Chapter 23
Biopharmaceutical Products from Animal Cell Culture

Darrin Kuystermans and Mohamed Al-Rubeai

Abstract Animal cell culture bioprocesses based on mammalian expression systems have given the pharmaceutical industry a means to produce complex glycosylated therapeutic proteins that is projected to be at least a US$500 billion dollar market by 2020. Medicinal products produced by mammalian cell cultures include hormones, enzymes, cytokines, bone morphogenic proteins, clotting factors, antibodies, and fusion protein therapeutics. Activase®, a recombinant thrombolytic enzyme, was the first approved mammalian cell culture drug to be produced from Chinese hamster ovary cell culture and marketed to the public. Over time, other mammalian derived products followed and have evolved from simple replicas of endogenous proteins to complex engineered bio-molecules. Among the existing mammalian expressed biological drugs discussed, that have been produced in the USA and EU till early 2014, monoclonal antibody therapeutics have become the top earning products being over 40 % of products produced. The development of chimeric, humanized and eventually fully human antibodies has also decreased immunogenic reactions in human patients to below 10 % for the majority of engineered monoclonals with some even reaching below 1 %. Enbrel®, the first Fc-fusion protein introduced onto the market has also led to engineered therapeutic proteins with a longer half-life and multiple functions as with the introduction of bispecific antibody therapeutics. The introduction of the first biosimilars, starting in 2007, can further lower the cost of access to mammalian produced biologics and has also meant a further increase in the overall mammalian cell culture capacity around the globe.

Keywords Biopharmaceuticals • Monoclonal antibodies • Fusion proteins • Mammalian cell culture • Biosimilars • EMA • FDA • Drug approvals

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23.1 Introduction

Over the recent years the small molecule pharmaceutical industry has slowed and shifted emphasis towards biopharmaceuticals. This shift is due to thinning pipelines and higher success rates of clinical trials for biopharmaceuticals compared to small molecules drug entities, but also pressure from the generics industry lowering the profit margins of blockbuster chemical compounds. The global biopharmaceutical market was estimated to be around US$199.7 billion in 2013 and was further projected to reach close to US$500 billion by 2020 growing at a compound annual growth rate (CAGR) of 13.5 % CAGR between 2010 and 2020 (Research and Markets 2013) compared to the 0.6 % growth rate of more small-molecules entities in the pharmaceutical market (Beck et al. 2008). The United States biopharmaceutical market alone is estimated to reach US$144 billion by 2016 due to an increase in new product launches but also due to an aging population, with a large majority reaching 65 years and above and the approval of new indications for existing drugs (Markets and Markets 2011). In 2012, 58 % of United States (US) and/or European approved and marketed biopharmaceuticals are produced via a mammalian cell culture (Ecker and Ransohoff 2014). The area of monoclonal antibody (mAb) constitutes the largest growing segment of the market with an estimated share of 25.6 % in 2013, US$51.1 billion of the global market and predicted to reach sales of US$70 billion by 2015 (Chon and Zarbis-Papastoitsis 2011). Generally, over the last two decades the amount of therapeutic recombinant glycoproteins on the market produced by animal cell culture has increased significantly from only 18 approved products in the 1990s to approaching close to 200 approved products by the end of 2015 at the current approval rates.

The first biopharmaceuticals consisted of replacement hormones and first generation vaccines, followed by monoclonal antibodies, recombinant proteins and second generation vaccines that are now maturing in the industry. Monoclonal antibodies (mAbs) have taken the leading segment in biopharmaceuticals, and are also the fastest growing segment (Reichert et al. 2005; Reichert 2014) predominantly produced via mammalian cell culture processes. Over thirty new biological entities (NBE) produced by mammalian cell culture came onto the US and European Union (EU) market between 2010 and the first quarter of 2014 which is a slight increase from the 3 years prior, before 2010. The market for glycosylated biopharmaceuticals has had a ramp up in manufacturing since the year 2000 after the first monoclonal antibody entered the market in 1986, known as Orthoclone®, opening the gateway for the first generation recombinant glycosylated protein approvals. This first generation consisted, initially, of recombinant tissue plasminogen activator (rtPA) and recombinant erythropoietin followed by a plethora of recombinant human proteins that entered the market with a majority showing great success over the past 25 years.

The top best-selling biopharmaceuticals in 2013 all were produced from mammalian cell culture with the top three drugs, Humira® (Adalimumab, a fully human mAb), Remicade® (Infliximab, a chimeric mAb) and Enbrel® (Etanercept, a

Fc-fusion based) all treating autoimmune diseases such as rheumatoid arthritis (RA). Cancer drugs are also a big hit with the sixth best-selling biopharmaceutical drug being a chimeric mAb initially used to treat non-Hodgkin lymphomas and follicular lymphoma which can also be used to treat RA. In fact, the sales of non-antibody based biopharmaceuticals produced in mammalian cell culture has slowed over the recent years due to decreases in the development pipeline for these kind of drugs (Ecker and Ransohoff 2014). This slowdown has had little effect on the overall growth of mammalian cell culture produced biopharmaceuticals with the once small segment in the overall pharmaceutical product pipeline becoming a major global segment for the pharmaceutical industry.

This chapter will give an overview of the biopharmaceutical products manufactured using animal/mammalian cell culture that have been approved by the European Medicines Agency (EMA) and the United States Food and Drug Administration (FDA) to be introduced onto the market, and how these products have evolved from simple copies of endogenous proteins to the engineered proteins we see today. The arrival of biosimilars is discussed and along with current market trends, what might be the impact on biopharmaceutical cell culture capacity. The recombinant proteins manufactured via mammalian cell culture brought onto the EU and US market from 1989 till the first quarter of 2014 have also been tabulated for reference along with their respective platform host cell lines and indications.

23.2 Early Mammalian Based Biopharmaceuticals

Mammalian cells are well suited to carry-out post-translational modifications similar to the native proteins found in the human body as opposed to microbial and yeast systems at this moment in time. The superior ability to perform these modifications can become a necessity for retaining biological activity of complex proteins such as mAbs, thus the first therapeutic monoclonal antibody, a mouse IgG2a, was produced using the mouse ascites, in 1986, Muromonab which was marketed as OrthoClone OKT3®. The ascitic fluid was harvested from a peritoneal tumor in mice that was induced by injecting hybridoma cells into the peritoneum. This essentially makes the rodent a mini bioreactor for cell growth so that the hybridoma densities could increase as they secreted antibodies until a concentrated solution of mAb's (~1–10 mg/ml) could be harvested (McGuill and Rowan 1989). Apart from this in vivo method of mAb production from ascites causing pain and significant distress in mammals the economic implications for large scale production have directed pharmaceutical manufacturers to utilize in vitro cell culture processes.

Over the past decades several cell lines have become popular hosts of marketed recombinant protein products. The past and current cell lines utilized as a main platform for mammalian biopharmaceutical culture are murine myeloma lymphoblastoid type cells such as NS0 (Bebbington et al. 1992; Barnes et al. 2001) and Sp2/0-Ag14 (Shulman et al. 1978), Chinese Hamster Ovary

(CHO) (Cockett et al. 1990; Milbrandt et al. 1983), baby hamster kidney (BHK-21) (Carvalhal et al. 2001; Christie and Butler 1999; Geserick et al. 2000; Kirchhoff et al. 1996), and human embryonic kidney epithelial cells (HEK-293) (Baldi et al. 2005; Schlaeger and Christensen 1999). From these cell types, CHO cell lines were the host that dominated the first round of commercial mammalian cell culture produced drugs, with the first recombinant therapeutic protein produced from animal cell culture being a tissue plasminogen factor marketed in 1987, by Genentech, under the trade name Activase® (alteplase), FDA-approved for the treatment of myocardial infarctions (Walsh 2004; Cannon et al. 1998; Gillis et al. 1995; Kunadian and Gibson 2012). With CHO based biopharmaceuticals gaining regulatory approval, most manufacturers considered CHO an acceptable host system for intravenous drug production and naturally the popularity of CHO cells as hosts increased. Other factors such as the CHO being a robust cell line that is adaptable, with the ability to reach a high cell density suspension culture also reinforced the CHO cell line popularity amongst manufacturers as time progressed. As of early 2014, CHO based cell culture represents over 75 % of all mammalian expressed biopharmaceuticals produced (Fig. 23.1). Since a host of highly glycosylated protein could not be produced by microbial systems such as *Escherichia coli* (*E.coli*), tPA, EPO (erythropoietin), and factor VIII recombinant therapeutics (see Table 23.1) became attractive first time biopharmaceuticals produced in mammalian cell culture systems in the late 1980s and early 1990s.

The popularity of commercial suspension mammalian cell culture produced mAb based therapeutics and their derivatives started in the late 1980s, soon after the release of Orthoclone OKT3®. While the first round of biopharmaceuticals were mainly copies of recombinant anti-hemophilic factors (clotting factors), cytokines, and enzymes such as the thrombolytic agents (see Table 23.1), this

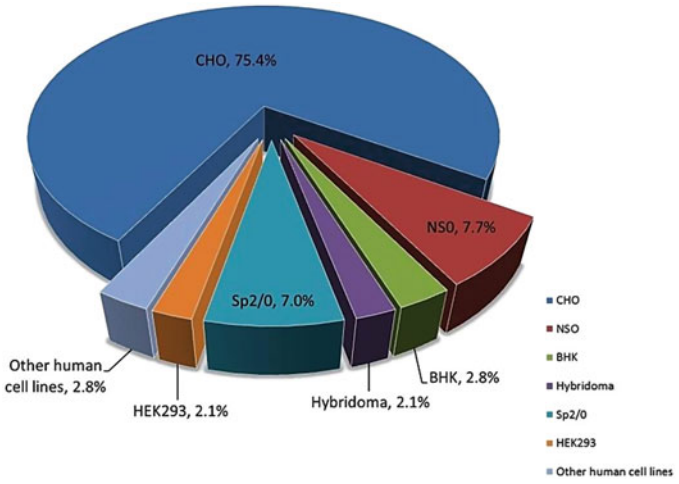

Fig. 23.1 Percentage of the type of mammalian cell lines used in commercial scale manufacturing of biopharmaceuticals from 1987 till April 2014

Table 23.1 The biopharmaceutical drug approvals in the EU and USA between 1989 and April 2014 which are produced by mammalian cell culture are summarized in table below. The table starts from the most recent approvals. Data was collected from several sources (European Medicines Agency 2014; United States Food and Drug Administration 2014). Those shaded in *green* are biosimilars and those shaded in *blue* indicates that the drug was already approved in another region the previous calendar year or earlier

