed in the 1970s, but recombinant DNA technology has had the greatest impact upon this sector. The biosynthesis of insulin in the human body and the procedure to enzymatically convert porcine insulin is nicely shown on the supplementary CD-ROM.

Initially produced in 1978, recombinant human insulin (trade name Humulin) was the first biopharmaceutical to gain approval in any world region (approved in 1982). Since then a number of additional recombinant insulin products have come on the market (Table 3). The modern insulin industry is dominated by Lilly, Novo and, to a lesser extent, Aventis, and these companies manufacture and market a range of both first-generation and engineered (second-generation) insulin products (Table 3). Engineered second-generation insulin analogs display an amino acid sequence altered in order to generate either "fast-acing" or "slow-acting" product. Unmodified human insulin molecules, when stored at typical commercial therapeutic dose concentrations (around 10^{-3} M), exist primarily in oligomeric form, as zinc-containing hexamers. Each hexamer consists of three identical dimers, exhibiting strong inter-subunit interactions. Three dimers are coordinated to central zinc ions. Upon s.c. administration, hexamers must first disassociate into monomeric form before entry into the bloodstream. As a result, injected insulin has a slower onset (and a longer duration) of action when compared to endogenous insulin secretion. A practical consequence is that such insulins must be administered to the diabetic 30 min or so before meal times and the planned meal time should not subsequently be altered. In addition to such traditional "short-acting" insulins, insulin may be formulated in order to actually retard the rate of insulin entry into the bloodstream from the injection site. Such "long-acting" insulins are usually administered (in combination with short-acting insulins) in order to mimic low baseline endogenous insulin levels.

Insulin lispro (sold under the trade names Humalog and Liprolog) exemplifies engineered short-acting insulin products. This product displays an amino acid sequence identical to native human insulin, with the exception that the natural proline–lysine sequence characteristics of positions 28 and 29 of the insulin B chain have been reversed. The sequence inversion leads to local conformational changes, eliminating hydrophobic interactions critical to dimer stabilization. As a result, deoligomerization occurs rapidly upon injection and the product can be administered at meal times rather than 30 min before. The different forms and formulations of insulin are shown on the supplementary CD-ROM.

Levemir is the trade name given to an unusual long-acting insulin product that has just recently gained marketing ap-

Table 3 Recombinant insulins/insulin analogs thus far approved

Product	Company	Therapeutic indication	Approved
Humulin (rhInsulin produced in *E. coli*)	Eli Lilly	diabetes mellitus	1982 (US)
Novolin (rhInsulin produced in *S. cerevisiae*)	Novo Nordisk	diabetes mellitus	1991 (US)
Humalog (Insulin lispro, an insulin analog produced in *E. coli*)	Eli Lilly	diabetes mellitus	1996 (US and EU)
Insuman (rhInsulin produced in *E. coli*)	Hoechst	diabetes mellitus	1997 (EU)
Liprolog (Bio Lysprol, short-acting insulin analog produced in *E. coli*)	Eli Lilly	diabetes mellitus	1997 (EU)
NovoRapid (Insulin Aspart, short-acting rhInsulin analog produced in *S. cerevisiae*)	Novo Nordisk	diabetes mellitus	1999 (EU)
Novomix 30 [contains insulin Aspart, short-acting rhInsulin analog produced in *S. cerevisiae* (see *NovoRapid*) as one ingredient]	Novo Nordisk	diabetes mellitus	2000 (EU)
Novolog (Insulin Aspart, short-acting rhInsulin analog produced in *S. cerevisiae*; see also *NovoRapid*)	Novo Nordisk	diabetes mellitus	2001 (US)
Novolog mix 70/30 (contains insulin Aspart, short-acting rhInsulin analog produced in *S. cerevisiae* as one ingredient; see also *Novomix 30*)	Novo Nordisk	diabetes mellitus	2001 (US)
Actrapid/Velosulin/Monotard/Insulatard/Protaphane/Mixtard/Actraphane/Ultratard (all contain rhInsulin produced in *S. cerevisiae* formulated as short/intermediate/long-acting product)	Novo Nordisk	diabetes mellitus	2002 (EU)
Lantus (Insulin glargine, long-acting rhInsulin analog produced in *E. coli*)	Aventis	diabetes mellitus	2000 (US and EU)
Optisulin (Insulin glargine, long-acting rhInsulin analog produced in *E. coli*, see *Lantus*)	Aventis	diabetes mellitus	2000 (EU)
Levemir (Insulin detemir, long-acting rhInsulin analog produced in *S. cerevisiae*)	Novo Nordisk	diabetes mellitus	2004 (EU)
Apidra (Insulin Glulisine, rapid-acting insulin analog produced in *E. coli*)	Aventis	diabetes mellitus	2004 (US)

proval (Table 3). The major structural alteration characteristic of this insulin analog is the attachment of a C14 fatty acid via the side-chain of lysine residue number 29 of the insulin B chain. This promotes binding of the insulin analog to albumin, both at the site of injection and in the plasma. In turn, this promotes a constant and prolonged release of free insulin into the blood, giving it a duration of action of up to 24 h.

6.4 Additional Recombinant Hormones

Nineteen additional recombinant hormones have been approved thus far. These include a number of recombinant versions of human growth hormone (hGH), various gonadotropins, glucagon, parathyroid hormone and calcitonin (Table 4). Amongst these "Somavert" is notable in that it is a recombinant PEGylated analog of hGH. It

Table 4 Additional recombinant hormones approved for general medical use

Product	Company	Therapeutic indication	Approved
Protropin (rhGH, differs from human hormone only by containing an additional N-terminal methionine residue; produced in *E. coli*)	Genentech	hGH deficiency in children	1985 (US)
Glucagen (rhGlucagon produced in *S. cerevisiae*)	Novo Nordisk	hypoglycemia	1998 (US)
Thyrogen (Thyrotrophin-*α*, rhTSH produced in CHO cells)	Genzyme	detection/treatment of thyroid cancer	1998 (US), 2000 (EU)
Humatrope (rhGH produced in *E. coli*)	Eli Lilly	hGH deficiency in children	1987 (US)
Nutropin (rhGH produced in *E. coli*)	Genentech	hGH deficiency in children	1994 (US)
Nutropin AQ (rhGH produced in *E. coli*)	Schwartz Pharma	growth failure, Turner's syndrome	2001 (EU)
BioTropin (rhGH produced in *E. coli*)	Biotechnology General	hGH deficiency in children	1995 (US)
Genotropin (rhGH produced in *E. coli*)	Pharmacia & Upjohn	hGH deficiency in children	1995 (US)
Saizen (rhGH produced in an engineered mammalian cell line)	Serono	hGH deficiency in children	1996 (US)
Serostim (rhGH produced in an engineered mammalian cell line)	Serono Laboratories	treatment of AIDS-associated catabolism/wasting	1996 (US)
Norditropin (rhGH produced in *E. coli*)	Novo Nordisk	treatment of growth failure in children due to inadequate GH secretion	1995 (US)

Table 4 (continued)