Product	Company	Target/Initial Therapeutic Indication	Product Category	Mammalian Cell Line	First Approved	Region
2014						
Entyvio™ Vedolizumab	Takeda Pharmaceutical Company Limited	Antibody that binds to integrin α4β7 blocking the α4β7 integrin resulting in gut-selective anti-inflammatory activity, for the treatment of ulcerative colitis and Crohn's disease.	Humanized Monoclonal Antibody	CHO	In Review	EU & USA
Cyramza™ (Ramucirumab)	Eli Lilly & Company	Binds to VEGFR2 blocking the binding of vascular endothelial growth factor (VEGF) to VEGFR2 indicated for the treatment of patients with advanced or metastatic, gastric or gastro-esophageal junction adenocarcinoma	Fully Human Monoclonal Antibody	NS0	23-Apr-14	USA
Sylvant™ Siltuximab	Janssen Biotech (Johnson & Johnson)	Antibody that specifically binds to and neutralizes human IL-6 with high affinity for the treament of adult patients with multicentric Castleman's disease (MCD)	Chimeric Monoclonal Antibody	CHO	23-Apr-14	USA
Alprolix™ (Coagulation Factor IX)	Biogen Idec.	Coagulation factor human IgG1 Fc fusion , which binds to the neonatal Fc receptor (FcRn) for treatment of hemophilia B	Clotting factor / Fc-Fusion Biologic	HEK293	28-Mar-14	USA
VIMIZIM™ (elosulfase alfa)	BioMarin Pharmaceutical Inc.	Recombinant N-acetylgalactosamine-6-sulfate sulfatase replacement therapy for treatment of Mucopolysaccharidosis type IVA (MPS IVA; Morquio A syndrome)	Enzyme	CHO	14-Feb-14	USA

Table. 23.1 (continued)

Product	Company	Target/Initial Therapeutic Indication	Product Category	Mammalian Cell Line	First Approved	Region
2013						
Obinutuzumab® (Gazyva GA101-CD20 mAb)	Genentech/Roche	Treatment of chronic lymphocytic leukemia (CLL) - Obinutuzumab targets CD20 and kills B cells	Humanized Monoclonal Antibody	CHO	01-Nov-13	USA
Novoeight®, Antihemophilic Factor VIII	Novo Nordisk	Recombinant factor VIII for the treatment and prophylaxis of bleeding in patients with haemophilia A (congenital factor VIII deficiency).	Clotting Factors	CHO	15-Oct-13	EU (13-Nov) & USA (15-Oct)
Ovaleap™ Follitropin	Teva Pharma B.V.	A biosimilar of follitropin alfa, recombinant follicle-stimulating hormone for use in conjunction with assisted reproductive technologies, such as in vitro fertilization by the induction of ovulation	Hormone	CHO	27-Sep-13	EU
Inflectra™ Infliximab	Hospira	A biosimilar to Remicade, against tumour necrosis factor alpha (TNF-α) used to treat Crohn's disease, ankylosing spondylitis, alcerative Colitis, psoriatic arthritis, plaque psoriasis and rheumatoid arthritis	Chimeric Monoclonal Antibody	Sp2/0	10-Sep-13	EU
Remsima™ Infliximab	Celltrion	A biosimilar to Remicade, against tumour necrosis factor alpha (TNF-α) used to treat Crohn's disease, ankylosing spondylitis, alcerative Colitis, psoriatic arthritis, plaque psoriasis and rheumatoid arthritis	Chimeric Monoclonal Antibody	Sp2/0	10-Sep-13	EU
Simponi® Aria™ Golimumab	Janssen Biotech (Johnson & Johnson)	Inhibits inflammatory response by suppressing tumor necrosis factor (TNF) as a treatment of moderately to severely active rheumatoid arthritis	Fully Human Monoclonal Antibody	CHO	18-Jul-13	USA
Rixubis®- (Coagulation Factor IX)	Baxter Interantional Inc.	Recombinant Coagulation Factor IX for treatment of hemophilia B, indicated for the control and prevention of bleeding episodes (prophylaxis)	Clotting Factors	CHO	27-Jun-13	USA
Kadcyla® Ado-Trastuzumab Emtansine, T-DM1	Genentech/Roche	Trastuzumab alone stops growth of cancer cells by binding to the HER2/neu receptor, whereas mertansine enters cells and destroys them by binding to tubulin	Humanized Monoclonal Antibody	CHO	22-Feb-13	EU (15-Nov) & USA (22-Feb)
Zaltrap™ Aflibercept	Regeneron/Sanofi Aventis	Fc fusion protein consisting IgG1 Fc fused with VEGF-binding portions from the extracellular domains of human VEGF receptors 1 and 2 for the treatment of metastatic colorectal cancer as Zaltrap (aka Elyea)	Fc-Fusion Biologic	CHO	01-Feb-13	EU (already approved in the USA in 2012)

Table. 23.1 (continued)

Product	Company	Target/Initial Therapeutic Indication	Product Category	Mammalian Cell Line	First Approved	Region
2012						
Abthrax® Raxibacumab injection	GlaxoSmithKline (GSK)	Monoclonal antibody that neutralizes the toxins produced by the anthrax bacterium Bacillus anthracis intended for the prophylaxis and treatment of inhaled anthrax	Human Monoclonal Antibody	NS0	14-Dec-12	USA
Eylea™ Aflibercept	Regeneron/Bayer	Fusion protein with VEGF-binding portions from the extracellular domains of human VEGF receptors 1 and 2 for the treatment of neovascular (wet) Age-related Macular Degeneration (AMD) as Eylea (aka Zaltrap)	Fc-Fusion Biologic	CHO	22-Nov-12	EU (already approved in the USA in 2011)
Adcetris™ Brentuximab vedotin	Millennium Pharmaceuticals/Takeda & Seattle genetics	Antibody that which targets the cell-membrane protein CD30 linked to cathepsin cleavable linker for the treament of Hodgkin lymphoma (HL) and systemic anaplastic large cell lymphoma (sALCL).	Chimeric Monclonal Antibody	CHO	25-Oct-12	EU (already approved in the USA in 2011)
Zaltrap™ ziv-aflibercept	Regeneron/Sanofi Aventis	Fusion protein consisting IgG1 Fc fused with VEGF-binding portions from the extracellular domains of human VEGF receptors 1 and 2 for the treatment of metastatic colorectal cancer as Zaltrap (aka Elyea)	Fc-Fusion Biologic	CHO	03-Aug-12	USA
Perjeta® Pertuzumab	Genentech/ Roche	Indicated for the treatment of HER2-positive breast cancer, in combination with trastuzumab and docetaxel.	Humanized Monoclonal Antibody	CHO	04-Mar-12	EU (4-Mar) and USA (8-Jun)

Table. 23.1 (continued)

Product	Company	Target/Initial Therapeutic Indication	Product Category	Mammalian Cell Line	First Approved	Region
2011						
Eylea™ Aflibercept	Regeneron/Bayer	Fusion protein with VEGF-binding portions from the extracellular domains of human VEGF receptors 1 and 2 for the treatment of neovascular (wet) Age-related Macular Degeneration (AMD) as Eylea (aka Zaltrap)	Fc-Fusion Biologic	CHO	18-Nov-11	USA
Adcetris™ Brentuximab vedotin	Millennium Pharmaceuticals/Takeda & Seattle genetics	Antibody that which targets the cell-membrane protein CD30 linked to cathepsin cleavable linker for the treament of Hodgkin lymphoma (HL) and systemic anaplastic large cell lymphoma (sALCL).	Chimeric Monclonal Antibody	CHO	19-Aug-11	USA
Xgeva® Denosumab	Amgen	Inhibits RANK ligand, which acts as the primary signal for bone removal for the treatment of bone loss due to cancer	Fully Human Monoclonal Antibody	CHO	13-Jul-11	EU (already approved in the USA in 2010)
Nulojix® Belatacept	Bristol-Myers Squibb (BMS)	Fc fragment of a human IgG1 linked to the extracellular domain of CTLA-4 blocking the process of T-cell activation for the prevention of acute rejection in adult kidney transplants	Fc-Fusion Biologic	CHO	15-Jun-11	EU (17-Jun) & USA (15-Jun)
Yervoy® Ipilimumab	Bristil-Myers Squibb (BMS)	Activates the immune system by targeting CTLA-4 for the treatment of late-stage melanoma	Fully Human Monoclonal Antibody	CHO	25-Mar-11	EU (13-Jul) & USA (25-Mar)
Benlysta® Belimumab	GlaxoSmithKline (GSK)	Inhibits B-cell activating factor, also known as B-lymphocyte stimulator for treatment of treatment of adults with active, autoantibody-positive systemic lupus erythematosus	Fully Human Monoclonal Antibody	NS0	09-Mar-11	EU (13-Jul) & USA (9-Mar)

Table. 23.1 (continued)

Product	Company	Target/Initial Therapeutic Indication	Product Category	Mammalian Cell Line	First Approved	Region
2010						
Xgeva® Denosumab	Amgen	Inhibits RANK ligand, which acts as the primary signal for bone removal for the treatment of bone loss due to cancer	Fully Human Monoclonal Antibody	CHO	18-Nov-10	USA
Prolia® Denosumab	Amgen	Inhibits RANK ligand, which acts as the primary signal for bone removal for the treatment of osteoporosis, bone metastases, multiple myeloma, and tumors of the bone	Fully Human Monoclonal Antibody	CHO	01-Jun-10	EU (26-Mar) & USA (1-Jun)
Lumizyme® Alglucosidase alfa	Genzyme Corporation	Lysosomal glycogen-specific enzyme indicated for patients 8 years and older with late (non-infantile) onset Pompe disease (replaces Myozyme®)	Enzyme	CHO	24-May-10	USA
Arzerra® Ofatumumab	GlaxoSmithKline (GSK)	CD20 monoclonal antibody for the treatment for chronic lymphocytic leukaemia in patients who have not responded to Campath (alemtuzumab) or fludarabine	Fully Human Monoclonal Antibody	NS0	19-Apr-10	EU (already approved in the USA in 2009)
VPRIV® Velaglucerase alfa	Shire Pharmaceuticals Ireland Ltd	VPRIV® is used as a long term treatment as an enzyme replacement in patients with Type I Gaucher disease.	Enzyme	Human Fibroblasts	26-Feb-10	EU (26-Aug) and USA (26-Feb)
Elonva® Corifollitropin alfa	Merck Sharp & Dohme Limited	A modified rhFSH in which the carboxy-terminal peptide of the beta subunit of hCG is fused to the FSH beta chain indicated for the controlled stimulation of the ovaries	Hormone	CHO	25-Jan-10	EU
Actemra ® Tocilizumab	Genentech/ Roche	Targets interleukin-6 receptor (IL-6R) for the treatment of rheumatoid arthritis (RA) and systemic juvenile idiopathic arthritis	Humanized Monoclonal Antibody	CHO	08-Jan-10	USA