Product	Company	Therapeutic indication	Approved
Gonal F (rhFSH produced in CHO cells)	Serono	anovulation and superovulation	1995 (EU), 1997 (US)
Puregon (rhFSH produced in CHO cells)	Organon	anovulation and superovulation	1996 (EU)
Follistim (Follitropin-*β*, rhFSH produced in CHO cells)	Organon	some forms of infertility	1997 (US)
Luveris (lutropin-*α*; rhLH produced in CHO cells)	Ares-Serono	some forms of infertility	2000 (EU)
Ovitrelle (also termed **Ovidrelle**; rhCG produced in CHO cells)	Serono	used in selected assisted reproductive techniques	2001 (EU), 2000 (US)
Forcaltonin (rSalmon calcitonin produced in *E. coli*)	Unigene	Paget's disease	1999 (EU)
Forteo (**Forsteo** in EU; teriparatide; recombinant shortened form of human parathyroid hormone, produced in *E. coli*).	Eli Lilly	treatment of osteoporosis in selected postmenopausal women	2002 (US), 2003 (EU)
Somavert [pegvisomant; recombinant engineered hGH analog (antagonist), produced in *E. coli*]	Pharmacia Enterprises	treatment of selected patients suffering from acromegaly	2002 (EU)

has been engineered so as to introduce nine mutations into the hGH amino acid sequence. It binds the hGH cell surface receptor, but fails to trigger an intracellular response. As such it functions in an antagonistic fashion, reducing the effects of endogenous hGH, underlining its use for the treatment of acromegaly. The molecule is PEGylated so as to increase its serum half-life (see also Part VI, Chapter 2).

6.5 Recombinant Hematopoietic Growth Factors

Recombinant hematopoietic growth factors consist of several EPOs and colony-stimulating factors (Table 5). EPO represents the single most lucrative biopharmaceutical of all and is indicated for the treatment of anemia associated with various medical conditions (see also Part VIII, Chapter 3). As previously described, "Nespo" (Aranesp in the US) is an engineered EPO analog displaying an extended serum half-life. Leukine (owned by Schering) is a recombinant human GM-CSF, a 127-amino-acid glycosylated hematopoietic growth factor produced in an engineered strain of *S. cerevisiae*. It was initially approved for use following induction of chemotherapy in adult patients with acute myelogenous leukemia (or acute nonlymphocytic leukemia) in order to shorten time to neutrophil recovery and reduce the incidence of severe infection. Neupogen (filgrastim) is a recombinant human G-CSF (see also Part VIII,

Table 5 Recombinant hemopoietic growth factors approved for general medical use

Product	Company	Therapeutic indication	Approved
Epogen (rhEPO produced in a mammalian cell line)	Amgen	treatment of anemias	1989 (US)
Procrit (rhEPO produced in a mammalian cell line)	Ortho Biotech	treatment of anemias	1990 (US)
Neorecormon (rhEPO produced in CHO cells)	Boehringer Mannheim	treatment of anemias	1997 (EU)
Aranesp (Darbepoetin-; long-acting rEPO analog produced in CHO cells)	Amgen	treatment of anemia	2001 (EU and US)
Nespo (Darbepoetin-; see also *Aranesp*; long-acting rEPO analog produced in CHO cells)	Dompe Biotec	treatment of anemia	2001 (EU)
Leukine (rGM-CSF, differs from the native human protein by 1 amino acid, Leu23; produced in *S. cerevisiae*)	Immunex	autologous bone marrow transplantation	1991 (US)
Neupogen (Filgrastim, rG-CSF, differs from human protein by containing an additional N-terminal methionine; produced in *E. coli*)	Amgen	chemotherapy-induced neutropenia	1991 (US)
Neulasta (Pegfilgrastim, recombinant PEGylated filgrastim – see *Neupogen*; also marketed in the EU as **Neupopeg**)	Amgen	neutropenia	2002 (US and EU)

Chapter 3) produced by recombinant means in *E. coli*. It regulates the production of neutrophils and is indicated for the treatment of neutropenia associated with various medical conditions. Neulasta is a PEGylated form filgrastim, also used to treat neutropenia. It exhibits an extended duration of action, due to the PEG-mediated reduction in product renal clearance rate.

6.6 Recombinant IFNs and ILs

Quite a number of IFN-based products have gained marketing approval over the last decade and a half (Table 6). The α IFNs have found application mainly in the treatment of certain cancer types and viral diseases. The trend in this case is toward the development of PEGylated forms of these products. As described earlier, the extended plasma half-life associated with such PEGylated forms renders possible their administration as single as apposed to thrice weekly injection. IFN-β preparations (Betaferon, Schering) have found application in the treatment of multiple sclerosis (MS), a chronic disabling disease of the central nervous system. The majority of MS patients develop significant disabilities, either gradually or due to relapsing/remitting symptoms, which involves a worsening of the disease followed by a temporary recovery (see also Part V, Chap-

Table 6 Recombinant IFNs and ILs approved for general medical use

Product	Company	Therapeutic indication	Approved
Intron A (rIFN-α2b produced in *E. coli*)	Schering Plough	cancer, genital warts, hepatitis	1986 (US), 2000 (EU)
PegIntron A (PEGylated rIFN-α2b produced in *E. coli*)	Schering Plough	chronic hepatitis C	2000 (EU), 2001 (US)
Viraferon (rIFN-α2b produced in *E. coli*)	Schering Plough	chronic hepatitis B and C	2000 (EU)
ViraferonPeg (PEGylated rIFN-α2b produced in *E. coli*)	Schering Plough	chronic hepatitis C	2000 (EU)
Roferon A (rhIFN-α2a, produced in *E. coli*)	Hoffmann-La Roche	hairy cell leukemia	1986 (US)
Actimmune (rhIFN-γ1b produced in *E. coli*)	Genentech	chronic granulomatous disease	1990 (US)
Betaferon (rIFN-β1b, differs from human protein in that Cys17 is replaced by Ser; produced in *E. coli*)	Schering	MS	1995 (EU)
Betaseron (rIFN-β1b, differs from human protein in that Cys17 is replaced by Ser; produced in *E. coli*)	Berlex Laboratories and Chiron	relapsing/remitting MS	1993 (US)
Avonex (rhIFN-β1a, produced in CHO cells)	Biogen	relapsing MS	1997 (EU), 1996 (US)
Infergen (rIFN-*a*, synthetic type I IFN produced in *E. coli*)	Amgen (US) and Yamanouchi Europe (EU)	chronic hepatitis C	1997 (US), 1999 (EU)
Rebif (rh IFN-β1a produced in CHO cells)	Ares-Serono	relapsing/remitting MS	1998 (EU), 2002 (US)
Rebetron (combination of ribavirin and rhIFN-α2b produced in *E. coli*)	Schering Plough	chronic hepatitis C	1999 (US)
Alfatronol (rhIFN-α2b produced in *E. coli*)	Schering Plough	hepatitis B and C, and various cancers	2000 (EU)
Virtron (rhIFN-α2b produced in *E. coli*)	Schering Plough	hepatitis B and C	2000 (EU)
Pegasys (Peginterferon-α2a produced in *E. coli*)	Hoffmann-La Roche	hepatitis C	2002 (EU and US)
Proleukin (rIL-2, differs from human molecule in that it is devoid of an N-terminal alanine and Cys-125 has been replaced by a Ser; produced in *E. coli*)	Chiron	renal cell carcinoma	1992 (US)
Neumega (rIL-11, lacks N-terminal proline of native human molecule; produced in *E. coli*)	Genetics Institute	prevention of chemotherapy-induced thrombocytopenia	1997 (US)
Kineret (anakinra; rIL-1 receptor antagonist produced in *E. coli*)	Amgen	rheumatoid arthritis	2001 (US)

ter 3). The underlining mechanism is not completely understood, but it is known that it is mediated by specific cell receptors and involves the modulation of the immune response.

In addition to IL-2 and -11, an IL-1 receptor antagonist (trade name Kineret) has also gained general marketing approval in the US for the treatment of rheumatoid arthritis (Table 6). Kineret binds IL-1α and IL-1β cell surface receptors, but without inducing a biological response. The product therefore blocks IL-1 biological activity, which is a critical mediator of the inflammation and joint damage characteristic of this condition.

6.7 Vaccines

A number of recombinant vaccines have also gained marketing approval for general medical use. By far the most prominent example is that of rHBsAg, which is used to vaccinate against hepatitis B. While the recombinant antigen can be used on its

Table 7 Recombinant vaccines approved for general medical use

Product	Company	Therapeutic indication	Approved
Recombivax (rHBsAg produced in *S. cerevisiae*)	Merck	hepatitis B prevention	1986 (US)
Comvax (combination vaccine, containing rHBsAg produced in *S. cerevisiae* as one component)	Merck	vaccination of infants against hemophilus influenzae type B and hepatitis B	1996 (US)
Engerix B (rHBsAg produced in *S. cerevisiae*)	SmithKline Beecham	vaccination against hepatitis B	1998 (US)
Tritanrix-HB (combination vaccine, containing rHBsAg produced in *S. cerevisiae* as one component)	SmithKline Beecham	vaccination against hepatitis B, diphtheria, tetanus and pertussis	1996 (EU)
Lymerix (rOspA, a lipoprotein found on the surface of *Borrelia burgdorferi*, the major causative agent of Lyme's disease; produced in *E. coli*)	SmithKline Beecham	Lyme disease vaccine	1998 (US)
Infanrix-Hep B (combination vaccine, containing rHBsAg produced in *S. cerevisiae* as one component)	SmithKline Beecham	immunization against diphtheria, tetanus, pertussis and hepatitis B	1997 (EU)
Infanrix–Hexa (combination vaccine, containing rHBsAg produced in *S. cerevisiae* as one component)	SmithKline Beecham	immunization against diphtheria, tetanus, pertussis, polio, hemophilus influenzae B and hepatitis B	2000 (EU)
Infanrix-Penta (combination vaccine, containing rHBsAg produced in *S. cerevisiae* as one component)	SmithKline Beecham	immunization against diphtheria, tetanus, pertussis, polio and hepatitis B	2000 (EU)

Table 7 (continued)

Product	Company	Therapeutic indication	Approved
Ambirix (combination vaccine, containing rHBsAg produced in *S. cerevisiae* as one component)	Glaxo SmithKline	immunization against hepatitis A and B	2002 (EU)
Twinrix (adult and pediatric formsin EU; combination vaccine containing rHBsAg produced in *S. cerevisiae* as one component)	SmithKline Beecham (EU) and Glaxo SmithKline (US)	immunization against hepatitis A and B	1996 (EU, adult), 1997 (EU, pediatric), 2001 (US)
Primavax (combination vaccine, containing rHBsAg produced in *S. cerevisiae* as one component)	Pasteur Merieux MSD	immunization against diphtheria, tetanus and hepatitis B	1998 (EU)
Pediarix (combination vaccine, containing rHBsAg produced in *S. cerevisiae* as one component)	Glaxo SmithKline	immunization of chil dren against various conditions, including hepatitis B	2002 (US)
Procomvax (combination vaccine, containing rHBsAg as one component)	Pasteur Merieux MSD	immunization against hemophilus influenzae type B and hepatitis B	1999 (EU)
Hexavac (combination vaccine, containing rHBsAg produced in *S. cerevisiae* as one component)	Aventis Pasteur	immunization against diphtheria, tetanus, pertussis, hepatitis B, polio and hemophilus influenzae type B	2000 (EU)
Triacelluvax [combination vaccine containing recombinant (modified) pertussis toxin]	Chiron	immunization against diphtheria, tetanus and pertussis	1999 (EU)
Hepacare [rS, pre-S and pre-S2 HBsAgs, produced in a mammalian (murine) cell line]	Medeva	immunization against hepatitis B	2000 (EU)
HBVAXPRO (rHBsAg produced in *S. cerevisiae*)	Aventis	immunization of children and adolescents against hepatitis B	2001 (EU)
Dukoral (*Vibrio cholerae* and recombinant cholera toxin B subunit)	SBL Vaccine	active immunization against disease caused by *V. cholerae* subgroup O1	2004 (EU)

own, it more generally represents one component of multicomponent vaccine preparations (Table 7). The emphasis upon hepatitis B no doubt reflects the global significance of this condition. Two billion people are infected worldwide, with 350 million individuals suffering from lifelong chronic infections. In excess of 1 million sufferers die each year from liver cancer and/or cirrhosis triggered by the condition.

6.8 Monoclonal and Engineered Antibodies

Antibody-based products represent the single largest category of biopharmaceutical approved for general medical use (Table 8) (see also Part IV, Chapter 16 and Part V, Chapters 1 and 2). As previously outlined, first-generation products were invariably murine monoclonals produced by classical hybridoma technology. The development of engineered products, i.e., chimeric and humanized antibodies, overcame many of the therapeutic difficulties associated with such early preparations and the majority of recently approved products are engineered in this way. The majority of antibody-based products are used to either detect or treat various forms of cancer. The antibodies are raised against specific tumor-associated antigens found on the surface of specific cancer types and therefore will bind specifically to those cell types upon administration. Conjugation of radioisotopes, toxins or other chemotherapeutic agents should allow selective delivery of these agents directly to the tumor surface, courtesy of antibody-binding specificity (see also Part II, Chapter 5 and Part V, Chapter 6). In practice, some difficulties can arise with this approach, e.g., due to antibody cross-reactivity with nontransformed cells or the presence of the tumor-associated antigen, even in low numbers, on the surface of unrelated, healthy cells.

Herceptin is the trade name given to one such antibody-based cancer therapy product (Table 8). It is a humanized mAb with binding specificity for the human epidermal growth factor receptor 2 (HER2), which is overexpressed on the surface of 25–30% of metastatic breast cancers. HER2 overexpression induces abnormal proliferation of cells (see also Part I, Chapter 5). The mAb binds specifically to the tumor cells overexpressing HER2, thus inhibiting their proliferation and inducing antibody-directed cell-mediated cytotoxicity. This results in reducing metastasis while not affecting normal cells, therefore limiting side-effects.