Table. 23.1 (continued)

Product	Company	Target/Initial Therapeutic Indication	Product Category	Mammalian Cell Line	First Approved	Region
2009						
Eporatio® Epoetin theta	Ratiopharm GmbH (Biopoin® and Eporatio® are the same product under two brand names)	Recombinant erythropoietin that stimulates the production of red blood cells from the bone marrow and is used to treat anaemia, chronic renal failure, and in adults with non-myeloid cancer.	Cytokine	CHO	29-Oct-09	EU
Arzerra® Ofatumumab	GlaxoSmithKline (GSK)	CD20 monoclonal antibody for the treatment for chronic lymphocytic leukaemia in patients who have not responded to Campath (alemtuzumab) or fludarabine	Fully Human Monoclonal Antibody	NS0	26-Oct-09	USA
Arcalyst® Rilonacept	Regeneron Pharmceuticals Inc.	For treatment of two Cryopyrin-Associated Periodic Syndromes (CAPS) disorders: Familial Cold Auto-Inflammatory Syndrome (FCAS) and Muckle-Wells Syndrome (MWS).	Fc-Fusion Biologic	CHO	23-Oct-09	EU (already approved in the USA in 2008)
Biopoin® Epoetin theta	Teva GmbH (Biopoin® and Eporatio® are the same product under two brand names)	Recombinant erythropoietin that stimulates the production of red blood cells from the bone marrow and is used to treat anaemia, chronic renal failure, and in adults with non-myeloid cancer.	Cytokine	CHO	23-Oct-09	EU
Stelara™ Ustekinumab	Janssen Biotech (Johnson & Johnson)	Directed against interleukin 12 and interleukin 23, proteins that regulate the immune-mediated inflammatory disorders to decrease the inflammatory response	Fully Human Monoclonal Antibody	CHO	16-Jan-09	EU (16-Jan) & USA (25-Sep)
Ilaris® Canakinumab	Novartis Pharmaceuticals	Monoclonal antibody targeted at interleukin-1 beta for treatment of Cryopyrin Associated Periodic Syndrome (CAPS)	Fully Human Monoclonal Antibody	Sp2/0	17-Jun-09	EU (23-Oct) & USA (17-Jun)
Simponi® Golimumab	Janssen Biotech (Johnson & Johnson)	Inhibits inflammatory response by suppressing tumor necrosis factor (TNF) as a treatment of moderately to severely active rheumatoid arthritis	Fully Human Monoclonal Antibody	CHO	24-Apr-09	EU (1-Oct) & USA (24-Apr)
Removab® Catumaxomab	Neovii Biotech GmbH /TRION Pharma	Attaches to two antigens: EpCAM, on cancer cells and CD3 on T cells forming a bridge for T-cells to attack the cancer and treat malignant ascites, peritoneal fluid accumulation caused by a cancer	Bispecific Antibody	Hybrid Hybridoma Cells	20-Apr-09	EU
Opgenra® Eptotermin alfa	Olympus Biotech International Limited	Recombinant version of osteogenic protein 1, also known as bone morphogenic protein 7 (BMP-7) which used for treating adults with spondylolisthesis	Bone Morphogenic Protein	CHO	19-Feb-09	EU
RoActemra® Tocilizumab	Genentech/ Roche	Targets interleukin-6 receptor (IL-6R) for the treatment of rheumatoid arthritis (RA) and systemic juvenile idiopathic arthritis	Humanized Monoclonal Antibody	CHO	16-Jan-09	EU

Table. 23.1 (continued)

Product	Company	Target/Initial Therapeutic Indication	Product Category	Mammalian Cell Line	First Approved	Region
2008						
Arcalyst® Rilonacept	Regeneron Pharmceuticals Inc.	For treatment of two Cryopyrin-Associated Periodic Syndromes (CAPS) disorders: Familial Cold Auto-Inflammatory Syndrome (FCAS) and Muckle-Wells Syndrome (MWS).	Fc-Fusion Biologic	CHO	27-Feb-08	USA
Xyntha® Factor VIII	Wyeth Pharmaceuticals/ Pfizer	Recombinant factor VIII (updated version of Refacto with no-animal origin raw materials used) for the treatment and prophylaxis of bleeding in patients with haemophilia A (congenital factor VIII deficiency).	Clotting Factors	CHO	21-Feb-08	USA
Recothrom® Thrombin	ZymoGenetics, Inc.	Recombinant thrombin used in the prevention of minor bleeding during surgery	Clotting Factors	CHO	17-Jan-08	USA
2007						
Silapo® Epoetin-Zeta	Stada Arzneimittel AG	A biosimilar medicine of Eprex/Erypo, a recombinant erythropoietin that stimulates erythropoiesis and is used to treat anemia, chronic renal failure related to cancer chemotherapy.	Cytokine	CHO	18-Dec-07	EU
Retacrit® Epoetin-Zeta	Hospira UK Limited	A biosimilar medicine of Eprex/Erypo, a recombinant erythropoietin that stimulates erythropoiesis and is used to treat anemia, chronic renal failure related to cancer chemotherapy.	Cytokine	CHO	18-Dec-07	EU
Vectibix® Panitumumab	Amgen	Monoclonal antibody specific to the epidermal growth factor receptor (EGFR)for the treatment of EGFR expressing metastatic colorectal cancer	Fully Human Monoclonal Antibody	CHO	03-Dec-07	EU
Abseamed® Epoetin alfa	Medice Arzneimittel Pütter GmbH & Co. KG	A biosimilar medicine of Eprex/Erypo, a recombinant erythropoietin that stimulates erythropoiesis and is used to treat anemia, chronic renal failure related to cancer chemotherapy.	Cytokine	CHO	28-Aug-07	EU
Binocrit® Epoetin alfa	Sandoz GmbH	A biosimilar medicine of Eprex/Erypo, a recombinant erythropoietin that stimulates erythropoiesis and is used to treat anemia, chronic renal failure related to cancer chemotherapy.	Cytokine	CHO	28-Aug-07	EU
Epoetin alfa Hexal®	Hexal AG	A biosimilar medicine of Eprex/Erypo, a recombinant erythropoietin that stimulates erythropoiesis and is used to treat anemia, chronic renal failure related to cancer chemotherapy.	Cytokine	CHO	28-Aug-07	EU

Table. 23.1 (continued)

Product	Company	Target/Initial Therapeutic Indication	Product Category	Mammalian Cell Line	First Approved	Region
2007						
Mircera® Methoxy polyethylene glycol-epoetin beta	Roche	Chemically linked erythropoietin with methoxy polyethylene glycol butanoic acid stimulating erythropoiesis and is used to treat anemia associated with chronic kidney failure.	Cytokine	CHO	20-Jul-07	EU (20-Jul) & USA (14-Nov)
Pergoveris® Follitropin alfa/Lutropin alfa	Merck Serono Europe Ltd.	Recombinant hormone combination indicated to stimulate the development of follicles in the ovaries of infertile adults with low FSH and LH.	Hormone	CHO	25-Jun-07	EU
Orencia® Abatacept	Bristol-Myers Squibb (BMS)	IgG1 Fc fusion to CTLA-4 binding with more avidity to CD80 (B7-1) than to CD86 (B7-2) for second-line treatment of rheumatoid arthritis in moderate to severe adult patients.	Fc-Fusion Biologic	CHO	21-May-07	EU
Soliris® Eculizumab	Alexion Pharmceuticals, Inc	Monoclonal antibody that is a terminal complement inhibitor and approved for the treatment of paroxysmal nocturnal hemoglobinuria (PNH)	Humanized Monoclonal Antibody	NS0	16-Mar-07	EU (20-Jun) & USA (16-Mar)
Elaprase® Idursulfase	Shire Pharmaceuticals	Recombinant lysosomal enzyme iduronate-2-sulfatase for the treatment of Hunter syndrome (mucopolysaccharidosis II; MPS II).	Enzyme	HT-1080	08-Jan-07	EU (already approved in the USA in 2006)
2006						
Vectibix® Panitumumab	Amgen	Monoclonal antibody specific to the epidermal growth factor receptor (EGFR)for the treatment of EGFR expressing metastatic colorectal cancer	Fully Human Monoclonal Antibody	CHO	27-Sep-06	USA
Elaprase® Idursulfase	Shire Pharmaceuticals	Recombinant lysosomal enzyme iduronate-2-sulfatase for the treatment of Hunter syndrome (mucopolysaccharidosis II; MPS II).	Enzyme	HT-1080	24-Jul-06	USA

Table. 23.1 (continued)

Product	Company	Target/Initial Therapeutic Indication	Product Category	Mammalian Cell Line	First Approved	Region
2006						
Tysabri® Natalizumab	Biogen Idec.	Monoclonal antibody against the cell adhesion molecule α4-integrin for treatment of multiple sclerosis	Humanized Monoclonal Antibody	NS0	27-Jun-06	EU
Myozyme® Alglucosidase alfa	Genzyme Corporation	Recombinant glucosidase for enzyme replacement therapy for the treatment of Pompe disease (Glycogen storage disease type II), a rare lysosomal storage disorder (LSD)	Enzyme	CHO	29-Mar-06	EU (29-Apr) & USA (28-Apr)
Naglazyme® galsulfase	BioMarin Pharmaceutical Inc.	Recombinant N-acetylgalactosamine 4-sulfatase increasing the catabolism of glycosaminoglycans (GAG)is indicated for patients with mucopolysaccharidosis VI (MPS VI)	Enzyme	CHO	24-Jan-06	EU
2005						
Orencia® Abatacept	Bristol-Myers Squibb (BMS)	IgG1 Fc fusion to CTLA-4 binding with more avidity to CD80 (B7-1) than to CD86 (B7-2) for second-line treatment of rheumatoid arthritis in moderate to severe adult patients.	Fc-Fusion Biologic	CHO	26-Dec-05	USA
Hylenex®, Cumulase® Hyaluronidase	Halozyme Therapeutics, Baxter Healthcare Inc.	Recombinant hyaluronidase for use as a "spreading agent" to enhance the delivery of local anesthesia, contrast agents, and for subcutaneous fluid replacement (hypodermoclysis)	Enzyme	CHO	05-Dec-05	USA
Xolair® Omalizumab	Genentech/ Novartis	Antibody that specifically binds to free human immunoglobulin E (IgE) used to reduce sensitivity to allergens for treatment of moderate-to-severe allergic asthma	Humanized Monoclonal Antibody	CHO	25-Oct-05	EU (already in the USA since 2003)
Naglazyme® galsulfase	BioMarin Pharmaceutical Inc.	Recombinant N-acetylgalactosamine 4-sulfatase increasing the catabolism of glycosaminoglycans (GAG)is indicated for patients with mucopolysaccharidosis VI (MPS VI)	Enzyme	CHO	31-May-05	USA
Avastin ® Bevacizumab	Genentech/ Roche	An angiogenesis inhibitor (inhibits VEGF-A)where the drug slows the growth of new blood vessels to treat colorectal, lung, breast, glioblastoma, kidney and ovarian cancers	Humanized Monoclonal Antibody	CHO	12-Jan-05	EU