Detection (as opposed to treatment) of tumors is facilitated by the conjugation of a γ-emitting radioactive tag to an appropriate antibody. The radioactivity congregated at the tumor site can penetrate outward from the body, facilitating its detection by equipment such as a planar γ-camera (see also Part V, Chapters 4, 5 and 7). Examples of such products approved include Leukoscan and Prostascint (Table 8).

Several antibody-based products are indicated for non-cancer applications. Zenapax, for example, is used for the prevention of acute kidney transplant rejection. The product is a humanized mAb that specifically binds the α-chain (also known as CD25 or Tac) of the IL-2 receptor. This receptor is expressed on the surface of activated lymphocytes. It acts as an antagonist of the receptor, thus blocking the binding of IL-2 that in turn prevents the stimulation of lymphocytes mediating organ rejection.

6.9 Additional Biopharmaceuticals

A number of additional products that do not fall into any of the categories discussed thus far have also gained marketing approval. Amongst these are several enzymes, including glucocerebrosidase (used for the treatment of Gaucher's disease, discussed previously), DNase (used to treat cystic fibrosis), α-galactosidase (used to treat Fabry disease) and enzymes for amino acid depletion (see also Part II, Chapter 6). Recombinant α-galactosidase is produced in mammalian cell lines. The

Table 8 Monoclonal/engineered antibody-based products approved for general medical use

Product	Company	Therapeutic indication	Approved
CEA-scan [Arcitumomab; murine mAb fragment (Fab), directed against human carcinoembryonic antigen]	Immunomedics	detection of recurrent/ metastatic colorectal cancer	1996 (US and EU)
MyoScint (Imiciromab-Pentetate; murine mAb fragment directed against human cardiac myosin)	Centocor	myocardial infarction imaging agent	1996 (US)
OncoScint CR/OV (Satumomab Pendetide; murine mAb directed against TAG-72, a high-molecular-weight tumor-associated glycoprotein)	Cytogen	detection/staging/ follow-up of colorectal and ovarian cancers	1992 (US)
Orthoclone OKT3 (Muromomab CD3; murine mAb directed against the T lymphocyte surface antigen CD3)	Ortho Biotech	reversal of acute kidney transplant rejection	1986 (US)
ProstaScint (Capromab Pentetate; murine mAb directed against the tumor surface antigen PSMA)	Cytogen	detection/staging/ follow-up of prostate adenocarcinoma	1996 (US)
ReoPro (Abciximab; Fab fragments derived from a chimeric mAb, directed against the platelet surface receptor $GPII_b/III_a$)	Centocor	prevention of blood clots	1994 (US)
Rituxan (Rituximab; chimeric mAb directed against CD20 antigen found on the surface of B lymphocytes)	Genentech/IDEC Pharmaceuticals	non-Hodgkin's lymphoma	1997 (US)
Verluma [Nofetumomab; murine mAb fragments (Fab) directed against carcinoma associated antigen]	Boehringer-Ingelheim/NeoRx	detection of small cell lung cancer	1996 (US)
Zenapax (Daclizumab; humanized mAb directed against the α chain of the IL-2 receptor)	Hoffmann-La Roche	prevention of acute kidney transplant rejection	1997 (US), 1999 (EU)
Simulect (Basiliximab; chimeric mAb directed against the α chain of the IL-2 receptor)	Novartis	prophylaxis of acute organ rejection in allogeneic renal transplantation	1998 (EU and US)
Remicade (Infliximab, chimeric mAb directed against TNF-α)	Centocor	treatment of Crohn's disease	1998 (US), 1999 (EU)
Synagis (Palivizumab; humanized mAb directed against an epitope on the surface of respiratory syncytial virus)	MedImmune (US) and Abbott (EU)	prophylaxis of lower respiratory tract disease caused by respiratory syncytial virus in pediatric patients	1998 (US), 1999 (EU)

Table 8 (continued)

Product	Company	Therapeutic indication	Approved
Herceptin (Trastuzumab; humanized antibody directed against HER2)	Genentech (US) and Roche Registration (EU)	treatment of metastatic breast cancer if tumor overexpresses HER2 protein	1998 (US), 2000 (EU)
Indimacis 125 (Igovomab; murine mAb fragment (Fab_2) directed against the tumor-associated antigen CA 125)	CIS Bio	diagnosis of ovarian adenocarcinoma	1996 (EU)
Tecnemab KI [murine mAb fragments (Fab/Fab_2 mix) directed against high-molecular-weight melanoma-associated antigen]	Sorin	diagnosis of cutaneous melanoma lesions	1996 (EU)
LeukoScan [Sulesomab; murine mAb fragment (Fab) directed against NCA 90, a surface granulocyte nonspecific cross-reacting antigen]	Immunomedics	diagnostic imaging for infection/inflammation in bone of patients with osteomyelitis	1997 (EU)
Humaspect (Votumumab; human mAb directed against cytokeratin tumor-associated antigen)	Organon Teknika	detection of carcinoma of the colon or rectum	1998 (EU)
Mabthera (Rituximab; chimeric mAb directed against CD20 surface antigen of B lymphocytes)	Hoffmann-La Roche (see also *Rituxan*)	non-Hodgkin's lymphoma	1998 (EU)
Mabcampath (EU) or **Campath** (US) (Alemtuzumab; humanized mAb directed against CD52 surface antigen of B lymphocytes)	Millennium and ILEX (EU), and Berlex, ILEX Oncology and Millennium Pharmaceuticals (US)	chronic lymphocytic leukemia	2001 (EU and US)
Mylotarg (Gemtuzumab zogamicin; humanized antibody-toxic antibiotic conjugate targeted against CD33 antigen found on leukemic blast cells)	Wyeth Ayerst	acute myeloid leukemia	2000 (US)
Zevalin (Ibritumomab Tiuxetan; murine mAb, produced in a CHO cell line, targeted against the CD20 antigen)	IDEC pharmaceuticals (US) and Schering (EU)	non-Hodgkin's lymphoma	2002 (US), 2004 (EU)
Humira [EU and US; also sold as **Trudexa** in EU; Adalimumab; recombinant (anti-TNF) human mAb created using phage display technology]	Cambridge Antibody Technologies and Abbott (US), and Abbott (EU)	rheumatoid arthritis	2002 (US), 2003 (EU)

429-amino-acid glycoprotein spontaneously dimerizes, yielding the 100-kDa biologically active enzyme. Fabry disease is a rare genetic condition characterized by a deficiency of the lysosomal enzyme α-galactosidase A. As a result, sufferers exhibit an inability to break down certain glycolipids, particularly the glycosphingolipid ceramide trihexoside or globotriaosylceramide (GL-3). Glycolipid accumulates in the walls of vascular cells, particularly in the kidney, heart and nervous system.

Regranex is an interesting product in that it is administered not by direct injection as is the case for most biopharmaceuticals. The active product ingredient is a recombinant human platelet-derived growth factor (PDGF). Active PDGF is a homodimer. Each 109-amino-acid, glycosylated polypeptide is aligned in an antiparallel fashion relative to the other, yielding the 24.5-kDa mature molecule. Regranex is produced by recombinant DNA technology in *S. cerevisiae* and the product is presented in a gel formulation containing 0.1% active ingredient for external topical use. Regranex is indicated for the treatment of chronic diabetic ulcers that do not

Table 9 Additional biopharmaceuticals approved for general medical use

Product	Company	Therapeutic indication	Approved
Beromun (rhTNF-α produced in *E. coli*)	Boehringer-Ingelheim	adjunct to surgery for subsequent tumor removal, to prevent or delay amputation	1999 (EU)
Revasc (anticoagulant; recombinant hirudin produced in *S. cerevisiae*)	Ciba Novartis Europharm	prevention of venous thrombosis	1997 (EU)
Refludan (anticoagulant; recombinant hirudin produced in *S. cerevisiae*)	Hoechst Marion Roussel (US) and Behringwerke (EU)	anticoagulation therapy for heparin-associated thrombocytopenia	1998 (US); 1997 (EU)
Cerezyme (rβ-glucocerebrosidase produced in CHO cells; differs from native human enzyme by 1 amino acid, Arg495 is substituted with a His, also has modified oligosaccharide component)	Genzyme	treatment of Gaucher's disease	1994 (US); 1997 (EU)
Pulmozyme (Dornase-α, rDNase produced in CHO cells)	Genentech	cystic fibrosis	1993 (US)
Fabrazyme (rhα-Galactosidase produced in CHO cells)	Genzyme	Fabry disease (α-galactosidase A deficiency)	2001 (EU)
Replagal (rhα-Galactosidase produced in a continuous human cell line)	TKT Europe	Fabry disease (α-galactosidase A deficiency)	2001 (EU)

Table 9 (continued)

Product	Company	Therapeutic indication	Approved
Fasturtec (Elitex in US; rasburicase; recombinant urate oxidase produces in *S. cerevisiae*)	Sanofi-Synthelabo	hyperuricaemia	2001 (EU), 2002 (US)
Aldurazyme (Laronidase; rh-α-L-iduronidase produced in an engineered CHO cell line)	Genzyme	long-term enzyme replacement therapy in patients suffering from mucopolysaccharidosis	2003 (EU)
Regranex (rhPDGF produced in *S. cerevisiae*)	Ortho-McNeil Pharmaceuticals (US) and Janssen-Cilag (EU)	lower extremity diabetic neuropathic ulcers	1997 (US), 1999 (EU)
Vitravene (Fomivirsen; an antisense oligonucleotide)	ISIS Pharmaceuticals	treatment of cytomegalovirus retinitis in AIDS patients	1998 (US)
Ontak (rIL-2-diphtheria toxin fusion protein which targets cells displaying a surface IL-2 receptor)	Seragen/Ligand Pharmaceuticals	cutaneous T cell lymphoma	1999 (US)
Enbrel (rTNF receptor–IgG fragment fusion protein produced in CHO cells)	Immunex (US) and Wyeth Europa (EU)	rheumatoid arthritis	1998 (US), 2000 (EU)
Osteogenic protein 1 (rhOsteogenic protein-1 : BMP-7 produced in CHO cells)	Howmedica (EU) and Stryker (US)	treatment of non-union of tibia	2001 (EU and US)
Infuse (rhBMP2 produced in CHO cells)	Medtronic Sofamor Danek	promotes fusion of vertebrae in lower spine	2002 (US)
Inductos (dibotermin-α; rBone morphogenic protein-2 produced in CHO cells)	Genetics Institute	treatment of acute tibia fractures	2002 (EU)
Xigris [drotrecogin-α; rh activated protein C produced in a mammalian (human) cell line]	Eli Lilly	severe sepsis	2001 (US), 2002 (EU)

heal with normal wound care practice. It is usually administered daily for up to a maximum of 20 weeks.

Bone morphogenic proteins (BMPs) represent another interesting class of biopharmaceutical (Table 9). As their name suggests, these proteins can promote the deposition and growth of new bone, and are often administered by implantation as part of a medical device. InductOs, for example, consists of a recombinant human BMP-2 that promotes the differentiation of mesenchymal cells into bone cells (see also Part I, Chapter 13). The biologically active form is a glycosylated heterodimer, consisting of 114- and 131-amino-acid

polypeptide subunits. It is produced in a CHO cell line. InductOs is used in skeletally mature patients for the treatment of acute tibia fractures in adjunct to standard care using fracture reduction and intramedullary nail fixation. It is applied during surgical procedure at the site of fracture. The use of a bovine collagen sponge ensures retention of the active substance at the site of the fracture for the time required for healing, with the matrix completely dissolving over time.

7 Products Approved for Veterinary Use

While the majority of pharmaceuticals produced by modern biotechnological means are destined for human use, several veterinary biopharmaceuticals have also gained approval (Table 10). One of the earliest such examples is bovine somatotropin (recombinant bovine GH), used to boost the milk yields of dairy cattle. The majority of veterinary biopharmaceuticals, however, are engineered vaccines. The importance of effective vaccination to prevent rapid spread of disease through high-density animal populations characteristic of modern agricultural practice is obvious and most vaccines are destined for use in agriculturally important species. Porcillis Porcoli, for example, is a multisubunit vaccine containing a combination of recombinant *E. coli*-derived adhesin proteins. These proteins are essential for colonization of the gut by pathogenic *E. coli*. Immunization of sows effectively provides passive immunity to progeny via colostrum for the first few days of life, when piglets are particularly susceptible to *E. coli* infections. Two additional veterinary biopharmaceuticals, which are particularly interesting, are

Table 10 Recombinant veterinary medicinal products approved in EU via the centralized application process

Product	Company	Therapeutic indication	Approved
Porcilis Porcoli (combination vaccine containing recombinant *E. coli* adhesins)	Intervet	active immunization of sows	1996
Fevaxyn Pentofel (combination vaccine containing recombinant feline leukemia viral antigen as one component)	Fort Dodge Laboratories	immunization of cats against various feline pathogens	1997
Neocolipor (vaccine containing four inactivated *E. coli* strains; two wild-type strains expressing *E. coli* adhesins F6 and F41, and two recombinant strains, engineered to express F4 and F5 adhesins)	Merial	reduction of neonatal enterotoxicosis of young piglets caused by *E. coli* strains expressing F4, F5, F6 or F41 adhesins	1998
Porcilis AR-T DF (combination vaccine containing a modified toxin from *Pasteurella multocida* expressed in *E. coli*)	Intervet	reduction in clinical signs of progressive atrophic rhinitis in piglets: oral administration	2000

Table 10 (continued)

Product	Company	Therapeutic indication	Approved
Porcilis pesti (vaccine containing recombinant classical swine fever virus E_2 subunit antigen produced in an insect cell baculovirus expression system)	Intervet	immunization of pigs against classical swine fever	2000
Ibraxion (vaccine consisting of an inactivated, BHV type 1 engineered by removal of the viral glycoprotein *gE* gene)	Merial	active immunization of cattle against infectious bovine rhinotracheitis	2000
Bayovac CSF E2 (vaccine consisting of recombinant classical swine fever virus E2 subunit antigen produced using a baculovirus vector system)	Bayer	immunization of pigs against classical swine fever virus	2001
Eurifel FELV (vaccine consisting of an engineered canarypox virus into which the *gag*, *env* and a partial *pol* gene of feline leukemia virus have been inserted)	Merial	Immunization of cats against feline leukemia virus	2000
Vibragen ω (rFeline IFN-ω)	Virbac	reduce mortality/clinical signs of canine parvovirusis	2001
Eurifel RCPFEVL (multicomponent vaccine containing as one component an engineered canarypox virus into which the *gag*, *env* and a partial *pol* gene of feline leukemia virus have been inserted (see Eurifel FELV above)	Merial	active immunization of cats against viral pathogens, including feline leukemia virus	2002
Gallivac HVT IBD (live multicomponent vaccine containing as one component an engineered herpes virus of turkeys housing a gene coding for the protective VP2 antigen of the infectious bursal disease virus)	Merial	active immunization of chickens against, amongst others, the viral causative agent of infectious bursal disease	2002

Ibraxion and Vibragen ω, as discussed below.