Table. 23.1 (continued)

Product	Company	Target/Initial Therapeutic Indication	Product Category	Mammalian Cell Line	First Approved	Region
2004						
Tysabri® Natalizumab	Biogen Idec.	Monoclonal antibody against the cell adhesion molecule α4-integrin for treatment of multiple sclerosis	Humanized Monoclonal Antibody	NS0	24-Nov-04	USA
Raptiva® Efalizumab	Genentech	Binds to the CD11a subunit of lymphocyte function-associated antigen 1 and acts as an immunosuppressant for treatment of autoimmune diseases, originally marketed to treat psoriasis	Humanized Monoclonal Antibody	CHO	09-Sep-04	USA
Neutrospec® Fanolesomab	Palatin Technologies, Ben Venue Labs, Mallinckrodt Inc.	Indication to aid in the diagnosis of appendicitis. It is labeled with a radioisotope, technetium-99m (99mTc). Withdrawn in Dec-2005	Murine Monoclonal Antibody	Hybridoma	30-Jun-04	USA
Luveris® LH	EMD Serono, Inc.	Luteinizing hormone (recombinant human LH) for the treatment of female infertility and it is indicated for use in combination with human follicle-stimulating hormone (Gonal-f®).	Hormone	CHO	24-May-04	USA
Advate® Antihemophilic Factor	Baxter Interantional Inc.	Recombinant factor VIII for the treatment and prophylaxis of bleeding in patients with haemophilia A (congenital factor VIII deficiency).	Clotting Factors	CHO	02-Mar-04	EU (already in the USA since 2003)
Avastin ® Bevacizumab	Genentech/ Roche	An angiogenesis inhibitor (inhibits VEGF-A)where the drug slows the growth of new blood vessels to treat colorectal, lung, breast, glioblastoma, kidney and ovarian cancers	Humanized Monoclonal Antibody	CHO	26-Feb-04	USA
Erbitux® Cetuximab	Bristol-Myers Squibb, Eli Lilly and Company, Merck KGaA	Mouse/human antibody for treatment of patients with epidermal growth factor receptor (EGFR)-expressing, metastatic colorectal cancer, head and neck cancer	Chimeric Monoclonal Antibody	Sp2/0	12-Feb-04	EU (29-Jun) & USA (12-Feb)
Zevalin® Ibritumomab tiuxetan	Spectrum Pharmaceuticals /Biogen Idec	Radioimmunotherapy treatment for relapsed or refractory, low grade or transformed B cell non-Hodgkin's lymphoma, a lymphoproliferative disorder	Murine Monoclonal Antibody	CHO	16-Jan-04	EU (already approved in the USA in 2002)

Table. 23.1 (continued)

Product	Company	Target/Initial Therapeutic Indication	Product Category	Mammalian Cell Line	First Approved	Region
2003						
Humira® Adalimumab	Abbott Laboratories (AbbVie)	Adalimumab binds to tumor necrosis factor-alpha (TNFα) and this TNFα inactivation has proven to be important in downregulating the inflammatory reactions	Fully Human Monoclonal Antibody	CHO	08-Sep-03	EU (already approved in the USA in 2002)
Advate® Antihemophilic Factor	Baxter Interantional Inc.	Recombinant factor VIII for the treatment and prophylaxis of bleeding in patients with haemophilia A (congenital factor VIII deficiency).	Clotting Factors	CHO	25-Jul-03	USA
Bexxar® Tositumomab-I131	GlaxoSmithKline (GSK)	Tositumomab binds to the CD20 antigen, which is predominantly expressed on mature B cells for treatment of non-Hodgkin's lymphoma (CD20 positive, follicular)	Murine Monoclonal Antibody	Hybridoma	27-Jun-03	USA
Xolair® Omalizumab	Genentech/ Novartis	Antibody that specifically binds to free human immunoglobulin E (IgE) used to reduce sensitivity to allergens for treatment of moderate-to-severe allergic asthma	Humanized Monoclonal Antibody	CHO	20-Jun-03	USA
Aldurazyme® Laronidase	Genzympe Corporation	Recombinant alpha-L-iduronidase used in enzyme replacement therapy for the treatment of Mucopolysaccharidosis I (MPS I)	Enzyme	CHO	30-Apr-03	USA
Fabrazyme® Agalsidase	Genzyme Corporation	Recombinant alpha-galactosidase A or alpha-GAL for enzyme replacement indicated for the treatment of Fabry disease	Enzyme	CHO	24-Apr-03	USA (already approved in the EU since 2001)
Amevive® Alefacept	Astellas Pharma Inc.	Inhibits the activation of CD4+ and CD8+ T cells by interfering with CD2 on the T cells and it is used for treatment of moderate-to-severe chronic plaque psoriasis	Fc-Fusion Biologic	CHO	30-Jan-03	USA

Table. 23.1 (continued)

Product	Company	Target/Initial Therapeutic Indication	Product Category	Mammalian Cell Line	First Approved	Region
2002						
Humira® Adalimumab	Abbott Laboratories (AbbVie)	Adalimumab binds to tumor necrosis factor-alpha (TNFα) and this TNFα inactivation has proven to be important in downregulating the inflammatory reactions	Fully Human Monoclonal Antibody	CHO	30-Dec-02	USA
Inductos® dibotermin alfa	Medtronic BioPharma B.V.	Acts on the bone structure since it is a recombinant version of bone morphogenetic protein 2 (BMP-2) stimulating bone formation to help heal fractures and aid in lower-back spine fusion surgery	Bone Morphogenic Protein	CHO	09-Sep-02	EU
Xigris® Drotrecogin Alpha	Eli Lilly & Company	Recombinant form of human activated protein C that has anti-thrombotic, anti-inflammatory, and profibrinolytic properties for the treatment of sepsis (withdrawn)	Clotting Factors	HEK293	22-Aug-02	EU
InFUSE™ Bone Graft/LT-CAGE™	Medtronic Sofamor Danek	Acts on the bone structure since it is a recombinant version of bone morphogenetic protein 2 (BMP-2) stimulating bone formation to help heal fractures and aid in bone graft surgery	Bone Morphogenic Protein	CHO	02-Jul-02	USA
Rebif® Interferon Beta-1a	Pfizer, EMD Serono	Recombinant interferon beta 1a for the treatment of relapsing forms of multiple sclerosis	Cytokine	CHO	07-Mar-02	USA (already luanched in the EU in 1998)
Zevalin® Ibritumomab tiuxetan	Spectrum Pharmaceuticals /Biogen Idec	Radioimmunotherapy treatment for relapsed or refractory, low grade or transformed B cell non-Hodgkin's lymphoma, a lymphoproliferative disorder	Murine Monoclonal Antibody	CHO	19-Feb-02	USA

Table. 23.1 (continued)

Product	Company	Target/Initial Therapeutic Indication	Product Category	Mammalian Cell Line	First Approved	Region
2001						
Xigris® Drotrecogin Alpha	Eli Lilly & Company	Recombinant form of human activated protein C that has anti-thrombotic, anti-inflammatory, and profibrinolytic properties for the treatment of sepsis (withdrawn)	Clotting Factors	HEK293	21-Nov-01	USA
Aranesp® Darbepoetin alfa	Amgen	Recombinant erythropoetin to simulate erythropoiesis (increases red blood cell levels) and is used to treat anemia, commonly associated with chronic renal failure and cancer chemotherapy	Cytokine	CHO	17-Sep-01	EU (8-Jun) & USA (17-Sep)
Fabrazyme® Agalsidase	Genzyme Corporation	Recombinant alpha-galactosidase A or alpha-GAL for enzyme replacement indicated for the treatment of Fabry disease	Enzyme	CHO	03-Aug-01	EU
Replagal® agalsidase beta	Shire Human Genetic Therapies AB	Recombinant alpha-galactosidase A or alpha-GAL for enzyme replacement indicated for the treatment of Fabry disease	Enzyme	CHO	03-Aug-01	EU
Nespo® Darbepoetin alfa	Dompé Biotec S.p.A.	Recombinant erythropoetin to simulate erythropoiesis (increases red blood cell levels) and is used to treat anemia, commonly associated with chronic renal failure and cancer chemotherapy	Cytokine	CHO	08-Jun-01	EU
Campath®, Mabcampath® Alemtuzumab	Genzyme Corporation	Binds to CD52, on the surface of mature lymphocytestreatment of chronic lymphocytic leukemia (CLL), cutaneous T-cell lymphoma (CTCL) and T-cell lymphoma	Humanized Monoclonal Antibody	CHO	07-May-01	EU (6-Jul) & USA (7-May)
Metalyse® Tenecteplase	Boehringer Ingelheim	Recombinant fibrin-specific plasminogen activator indicated for use in the reduction of mortality associated with acute myocardial infarction (AMI).	Enzyme	CHO	23-Feb-01	EU
Ovitrelle® Choriogonadotropin Alpha	Merck Serono Europe Limited	Ovidrel (recombinant Chorionic Gonadotropin (Hcg) used as part of a treatment program for certain fertility problems in women and generally in combination with another hormone (FSH).	Hormone	CHO	02-Feb-01	EU (already launched in the year 2000 in the USA)

Table. 23.1 (continued)

Product	Company	Target/Initial Therapeutic Indication	Product Category	Mammalian Cell Line	First Approved	Region
2000						
Ovidrel® Choriogonadotropin Alpha	EMD Serono, Inc.	Ovidrel (recombinant Chorionic Gonadotropin (Hcg) used as part of a treatment program for certain fertility problems in women and generally in combination with another hormone (FSH).	Hormone	CHO	20-Sep-00	USA
Herceptin® Trastuzumab	Genentech/ Roche	A treatment that mainly interferes with the HER2/neu receptor to treat certain breast cancers.	Humanized Monoclonal Antibody	CHO	28-Aug-00	EU (already approved in the USA in 1998)
Helixate FS® / NexGen® Factor VIII octocog alfa	CSL Behring	A recombinant antihemophilic factor that is indicated for the control and prevention of bleeding episodes in patients with hemophilia A	Clotting Factors	BHK	04-Aug-00	EU
Kogenate Bayer® Factor VIII, octocog alfa	Bayer Healthcare	A recombinant antihemophilic factor that is indicated for the control and prevention of bleeding episodes in patients with hemophilia A	Clotting Factors	BHK	04-Aug-00	EU
TNKase® Tenecteplase	Genentech/Roche	Recombinant fibrin-specific plasminogen activator indicated for use in the reduction of mortality associated with acute myocardial infarction (AMI).	Enzyme	CHO	02-Jun-00	USA
Mylotarg® Gemtuzumab ozogamicin	Wyeth Pharmaceuticals	An antibody-drug conjugate targeting CD33 for the treatment of acute myelogenous leukemia (withdrawn 2010)	Humanized Monoclonal Antibody	NS0	17-May-00	USA
'Thyrogen® (thyrotropin alfa)	Genzyme Europe B.V.	Recombinant form of human thyroid-stimulating hormone (TSH) which binds to TSH receptors used as a diagnostic tool for patients that may have thyriod cancer	Hormone	CHO	09-Mar-00	EU (already luanched since 1998 in the USA)
ReFacto® Antihemophilic Factor	Wyeth Pharmaceuticals/ Pfizer	Recombinant factor VIII for the treatment and prophylaxis of bleeding in patients with haemophilia A (congenital factor VIII deficiency).	Clotting Factors	CHO	05-Mar-00	USA (already launched 1999 in EU)
Enbrel® Etanercept	Pfizer	Tumor necrosis factor receptor2-immune globlulin G1 Fc fusion protein for treatment of rheumatoid arthritis, psoriasis, and ankylosing spondylitis.	Fc-Fusion Biologic	CHO	03-Feb-00	EU

Table. 23.1 (continued)

Product	Company	Target/Initial Therapeutic Indication	Product Category	Mammalian Cell Line	First Approved	Region
1999						
Remicade® Infliximab	Janssen Biotech (Johnson & Johnson)	Chimeric monoclonal antibody against tumour necrosis factor alpha (TNF-α) used to treat autoimmune diseases such as Crohn's disease and rheumatoid arthritis	Chimeric Monoclonal Antibody	Sp2/0	13-Aug-99	EU
Synagis® Palivizumab	MedImmune Inc.	Monoclonal antibody (IgG) directed against an epitope in the A antigenic site of the F protein of respiratory syncytial virus (RSV) for treatment of RSV infections	Humanized Monoclonal Antibody	NS0	13-Aug-99	EU
ReFacto® Antihemophilic Factor	Wyeth Pharmaceuticals/ Pfizer	Recombinant factor VIII for the treatment and prophylaxis of bleeding in patients with haemophilia A (congenital factor VIII deficiency).	Clotting Factors	CHO	13-Apr-99	EU
NovoSeven® Factor VIIa	Novo Nordisk	Treating or preventing bleeding episodes in certain patients with bleeding problems such as hemophilia A or B, acquired hemophilia, or congenital FVII deficiency.	Clotting Factors	CHO	25-Mar-99	USA (already launched 1996 in EU)
Zenapax® Daclizumab	Roche	First humanized antibody that binds to CD25, the alpha subunit of the IL-2 receptor of T cells for treatment of organ transplant rejection and investigated for treatment of multiple sclerosis.	Humanized Monoclonal Antibody	Sp2/0	26-Feb-99	EU

Table. 23.1 (continued)

Product	Company	Target/Initial Therapeutic Indication	Product Category	Mammalian Cell Line	First Approved	Region
1998						
Thyrogen® (thyrotropin alfa)	Genzyme Corporation	Recombinant form of human thyroid-stimulating hormone (TSH) which binds to TSH receptors used as a diagnostic tool for patients that may have thyriod cancer	Hormone	CHO	30-Nov-98	USA
Enbrel® Etanercept	Amgen	Tumor necrosis factor receptor2-immune globlulin G1 Fc fusion protein for treatment of rheumatoid arthritis, psoriasis, and ankylosing spondylitis.	Fc-Fusion Biologic	CHO	02-Nov-98	USA
Herceptin® Trastuzumab	Genentech/ Roche	A treatment that mainly interferes with the HER2/neu receptor to treat certain breast cancers.	Humanized Monoclonal Antibody	CHO	25-Sep-98	USA
Humaspect® Votumumab	KS Biomedix Limited	Diagnostic radiopharmaceutical for detection of carcnima of the colon or rectum	Human Monoclonal Antibody	Human Lymphoblastoid Cell Line	25-Sep-98	EU
Remicade® Infliximab	Janssen Biotech (Johnson & Johnson)	Chimeric monoclonal antibody against tumour necrosis factor alpha (TNF-α) used to treat autoimmune diseases such as Crohn's disease and rheumatoid arthritis	Chimeric Monoclonal Antibody	Sp2/0	24-Aug-98	USA
Synagis® Palivizumab	MedImmune Inc.	Monoclonal antibody (IgG) directed against an epitope in the A antigenic site of the F protein of respiratory syncytial virus (RSV) for treatment of RSV infections	Humanized Monoclonal Antibody	NS0	19-Jun-98	USA
Mabthera® (Rituxan®) Rituximab	Genentech (Roche)/Biogen Idec	Monoclonal antibody against the protein CD20 found primarily on B cells allowing for the treament of diseases with excessive or dysfunctional B cells	Chimeric Monoclonal Antibody	CHO	02-Jun-98	EU (already in USA since 1997)
Simulect® Basiliximab	Novartis Pharmaceuticals	Mouse-human monoclonal antibody to the α chain (CD25) of the IL-2 receptor to prevent organ transplantation rejection	Chimeric Monoclonal Antibody	Sp2/0	12-May-98	USA
Rebif® Interferon Beta-1a	Merck Serono Europe Ltd.	Recombinant interferon beta 1a for the treatment of relapsing forms of multiple sclerosis	Cytokine	CHO	04-May-98	EU

Table. 23.1 (continued)

Product	Company	Target/Initial Therapeutic Indication	Product Category	Mammalian Cell Line	First Approved	Region
1997						
Zenapax® Daclizumab	Roche	First humanized antibody that binds to CD25, the alpha subunit of the IL-2 receptor of T cells for treatment of organ transplant rejection and investigated for treatment of multiple sclerosis.	Humanized Monoclonal Antibody	Sp2/0	10-Dec-97	USA
Rituxan® Rituximab	Genentech (Roche)/Biogen Idec	Monoclonal antibody against the protein CD20 found primarily on B cells allowing for the treament of diseases with excessive or dysfunctional B cells	Chimeric Monoclonal Antibody	CHO	26-Nov-97	USA
Cerezyme® Imiglucerase	Genzyme Europe B.V.	Recombinant analogue of human β-glucocerebrosidase used in the treatment of Gaucher's disease, in which a fatty substance (lipid) accumulates in cells and certain organs	Enzyme	CHO	17-Nov-97	EU
Follistim ® Follitropin-Beta	Merck & Co	Recombinant follicle-stimulating hormone for the treatment of infertility by stimulating ovaries to produce one or more eggs during each treatment.	Hormone	CHO	29-Sep-97	USA
Gonal-F® Follitropin-alfa	EMD Serono, Inc.	Recombinant follicle-stimulating hormone for use in conjunction with assisted reproductive technologies, such as in vitro fertilization by the induction of ovulation	Hormone	CHO	29-Sep-97	USA
NeoRecormon® Epoetin-Beta	Roche	Recombinant erythropoietin. It stimulates erythropoiesis and is used to treat anemia, commonly associated with chronic renal failure and cancer chemotherapy.	Cytokine	CHO	16-Jul-97	EU (already launched in the USA in 1996)
Avonex® Interferon Beta-1a	Biogen Idec, Inc.	Recombinant interferon beta 1a is a drug in the interferon family used to treat multiple sclerosis (MS) by balancing the expression of pro- and anti-inflammatory agents in the brain.	Cytokine	CHO	13-Mar-97	EU
BeneFIX® Factor IX	Wyeth Pharmaceuticals/ Pfizer	For the control and prevention of hemorrhagic episodes in patients with hemophilia B (congenital factor IX deficiency and Christmas disease), including control and prevention of bleeding in surgical settings	Clotting Factors	CHO	11-Feb-97	EU (27-Aug) & USA (11-Feb)

Table. 23.1 (continued)

Product	Company	Target/Initial Therapeutic Indication	Product Category	Mammalian Cell Line	First Approved	Region
1996						
Cathflo®, Activase®, Actilyse® (Alteplase)	Genentech, Boerhringer Ingelheim	Recombinant tissue plasminogen activator that is responsible for clot breakdown an indicated for treating heart attacks and acute massive pulmonary embolism such as in strokes.	Enzyme	CHO	18-Jun-96	USA
NeoRecormon® Epoetin-Beta	Roche	Recombinant erythropoietin. It stimulates erythropoiesis and is used to treat anemia, commonly associated with chronic renal failure and cancer chemotherapy.	Cytokine	CHO	14-Jun-96	USA
Avonex® Interferon Beta-1a	Biogen Idec, Inc.	Recombinant interferon beta 1a is a drug in the interferon family used to treat multiple sclerosis (MS) by balancing the expression of pro- and anti-inflammatory agents in the brain.	Cytokine	CHO	17-May-96	USA
Puregon® Follitropin beta	N.V. Organon	Recombinant follicle-stimulating hormone for the treatment of infertility by stimulating ovaries to produce one or more eggs during each treatment.	Hormone	CHO	03-May-96	EU
NovoSeven® Factor VIIa	Novo Nordisk	Treating or preventing bleeding episodes in certain patients with bleeding problems such as hemophilia A or B, acquired hemophilia, or congenital FVII deficiency.	Clotting Factors	CHO	23-Feb-96	EU
1995						
Gonal-F® Follitropin-alfa	Merck Serono Europe Ltd	Recombinant follicle-stimulating hormone for use in conjunction with assisted reproductive technologies, such as in vitro fertilization by the induction of ovulation	Hormone	CHO	20-Oct-95	EU

Table. 23.1 (continued)