The advent of genetic engineering has facilitated the development of engineered vaccines capable of allowing subsequent immunological differentiation between infected and vaccinated animals. Using this form of vaccination allows veterinary inspectors to tell if a seropositive animal has simply been vaccinated (and is noninfectious) or if it has been infected with the wild-type pathogen (and is likely to be infectious, thereby requiring treatment/isolation).

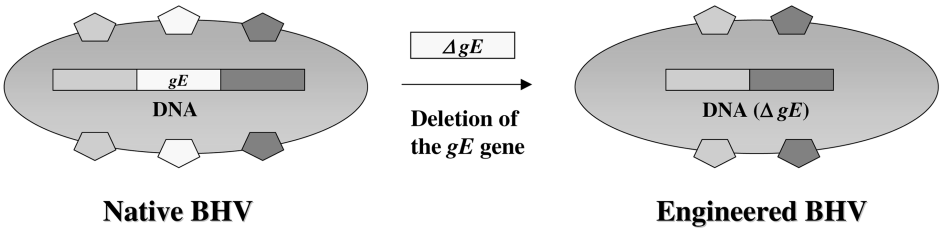

Fig. 3 Diagrammatic representation of the alteration made to the engineered BHV in order to produce the product Ibraxion. By means of genetic engineering, the structural gene *gE* is deleted from the genome. BHV induces infectious bovine rhinotracheitis, a condition characterized by losses in animal production and abortions. Ibraxion induces immunological protection in cattle, but the serum of Ibraxion-vaccinated animals is devoid of anti-gE antibodies, whereas infected animals will have high titers of such antibodies.

Ibraxion is an example of such an engineered vaccine. It is an engineered bovine herpes virus (BHV) from which one structural gene (the *gE* gene) has been deleted. BHV induces infectious bovine rhinotracheitis, a condition characterized by losses in animal production and abortions. Ibraxion induces immunological protection in cattle, but the serum of Ibraxion-vaccinated animals is devoid of anti-gE antibodies, whereas infected animals will have high titers of such antibodies (Fig. 3).

Vibragen ω is IFN-ω, a novel type 1 IFN. Like other type 1 IFNs, it displays antiviral activity which is the basis of its use in treating parvoviral infections, especially in young dogs – for whom such an infection can be fatal. Vibragen ω is also somewhat unusual in that it is manufactured using insect-based biosynthesis occurring in whole silkworms (Fig. 4). The process entails the use of the silkworm nuclear polyhedrosis virus (NPV), engineered to carry cDNA for feline IFN-ω. Initial viral amplification is first undertaken to produce sufficient quantities of virus to seed the process. Amplification is undertaken by viral incubation with an insect cell line (originated from *Bombix mori*) grown in conventional culture flasks. The product is then manufactured by rearing several thousand silkworms on heat-treated, synthetic chow in sterile cabinets. After 24–48 h each silkworm is inoculated with NPV using an automatic microdispenser. Five days later the silkworms are mechanically incised and the acid-stable IFN is extracted from the body parts. Downstream processing is more conventional, and employs both dye and metal affinity chromatography to achieve product purification.

8 Likely Future Directions

The main aim of this Introduction is to provide the reader with a snapshot of the profile of biopharmaceutical products approved to date. How the sector will develop over the next decade or two will likely echo, at least in part, many of the innovations discussed within the remaining chapters of this book. A summary overview of some such likely innovations and directions is presented below.

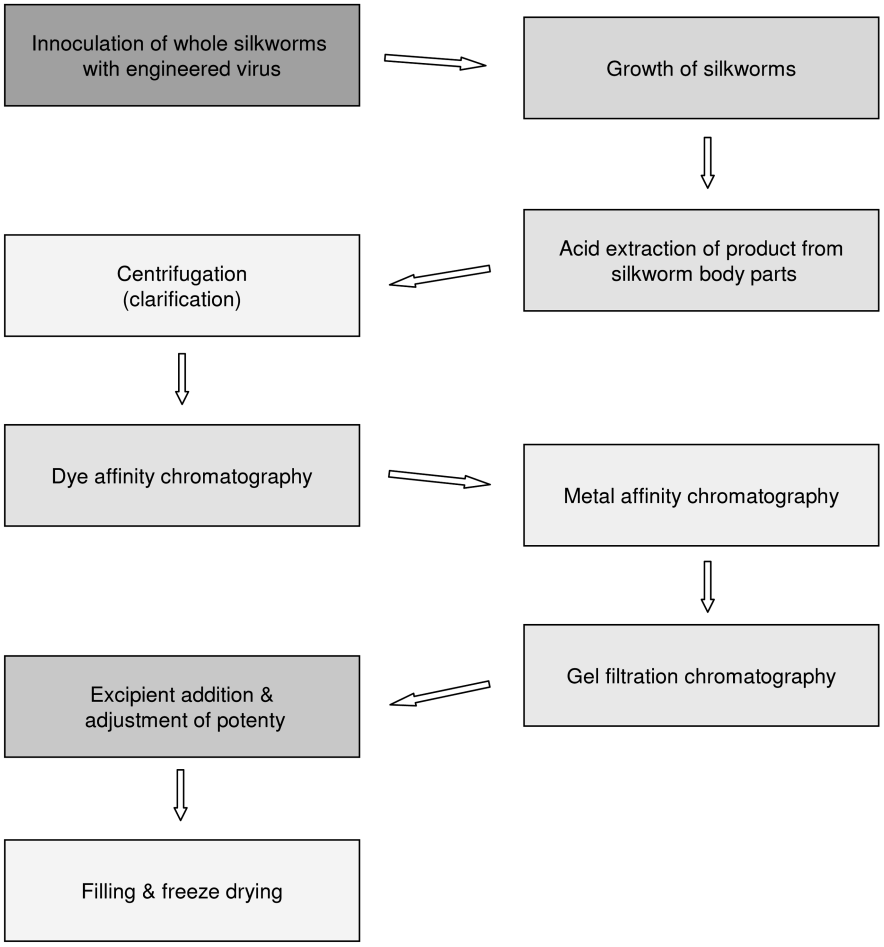

Fig. 4 Overview of the manufacture of the veterinary medicinal product Vibragen ω. The process entails inoculation of whole silkworms (grown on synthetic food in pre-sterile cabinets) with an engineered silkworm NPV housing the feline IFN-ω gene. Product extraction, purification and formulation ensues.