Product	Company	Target/Initial Therapeutic Indication	Product Category	Mammalian Cell Line	First Approved	Region
1994						
Reopro® Abciximab	Janssen Biotech and Eli Lilly and Company	Antibody fragment and a platelet aggregation inhibitor mainly used during and after coronary artery procedures like angioplasty to prevent thrombus (blood clots)	Chimeric Fab Antibody	Sp2/0	14-Nov-94	EU (country specific) & USA
Cerezyme® Imiglucerase	Genzyme Corporation	Recombinant analogue of human β-glucocerebrosidase used in the treatment of Gaucher's disease, in which a fatty substance (lipid) accumulates in cells and certain organs	Enzyme	CHO	06-Jun-94	USA
1993						
Bioclate™ Factor VIII	Aventis Behring	A recombinant antihemophilic factor that is indicated for the control and prevention of bleeding episodes in patients with hemophilia A	Clotting Factors	CHO	31-Dec-93	USA
Pulmozyme® human deoxyribonuclease I (rhDNase)	Genentech/ Roche	Recombinant human deoxyribonuclease I (rhDNase) hydrolyzes the DNA present in sputum/mucus of cystic fibrosis patients and reduces viscosity in the lungs, promoting improved clearance of secretions.	Enzyme	CHO	30-Dec-93	USA
Kogenate FS® Factor VIII, octocog alfa	Bayer Healthcare	A recombinant antihemophilic factor that is indicated for the control and prevention of bleeding episodes in patients with hemophilia A	Clotting Factors	BHK	28-Feb-93	USA
Helixate FS® Factor VIII octocog alfa	CSL Behring	A recombinant antihemophilic factor that is indicated for the control and prevention of bleeding episodes in patients with hemophilia A	Clotting Factors	BHK	28-Feb-93	USA

Table. 23.1 (continued)

Product	Company	Target/Initial Therapeutic Indication	Product Category	Mammalian Cell Line	First Approved	Region
		1992				
Recombinate™ Antihemophilic Factor VIII	Baxter Interantional Inc.	A recombinant antihemophilic factor that is indicated for the control and prevention of bleeding episodes in patients with hemophilia A	Clotting Factors	CHO	21-Dec-92	USA
		1989				
Procrit® , Epogen® (Epoetin-Alfa)	Amgen, Janssen Biotech (Johnson & Johnson)	Recombinant erythropoietin that stimulates erythropoiesis and is used to treat anemia, commonly associated with chronic renal failure and cancer chemotherapy.	Cytokine	CHO	01-Jun-89	USA (already approved in Europe)
		1988				
Eprex® , Erypo® (Epoetin-Alfa)	Janssen Biotech (Johnson & Johnson)	Recombinant erythropoietin that stimulates erythropoiesis and is used to treat anemia, commonly associated with chronic renal failure and cancer chemotherapy.	Cytokine	CHO	04-Aug-88	Europe
		1987				
Activase® Alteplase	Genentech	Recombinant tissue plasminogen activator that is responsible for clot breakdown an indicated for treating acute mycardial infarctions.	Enzyme	CHO	13-Nov-87	USA
		1986				
Orthoclone OKT3® Muromonab	Ortho Biotech (Janssen Biotech)	Immunosuppressant drug given to reduce acute rejection in patients with organ transplants (First monoclonal antibody drug to be approved for clinal use in humans)	Monoclonal Antibody	Mouse Ascites	19-Jun-86	USA

changed in 1994, when the first approved and successfully marketed antibody drug produced via mammalian cell cultured was manufactured by Centocor (now known as Janssen Biotech from Johnson & Johnson) with the trade name ReoPro® (Abciximab) (Tam et al. 1998). Abciximab is a fragment antigen-binding (Fab) fragment of the chimeric human murine monoclonal antibody 7E3 which was designed to overcome the obstacles of murine based antibodies for human therapeutics since murine antibodies can have glycosylation patterns that are highly immunogenic to humans (Butler 2005; Jenkins et al. 1996) as can be seen with Orthoclone OKT3 studies where 50 % patients have experienced a potentially lethal human anti-mouse antibody (HAMA) response (Niaudet et al. 1993; Richards et al. 1999). While Orthoclone OKT3® can be considered a first generation biopharmaceutical, along with protein drugs that are simply engineered copies of native endogenous proteins. Abciximab is also one of the first efforts of designing a second generation mAb biopharmaceutical produced from mammalian cells. Second generation biopharmaceuticals have been engineered to improve their performance by a combination or single alteration of the following; reengineering the amino acid sequence or glycoproteins, the addition of chemical conjugates or the creation of fused protein structures that improve drug function such as stability and targeting. Abciximab was specifically designed to reduce immunogenicity, (Tam et al. 1998). To reduce possible complement-activating and immunogenicity reactions from Abciximab, the Fc fragment is removed from the complete antibody so that the fragment antigen binding structure is only left (Knight et al. 1995).

Years prior to Abciximab, Centocor had almost reached the brink of bankruptcy due to approval denial for an IgM antibody drug expressed from a Sp2/0 cell culture in an industrial scale perfusion process. The drug, known as nebacumab (Centoxin®), was already approved in The Netherlands, Britain, Germany and France in 1991, where it was indicated as a treatment for Gram-negative sepsis but soon after the FDA rejected approval in the USA due to new clinical trial data that eventually led to the discontinuation of Centoxin® from the market. The lessons learned and the bioprocesses developed from Centoxin® allowed Centocor in partnership with Eli Lilly to develop the perfusion process and gain marketing approval of ReoPro®(Marks 2012), 8 years after the first antibody based drug was introduced into the market.

After ReoPro® showed success, a flood of chimeric and humanized monoclonal antibody drugs that have the ability to trigger effector functions in humans, longer circulatory half-life, and decreased immunogenicity compared to murine antibodies came to the market (see Table 23.1). These engineered antibodies appeared first in 1997 starting with the chimeric molecule Rituximab under the trade names Rituxan® and Mabthera®. Rituximab was conceived and developed by IDEC Pharmaceutical Corporation, San Diego, CA (now known as Biogen Idec) under the development name IDEC-C2B8 (Maloney et al. 1997). The drug was brought to market in collaboration with Genentech, Inc., South San Francisco, CA and F. Hoffman-LaRoche (Nutley, NJ) as the first mAb approved for the treatment of cancer, specifically the treatment non-Hodgkin's lymphoma. Rituximab was also the first mAb approved for the treatment of cancer, specifically the treatment

non-Hodgkin's lymphoma (Grillo-Lopez 2000). To generate rituximab, the variable regions of a murine anti-human CD20 that are found on the surface of malignant and normal B cells were fused to the human IgG and kappa-constant regions (Silverman and Weisman 2003). Rituximab is designed to promote antibody-dependent cellular cytotoxicity (ADCC) with human effector cells and mediate complement-dependent cell lysis. The U.S. patent for rituximab was issued in 1998 and will expire in 2015. Apart from Rituximab Roche also had the first humanized MAb approved for marketing in 1997, a few months after rituximab, known as daclizumab (Zenapax®) and used in the treatment of organ transplant rejection similar to Orthoclone OKT3®. Humanization of an antibody, usually involves reengineering of antibodies, where the complimentary determining regions from the rodent antibody V-regions are combined with framework regions from human V-regions in an attempt to decrease immunogenicity even further than chimeric antibodies. Soon after Zenapax®, in 1998, Novartis got marketing approval for Simulect® (basiliximab) and Johnson & Johnson got approval for Remicade® (Infliximab), both chimeric mAbs (see Table 23.1). In the case of the humanized mAbs that followed Zenapax®, it was followed a year later with Synagis® (Astra Zeneca) and Herceptin® (Roche) in 1998. Currently, a plethora of chimeric and humanized antibodies have been approved (reviewed in Table 23.1), but with the advent of technology to produce fully human antibodies Abbot was able to create the first fully human mAb drug, marketed as Humira® (adalimumab) in 2002. The fully human antibody is another variant of engineered mAbs harnessed as a therapeutic drug that can provide reduced immunogenicity and a longer half-life compared to the use of murine antibodies.

The early mammalian cell culture based production processes had very low yields of recombinant protein product, sometimes a 100 times less when compared to today's processes. This was due to both upstream and downstream processes lacking optimization. Firstly the bioreactor cultures gave low titers of <50 mg/l for mammalian cells, a far cry from today's average of 2,000–5,000 mg/L for fed batch process and beyond that titer for perfusion processes where up to 25 g/l has been reported (Kelley 2009; Chon and Zarbis-Papastoitsis 2011). On top of that the purification steps were sometimes giving yields below 20 % and these aspects meant that the manufacturer had to increase the scale of the process in order to achieve enough products to serve the market with reasonable economics. To meet market demand with low yielding processes, large 10,000–15,000 L bioreactor capacity facilities were build, usually designed for mono-product operations making it difficult to transfer different products across facilities (Werner 2013). Over time the optimizations of cell productivity due to improved cell line development and selection methods, optimization of bioreactor designs and configurations (Kuystermans and Al-Rubeai 2011), and improved downstream process technology and design has facilitated increases in efficiency of bioprocesses (Low et al. 2007; Shukla et al. 2007) affording them to operate at a smaller scale to satisfy market demand. These smaller scales can operate with disposable systems in conjunction with high yielding engineered cell lines to allow for flexible multi-product operating facilities. In addition and concurrently, market demand for complex

glycosylated recombinant protein drugs has increased at such a pace that large scale facilities with multi-product capabilities are still feasible options in order to supply today's market for certain high demand products. Although, there is still a considerable amount of further development expected in the areas of bioprocess optimizations, much has already been done over the last two decades and contributed to reduced production costs (Shukla and Thömmes 2010). With reported research of fed batch CHO culture reaching titers of more than 10 g/l (Huang et al. 2010), continued development of mammalian cell culture processes for high value drug production will reduce the cost to market even further and enable wider access to biopharmaceuticals around the world.

23.3 Monoclonal Antibodies as Drugs

From the data shown in Table 23.1, it is apparent that monoclonal antibody drugs have become important driver in the biopharmaceutical market that is now dominated by biomolecular manufactured drugs as the fastest growing source of innovation and revenue with a total of more than 140 mammalian cell culture derived drugs approved between 1986 and 2014 in the EU and USA. As antibody drugs evolved from murine to chimeric, humanized, and finally fully human antibodies, the concerns regarding immunogenicity, weak efficacy, and short serum half-life, has been reduced significantly. For example Orthoclone OKT3 had a serum half-life of 0.3–0.75 days and immunogenicity chance of 50 % compared to the fully human mAb drug approved in 2010 with a serum half-life of 26 days and the chance of immunogenicity being >1.6 % marketed under the trade name Ilaris® (Canakinumab) from Novartis Pharmaceuticals (Wilde and Goa 1996; Yoon et al. 2010). Apart from Orthoclone OKT3, two other murine mAb produced therapeutics have been approved for marketing, named Zevalin® (Ibritumomab tiuxetan) and Bexxar® (Tositumomab-I131) as they showed a lower immunogenicity, with below 8 % of patients only having HAMA responses, whereas normally it is observed that with murine therapeutic antibodies HAMA responses can range within the 50–100 % range for the majority (Hwang and Foote 2005). Zevalin® was approved in 2002 as a CD20 targeting IGg1 conjugate drug for radio-immunotherapy therapy for difficult to manage low grade or transformed B cell non-Hodgkin's lymphoma, a type of lymphoproliferative disorder. The murine antibody is conjugated to the radioactive isotope yttrium 90 via the chelate tiuxetan and has a half-life of 1.25 days. Bexxar® is also targeting the B cell marker CD20 to treat non-Hodgkin's lymphoma with an Iodine 131 conjugate for radio-immunotherapy treatments, although this time; the isotope is linked directly to the antibody instead of through a chelate. Again, Bexxar® like Zevalin® has a short half-life, being only 2.7 days, something that is desirable to avoid excess exposure to the antibody and conjugate (Leveque et al. 2005).