8.1 What is in the Pipeline?

Globally, in excess of 500 candidate biopharmaceuticals are undergoing clinical evaluation. The Pharmaceutical Research and Manufacturers of America (PhRMA), which represents the US drug industry, estimates that some 371 biotech medicines are undergoing trials in the US [27]. Of these, around half (178) aim to treat cancer (see also Part II, Chapter 4), and other notable target indications include infectious diseases (47 products) (see also Part VI, Chapter 3), autoimmune disorders (26 products) (see also Part V, Chapter 3), neurological disorders (22 products) (see also Part I, Chapter 14) and AIDS/HIV related conditions (21 products) (see also Part II, Chapters 7 and 8). The single largest cate-

gory is vaccines, of which there are 98 in development (see also Part I, Chapter 7). Fifty-three of these vaccines aim to treat or prevent cancers, whereas an additional 29 aim to treat various infectious diseases, including hepatitis and HIV. The second largest product category is that of monoclonal/engineered antibodies (see also Part IV, Chapter 16 and Part V, Chapters 1 and 2). Of the 75 such products in development, 39 (52%) target cancers and 10 aim to treat various autoimmune conditions, most notably rheumatoid arthritis. The number of IL- and antibody-based products in trials has increased modestly over the past 2–3 years. The past few years has also witnessed a significant decrease in the number of growth factors and gene-therapy-based products undergoing clinical evaluation by PhRMA-associated companies, at least (see also Part VI, Chapter 6). The latter reflects the continued difficulties associated with making nucleic acid-based products a therapeutic reality [28, 29] (see also Part I, Chapters 6–9).

8.2 Alternative Production Systems for Biopharmaceuticals

Essentially all recombinant therapeutic proteins approved thus far are expressed either in *E. coli*, *S. cerevisiae* (see also Part IV, Chapters 12 and 13), or in an engineered animal cell line (see also Part IV, Chapters 1 and 4), hybridoma (mouse/human) cells (see also Part IV, Chapter 2) or even human cells (see also Part IV, Chapter 3).

Research continues into the development of alternative production systems and of particular note is the use of transgenic animals or plants (see also Part IV, Chapter 5). A number of recombinant therapeutic proteins (including α_1-antitrypsin, α-glucosidase and antithrombin III) have been successfully produced in transgenic animals, mainly in the milk of mice (proof-of-concept stage) or goats (putative production-scale systems) (see also Part IV, Chapter 11). While this approach has proven to be technically possible, a range of problems has thus far delayed/prevented approval of any product produced in this manner, although ATryn® (antithrombin III) will most likely be approved at the time when this book will be published. Difficulties have included modest/variable production levels, regulatory issues and cost. The major companies – besides GTC Biotherapeutics (US) – sponsoring this technology are PPL therapeutics (Scotland) and Pharming (The Netherlands). Work also continues on the development of transgenic plant-based systems for the production of, for example, oral vaccines or other therapeutic proteins. Again, regulatory and cost issues are complicating factors, as are issues such as the significant difference between glycosylation patterns characteristic of plant versus animal cell-based production systems. A comprehensive overview is given by Knäblein in two excellent reviews [30, 31]. Due to the importance of such emerging systems, this book has dedicated a complete section to alternative expressions systems for biopharmaceuticals – especially plant-based expression systems (see also Part IV, Chapter 6, Part IV, Chapters 7 and 8, Part IV, Chapter 9 and Part IV, Chapter 10). The section concludes with the engineering of plant expression systems for abiotic stress tolerance. By making plants tolerant for high salt concentrations, heat and drought, this might in the near future lead to growing plants in areas which today cannot be used for agriculture at all.

8.3 Alternative Delivery Methods for Biopharmaceuticals

Thus far, therapeutic proteins are invariably administered parenterally. Drug administration by nonparenteral means is generally less invasive, requires less technical training and is normally associated with improved patient compliance (see also Part VI, Chapter 1, and Part VI, Chapters 5 and 3). Some progress has also been recorded relating to the development of nonparenteral delivery routes for biopharmaceuticals. Most prominent in this regard is pulmonary delivery [32, 33]. Macromolecules are absorbed from the lung surprisingly well, likely due to the lung's large surface area, thin diffusional layer and the presence of proteolytic inhibitors. Nebulizer technology allows product delivery into the deep lung and drugs adsorbed in this way avoid first-bypass metabolism (see also Part VI, Chapter 4). Exubera is the name given to an insulin product administered by pulmonary means (see also Part IV, Chapter 13). Developed by Pfizer, Aventis and Nektar (see also Part VI, Chapter 2), this product has completed phase III clinical trials, although additional safety studies are currently being undertaken.

8.4 The Advent of Generic Biopharmaceuticals?

Patent protection for many early biopharmaceuticals, such as recombinant insulin, EPO, hGH and IFN-α, is now nearing or at an end (see also Part VIII, Chapter 3). Most of these are blockbuster products – each commands annual sales well in excess of $ 1 billion and hence these represent attractive targets for the fledgling biopharmaceutical generics industry [34] (see also Part II, Chapter 4 and Part VIII, Chapter 1). Companies producing generic biopharmaceuticals include Sicor (Irvine, CA), Ivax (Miami, FL), Dragon (Vancouver, Canada), Genemedix (Suffolk, UK) and BioGenerix (Mannheim, Germany). Sicor already markets hGH and α-IFN-α in Eastern Europe, whereas Genemedix markets a recombinant colony-stimulating factor in China and is soon to manufacture EPO (see also Part VIII, Chapter 3). Major generics companies such as Teva, Sandoz and Merck will also likely develop/consider developing biopharmaceutical portfolios. However, the regulatory framework required to underpin generic biopharmaceutical approvals within Europe and North America is not yet finalized, although it appears to be at a more advanced stage in Europe as compared to the US (see also Part VII, Chapter 4). The concept of "similar biological medicinal products" is one now enshrined in the EU regulatory framework. Within the US the regulatory terminology includes phrases such as "follow-on biologics" and "well-characterized protein". Although generics will probably be reviewed by regulators on a case-by-case basis, substantial *in vitro* work as well as some clinical data will almost certainly be required to show comparability/product equivalence.

8.5 Genomics and Proteomics

Most pharmaceutical companies have research programs in genomics/proteomics. The "omics" revolution was initially hailed as a revolution in drug discovery. While these modern technologies may well help identify a host of putative new biopharmaceuticals (see also Part I, Chapters 4 and 5), they almost certainly will have a far more significant impact upon identifying

new drug targets as well as disease diagnostic markers (see also Part I, Chapters 2 and 3 and Part V, Chapter 8).