As indicated earlier, the introduction of chimeric full antibodies as drugs started with rituximab (Rituxan®) from Genentech in 1997, used to treat non- Hodgkin's

lymphoma, this antibody is only immunogenic in 1.1 % of patients with no secondary conditions (Yoon et al. 2010). The serum half-life of Rituximab is 22 days (Genentech 1997) due to the increased stability of a human Fc region. Chimeric antibodies have generally shown varied immunogenicity with chimeric antibodies such as basiliximab and infliximab both demonstrating immunogenicity in patients of up to 44 % and 37 % respectively (Leveque et al. 2005), rituximab, demonstrates much lower immunogenicity. With humanized and fully human mAb drugs, there has been a further decrease in the average immunogenicity within patients, as the majority has shown immunogenicity's below 10 % (Yoon et al. 2010). By engineering antibodies with a reduced murine amino acid derived sequence makeup it is possible to reduce the immunogenicity. Humanized and fully human antibodies can still have immunogenicity risks since the variable regions can be murine derived such as the complementarity determining regions (CDR)-sequence that can contain these murine regions. Apart from the presence of murine amino acid sequences that can contribute to increased immunogenicity of mAb therapeutics, there can be several other intrinsic and even extrinsic factors that may increase immunogenicity for mAb therapeutics. It is known that the carbohydrate side-chains attached via glycosylation has a major impact on immunogenicity of an antibody and plays a major intrinsic role as well as other post translational events that may modify the antibody sequence such as oxidation, non-enzymatic glycosylation, and deamination of the amino side chains (Arnold et al. 2007; Sheeley et al. 1997) . It has also been found that antibodies that target insoluble factors, such as cell surface markers, may pose a risk of increased immunogenicity to the patient. Another intrinsic factor is the presence of CD4+ T helper epitopes that can lead to an immune response depending on the amino acid sequence (Harding et al. 2010) . Apart from a patients immunological status and the effects of co-medication (Harding et al. 2010; Hendrickson et al. 2006), extrinsic factors may arise due to the composition of the antibody drugs manufacturers formulate. Some formulations may be able to cause increased immunogenicity issues due to the presence of adjuvant-like contaminants and aggregates (Shire 2009; Rosenberg 2006).

Adalimumab, the first fully human antibody, was selected via phage display of the human variable heavy and light chain sequences, but it is also possible to produce fully human antibodies from an engineered mouse via a process known as XenoMouse technology. With XenoMouse technology, the immunoglobulin genes within the transgenic mouse are of human origin (Lonberg et al. 1994; Green 1999) making the possibility of natural in vivo affinity maturation of the sequences which may contribute to a further reduction in immunogenicity. The first therapeutic mAb to be approved for marketing that utilized the XenoMouse technology was panitumumab (Vectibix®), in 2006 (Jakobovits et al. 2007). Panitumumab has a very low immunogenicity of 3–4 %, due to the antibody development strategy employed, thus fully human derived antibodies can contain no murine sequences, unlike humanized antibodies, but immune responses can still occur. Thus the development of fully human antibodies are not a guarantee of non-immunogenicity, but it is possible that with further development steps

immunogenicity of engineered mAbs can be reduced or even eliminated by a combination of CDR-sequence engineering, optimized cell culture bioprocess development strategies, and formulation engineering to help fine tune the intrinsic and extrinsic factors that can reduce immunogenicity.

What has also been observed is that serum half-life can vary greatly with humanized and fully human antibody drugs compared to natural antibodies such as IgG which has a mean half-life of 25–32 days (Maarschalk-Ellerbroek et al. 2011). These engineered therapeutic antibodies have a serum half-life that varies greatly from a low of 7.5 days to a range similar to natural antibodies (Yoon et al. 2010). Varying serum half-life can also be the result of variations in post translational processing of these recombinant antibodies with the use of non-human originating cell lines including the culture conditions during manufacturing as we know that glycans also influence immunogenicity and efficacy (Ghaderi et al. 2012).

The serum half-life of mAb's is usually high compared to other recombinant proteins due the neonatal Fc receptor of IgG (FcRn). The FcRn is a MHC Class I like molecule that binds to the CH2-CH3 hinge region of IgG which starts a process that ultimately protects IgG from degradation thereby promoting the extended half-life of this class of antibody in the serum (Simister and Mostov 1989; Kuo et al. 2010). In further detail, IgG is bound to the Fc receptor of a cell within an acidic endosome that is destined to be internalized via pinocytosis, the IgG can be recycled to the cell surface and released back into a neutral pH environment preventing the faith of lysosomal degradation that unbound proteins face when taken in by the endosome. This recycling can extend the serum half- life of IgG (Rodewald 1976), although, further studies are required since studies have shown that an increase in binding affinity of an engineered IgG molecule to the FcRn is not proportional to half-life (Roopenian and Akilesh 2007). One study demonstrated this with variants of Mab drug, Herceptin™, from Genentech with 3 and 12-fold higher binding affinities for the FcRn that still had similar half-life compared to Herceptin at the end (Petkova et al. 2006). Currently, more than 20 glyco-engineered mAbs, with enhanced ADCC, are being evaluated in clinical studies. Two of these mAbs have already been approved, mogamulizumab (Poteligeo®) on March 30th 2012 for marketing in Japan, an antibody developed exclusively by Kyowa Hakko Kirin, and obinutuzumab (Gazyva®), approved on November 1st 2013 in the USA (see Table 23.1), confirming the success of this approach. Although mogamulizumab has not been approved in Europe or the USA as of this writing, it is under review for treatment peripheral T-cell lymphoma while clinical studies have shown that the engineered obinutuzumab has a half-life of 28 days (Reichert 2011). The glyco-engineered Fc region of obinutuzumab has a bisected, complex, non-fucosylated oligosaccharides attached to asparagine 297, that enhances the binding affinity to FcγRIII an Fc receptor (Mossner et al. 2010). The glycol-engineering of obinutuzumab has significantly improved the efficacy over earlier therapeutic molecules such as rituximab and earlier developed mAb in B-cell malignancies.

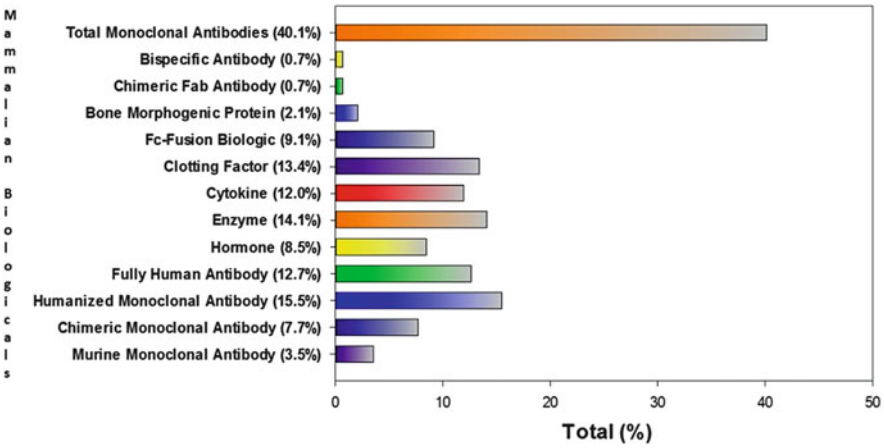

Fig. 23.2 A representation of the variety of mammalian cell culture products approved in the EU and USA, from the year 1987 till April 2014, revealing that monoclonal antibodies take 40.1 % of the approved mammalian biologicals on the market. Humanized and fully human antibodies make up the majority of the monoclonal antibodies approved

The majority of mammalian expressed biologics approved from 1987 up until April 2014, are monoclonal antibodies, with 40.1 % of the market (Fig. 23.2). In the month of January 2014, a total of 7 mAb therapeutics were undergoing their first regulatory review with the first submission of marketing applications being for; vedolizumab, siltuximab, ramucirumab, secukinumab, dinutuximab, nivolumab, and pembrolizumab. As of April 23th 2014, the FDA approved the chimeric antibody drug Sylvant™ (siltuximab) and the fully human antibody Cyramza™ (ramucirumab) for marketing (see Table 23.1). The median circulating half-life of siltuximab has been shown to be 17.8 days and siltuximab treatment was well tolerated and non-immunogenic according to in-house studies (Puchalski et al. 2010) and an external clinical lab (Kurzrock et al. 2013; van Rhee et al. 2010). Ramucirumab has a low immunogenicity with only 7.4 % of patients developing anti-ramucirumab antibodies in clinical trials when administered every 2 weeks. As screening and antibody engineering technologies improve, along with mammalian cell culture processes and cell line development techniques it is expected that the majority of mAb drugs will be non-immunogenic or have a very low chance of immunogenicity as well as a similar half-life to natural antibodies when required by their medicinal indication.

Apart from the typical antibody constructs used a therapeutic agents in 2009, the first bispecific antibody, under the trade name Removab® (catumaxomab), was approved in the EU for the treatment of cancer, malignant ascites, and peritoneal fluid accumulation caused by a cancer (European Medicine Agency 2014). The antibody structure consists of a mouse κ-light chain, a rat λ-light chain, a mouse IgG2a-heavy chain and a rat IgG2b-heavy chain that has two antigen binding sites where one mouse derived Fab region of the antibody binds an epithelial cell

adhesion molecule (EpCAM) and the second rat derived Fab region binds to CD3 (Walsh 2010). The hybrid antibody is manufactured via a rat-mouse hybrid-hybridoma cell culture process and the bispecific antibody functions by bringing together CD3-expressing T-cells, EpCAM-expressing tumor cells, and immune effector cells such as natural killer (NK) cells, macrophages, or dendritic cells that would bring about the destruction of the tumor cells through multiple immune system mechanisms.