8.6 Gene Therapy

There have been well in excess of 400 gene-therapy-based trials undertaken to date. The vast majority have reported a disappointing lack of efficacy. Safety concerns have also been raised and these concerns were heightened in 1999 when one participant in a gene-therapy trial died. Largely prompted by this event, the US National Institute of Health requested detailed information from a large number of trials and uncovered several hundred reports of serious trial-associated adverse events in the process. In addition, allegations ensued that other gene-therapy trial deaths went unreported/misreported, particularly in trials using retroviral-based delivery vectors. As a result, more stringent reporting requirements were introduced.

Gene therapy did receive a much-needed boost in 2002 when French scientists reported that they had apparently corrected severe combined immunodeficiency in a number of children using a retroviral-mediated protocol [35]. Severe combined immunodeficiency is a genetic condition caused by a deficiency of the enzyme adenosine deaminase, which triggers severe B and T lymphocyte dysfunction. However, celebrations were halted when a number of trial participants developed uncontrolled lymphoproliferation, a condition similar to leukemia. This halted – at least temporarily – several gene-therapy trials in various regions of the world [36].

Up until then retroviruses had been used as the delivery vectors in over 75% of all gene-therapy clinical trials, mainly because their molecular biology was well understood, the efficiency of gene transfer to sensitive cells was extremely high, subsequent gene expression is usually high and high level stocks of replication-deficient retroviral particles can be produced. Despite such undoubted advantages, retroviruses also display certain disadvantages in the context of gene delivery, including their ability to infect only actively replicating cells and the fact that their proviral DNA integrates randomly into the host chromosome [37].

The emphasis is now shifting somewhat away from retroviruses and towards alternative viral as well as nonviral vectors. Adenoviruses are receiving attention due to their stability, easy manufacture, ability to infect nondividing cells and their ability to promote high-level gene expression (see also Part I, Chapters 6 and 7). However, this category of vector is not without its own difficulties, as adenoviruses tend to be highly immunogenic in humans, display broad cell specificity and the duration of resultant gene expression can be transient. The most prominent nonviral vector type remains liposome based [38] (see also Part VI, Chapters 7 and 8).

While genetic diseases constitute an obvious target for gene therapy, cancer remains the major indication (see also Part I, Chapter 1). A wide range of strategies continue to be pursued in this regard, including selected delivery of toxins/tumor-suppressor genes/suicide genes into tumor cells, modifying tumor cells to increase their immunogenicity or modifying lymphocytes in order to enhance their antitumor activity [39].

8.7 Antisense and RNA Interference (RNAi)

Antisense technology is based upon the manufacture of short single-stranded

stretches of nucleic acids (DNA or RNA based) or chemically modified versions thereof (see also Part I, Chapter 8). The nucleotide sequence specificity of these antisense molecules allows them to bind to specific gene or (more commonly) mRNA sequences, thereby preventing gene expression by blocking either transcription or translation [40]. The therapeutic rationale underlining this approach stems from the fact that many diseases are triggered or are exacerbated by inappropriate expression/overexpression of specific genes. Antisense, in principle, provides a mechanism by which this can be blocked. While the underlining concept is straightforward, like gene therapy, it is proving more difficult to apply in practice. Major difficulties have arisen in relation to product nuclease sensitivity, product targeting, delivery and cellular uptake.

Vitravene (fomivirsen sodium, ISIS Pharmaceuticals; see Table 9) remains the only antisense-based biopharmaceutical approved for general medical use (see also Part III, Chapter 3). The product is a 21-base phosphorothioate nucleotide that displays a base sequence complementary to certain human cytomegaloviral mRNA transcripts. Its administration inhibits viral replication through an antisense mechanism. Approved in the US in 1998 and in the EU in 1999, the product is indicated for the treatment of cytomegalovirus retinitis by intraocular injection in AIDS patients. It was withdrawn from the EU market in May 2002 for commercial reasons.

RNAi represents an alternative and more recently pursued mechanism of downregulating gene expression ([41] and references therein) (see also Part II, Chapter 8 and Part I, Chapter 1 and 10). The RNAi pathway was first discovered in plants, but it is now known to function in most if not all eukaryotes. RNAi represents the sequence-specific post-translational inhibition of gene expression, induced ultimately by double-stranded RNA (dsRNA). Be it produced naturally or synthesized *in vitro* and introduced into a cell by researchers, the (sequence-specific) dsRNA is then cleaved into short (20- to 25-nt) fragments. The RNA strands therein are separated – one is degraded and the other binds to a cellular protein complex. This strand will bind to target (complementary) mRNA, which is then cleaved by an endonuclease within the complex. Because of its ability to downregulate gene expression, RNAi technology has obvious therapeutic potential, and initial therapeutic targets of RNAi include viral infection, neurological diseases and cancer therapy. The synthesis of dsRNA displaying the desired nucleotide sequence is straightforward. However, as in the case of additional nucleic acid-based therapeutic approaches, major technical hurdles remain to be overcome before RNAi becomes a therapeutic reality.

8.8 Stem Cell-based Therapies

The therapeutic application of stem cells has long been a dream of medical sciences, but recent discoveries and technical advances have brought this dream much closer to being a reality. Stem cells are usually defined as undifferentiated cells capable of self-renewal, which can differentiate into more than one specialized cell type. Pluripotent stem cells are capable of essentially differentiating into any cell type, whereas multipotent stem cells, often found (be it in low numbers) within specific organs, give rise to lineage-restricted, tissue-specific cell types (see also Part I, Chapter 13). Human embryonic stem cells, harvested from the inner mass

of the blastocyst, are the most convenient source of pluripotential cells. A quantum leap was taken in 2004 with the generation of an unlimited source of human embryonic stem cells by Woo Suk Hwang from Seoul University. Hwang et al. were able to obtain pluripotent embryonic stem cells from somatic cell nuclear transfer of reprogrammed human adult cells (see also Part I, Chapter 11). When cultured in the presence of various specific growth factors these pluripotential cells have be induced to differentiate into various mature cell types, including liver, hematopoietic cells, neurons, pancreatic, skeletal and endothelial cells. Therefore stem cells harbor the potential to form replacements for damaged/diseased body cells, tissue or even entire organs (see also Part I, Chapters 12 and 15). This technology could eventually give rise to cell-based therapies for various neurological diseases, kidney, heart, lung or other organ failure, diabetes, cardiac damage, etc. The scope and limitations currently underpinning stem cell technology are well beyond the scope of Introduction, but will be discussed in the respective chapters in this book. The interested reader is also referred elsewhere [42–45] for sources of additional information.

9 Concluding Remarks

Overall, the biopharmaceutical sector is one that is now maturing rapidly. The fact that biopharmaceuticals now generate in excess of $ 30 billion revenue annually – from a zero starting point just over 20 years ago – illustrates the medical and, indeed, commercial importance of these drugs. Even more excitingly, continued advances in both pure and applied medical research will fuel continued growth within the biopharmaceutical sector for many years to come.

Many of the concepts described in here are explored in more detail in subsequent chapters. It was a real pleasure for me to write this Introduction and I am impressed, because this book brings together world-class contributions from world-class scientists, drawn from both industry and academia. This compilation is one of the most comprehensive books published to date in this area and it is a "must-read" for everybody working in this field.

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