The future outlook of mAb drugs is bright, with the start of this decade having close to 300 mAb's in various stages of clinical development. Of these mAb's approximately 150 new monoclonal antibodies are in development for the area of oncology treatments and close to 70 mAb's are in clinical development for treatment of inflammatory and autoimmune diseases with the rest are for indications that include metabolic disorders, cardiovascular disorders, CNS disorders, infectious diseases, and transplant rejection (Norman 2011). Currently, mAbs are the strongest growing segment of the pharmaceutical market and is expected to further grow at a fast pace along with sub categories such as fusion protein drugs that use antibody components to carry-out their function.

23.4 Fusion Protein Drugs

Since the Fc region of antibody binds to the FcRn to confer longer circulatory half-life there has been great success with the use of this natural molecular process to engineer proteins that can take advantage of this. In 1998, Enbrel® (etanercept) was the first CHO cell culture produced Fc fusion biologic, to gain marketing approval by the FDA. Enbrel®, a recombinant human soluble tumor necrosis factor (TNF) receptor able to bind and inactivate soluble and cell bound TNF and lymphotoxin competing with the cellular TNF receptors for the treatment of rheumatoid arthritis. Enbrel® has been one of the most successful biopharmaceuticals on the market with global sales reaching $8.4 billion in 2013 just behind Humira® of $10.7 billion, the two of these being the most successful drugs the biopharmaceutical industry has ever developed. Enbrel® consists of an intracellular portion of the human p75 TNFR linked to the Fc portion of IgG1 to form a dimeric protein. The benefit of the Fc fusion bestows the etanercept molecule with an extended median half-life of 4.8 days, together with a high binding affinity this contributes to Enbrel® overall effectiveness as an arthritis drug compared to others on the market (Mohler et al. 1993) at the time of its approval. A CHO cell line is used as the host for expression of the 150 kDa dimeric etanercept molecule. Enbrel® is part of a class of biologics that work by inhibiting the binding of TNF such as adalimumab (Humira®), golimumab (Simponi®, Simponi ARIA®), and infliximab (Remicade®), this allows these biologics to suppress the cascade of reactions that lead to an inflammatory response within the body that can actually destroy joint tissue as is characteristic with rheumatoid arthritis.

After market approval and release of Enbrel®, several other Fc fusion molecules were approved in the EU and USA that required a mammalian cell culture process in order to produce their complex fusion molecules. In 2003, a second mammalian cell expressed fusion product was approved by the name of Amevive® (alefacept) (Krueger and Callis 2003; Krueger and Ellis 2003) which utilized the Fc portion for apoptosis induction apart from boosting half-life. This 91.4 kDa protein has a Fc region of IgG1 linked to human leukocyte function antigen 3 (LFA-3) that can bind, with high affinity, to CD2, a functionally important and widely distributed T lymphocyte surface glycoprotein. Upon human LFA-3/IgG1 fusion protein administration the LFa-3 binds to CD2 inhibiting T-cell activation and proliferation. The Fc portion extends the circulatory half-life to 11.25 days (Kimchi-Sarfaty et al. 2013) in addition to interacting with the FcγRIII receptor on the surface of NK cells which results in NK induced apoptosis of T-lymphocytes (Majeau et al. 1994). This overall effect suppresses the immune system and can be used in the treatment of psoriasis, a skin condition that causes skin redness and irritation.

The CHO cell line has been the favorite for Fc fused recombinant proteins produced as biopharmaceuticals since all except one of the Fc-biologics from mammalian cell culture have at the time of this writing been produced in CHO cells, sticking to the formula of not changing what already works well. Fc fusions have also benefited from extended half-life, similar to Enbrel®. For example, Orencia® (Abatacept), Arcalyst® (Rilonacept), Nulojix® (Belatacept), Eylea®/Zaltrap® (Aflibercept), and Alprolix™ (Coagulation Factor IX) have all had an increased half-life due to an Fc fusion. For example, Orencia® has a half-life of 13.1 days. The therapeutic is a second-line treatment of rheumatoid arthritis in moderate to severe adult patients but works via a different biological mechanism than Enbrel®, acting as the first in a new class of agents that acts as a co-stimulation modulator able to inhibit full T-cell activation (Quan et al. 2008). This fusion protein is engineered as an IgG1 Fc fusion to CTLA-4 binding that acts by binding to CD80 and CD86 on antigen-presenting cells which will inhibit interaction with CD28 on T cells suppressing T-cell activation. Orencia® has been shown, in vitro, to decrease T-cell proliferation and inhibit the production of tumor necrosis factor alpha, interferon-gamma and interleukin-2 (Vital and Emery 2006). The extended half-life provided by the benefit of the glycosylated FC fusion structure for Orencia® means that it will only need to be taken once a month at maintenance dosage by the patient compared to once weekly with Enbrel®. Arcalyst® is an orphan designated drug also known as an interleukin-1 (IL- 1) trap since the fusion protein inhibits IL-1 which in turn reduces inflammatory responses due to unbalanced IL-1 stimulation. As with other Fc fusions the half-life is considerably extended to 8.6 days (Hoffman et al. 2008). Nulojix® is another IgG1 Fc fusion to CTLA-4 immunosuppressive agent but this time approved for the prevention of kidney transplant rejection therapy. Belatacept only differs from abatacept by two amino acids and although half- life is considered reasonable, it has been shown to be a little shorter than abatacept, at 8–10 days.

Two of the Fc-fusion biologics are not solely immunosuppressive and these are Elyea® and Alprolix™. The drug Eylea® is an anti-angiogenic used in the

treatment of neovascular age-related macular degeneration, an eye disease due to blood vessels leaking fluid into the macula. Zaltrap® (named ziv-aflibercept for distinction) is the same drug but approved as an anti-cancer agent for treatment of metastatic colorectal cancer. Aflibercept is a vascular endothelial growth factor (VEGF) trap that consists of an Fc region fused with the VEGF-binding portions from the extracellular domains of human VEGF receptors 1 and 2. The VEGF trap has a highly variable half-life of 1.7–7.4 days depending on the dosage. Alprolix™, which was approved for marketing on the 28th of March 2014, is the only Fc-fusion protein produced in HEK293 cells that has been approved by the FDA and also the first fusion drug for the treatment of hemophilia B (Food and Drug Administration 2014). The Fc fusion increased the half-life of the drug to 3.6 days, a considerable increase over the other coagulation factor IX drugs, Rixubis® and BeneFIX®. Rixubis® was introduced in 2013 with a half-life more than three times less than Alprolix®, at 26 h, and BeneFIX® was approved in 1997 with a maximum half-life of 24 h (Pfizer 1997; Baxter 2014). The coagulation factor IX drugs activate the coagulation pathway to ultimately convert prothrombin to thrombin which converts fibrinogen to fibrin so that a clot can be formed for the treatment of bleeding episodes.

More innovative fusion drugs are also being introduced onto the US market, such as the fertility drug Elonva® (corifollitropin alfa), a fusion drug Merck is currently seeking FDA approval for and has already been approved in the EU in 2010 (Table 23.1). Corifollitropin alfa is a modified recombinant human follicle stimulating hormone (rhFSH) in which the carboxy-terminal peptide of the beta subunit of human chorionic gonadotropin (hCG) is fused to the FSH beta chain. This drug is the first long-acting hybrid molecule that has a prolonged half-life and a slower absorption to serum peak concentrations meaning sustained follicle stimulating activity for the controlled stimulation of the ovaries. The benefits of corifollitropin alfa compared to other follicle stimulating drugs on the market today is that it remains effective for 7 days whereas other approved recombinant human FSH drugs require daily injections (Bouloux et al. 2001; Duijkers et al. 2002; Fares et al. 1992).

23.5 Drug Approvals and Regulation

The actual approval process for a biopharmaceutical product to be able to come on the market has evolved over the years and since the EMA and the FDA have different regulatory systems for the review and approval of new drugs there has been some harmonization between the two agencies over the years. The FDA implements regulations based on legislative law in the USA and the majority of activities related to biopharmaceutical regulation and the marketing approval process is done via two FDA divisions known as the Center for Drug Evaluation and Research (CDER) and the Center for Biologics Evaluation and Research (CBER). Briefly, in the USA, a potential new drug has to undergo pre-clinical trials

and the data submitted as an investigational new drug (IND) application to either CDER or CBER and when a biopharmaceutical seeks marketing approval for a new biological entity (NBE) they would either submit an application to CDER as a new drug application (NDA) or to CBER as a biologics license application (BLA) (Walsh 2003). The EMA was originally known as the European Agency for the Evaluation of Medicinal Products from 1995 to 2004, and the agency was setup by funding from the individual member states in western Europe, the European Union (EU) and the pharmaceutical sector. Headquartered in London, the EMA was born after years of negotiations among EU governments and since individual member states have their own national medicine regulatory bodies the EMA was setup to work closely with the agencies in the 28 European Union Member States as well as the European Economic Area (EEA) countries such as Norway, Iceland and Liechtenstein (European Medicine Agency 2014). Since by law, a pharmaceutical company can only market a medicine once it has received a marketing authorization, the EMA allows pharmaceutical companies to submit a single marketing-authorization application to the EMA for approval for all the member states and EEA countries as part of a centralized system. Like the FDA, the EMA requires pre-clinical and clinical trial data before marketing authorization application can take place and thus a clinical trial authorization (CTA) application has to be filed with the relevant governing body of the country the trial is being conducted.

Over the past few years, the EMA and FDA have taken several steps to harmonize and align regulatory practices for the approval and marketing of drugs (Trotta et al. 2011). These efforts have brought the differences in the time required for approval of the same drug between the two agencies closer together as can be seen in Table 23.1. One important collaboration established between the two agencies in 2003 are arrangements to allow the exchange of confidential information between the EU and the FDA as part of their regulatory and scientific processes (European Medicine Agency 2014). Further collaboration has brought about the development of common procedures for good-manufacturing-practice (GMP) and good-clinical-practice (GCP) inspections including applications for orphan drug designations.

23.6 Biosimilars or Follow on Biologics Approvals

As competition drives the price of the generic products down in the pharmaceutical sector, the impact of biosimilars would be expected to have a similar affect. Once a patent expires generic drugs can be legally produced, although loop holes can exist in areas or countries where the patent is not enforceable or the patent can be proven invalid. While small-molecule formulated pharmaceuticals can have exact copies made that can pass the regulatory framework, biopharmaceutical manufactured products such as, recombinant proteins can have a high degree of molecular complexity that includes the post translational modification which are all affected by the manufacturing process. The term biosimilar, or follow-on biologic, was

introduced by the regulatory authorities in the EU and USA to imply that the newly introduced product would be similar to the original biologic, but might not be an identical molecular copy of the parent biologic. The FDA tends to use the term follow-on biologics while in the EU biosimilar is used by the EMA (European Medicine Agency 2014; Food and Drug Administration 2014). In order to prove a biological entity is a biosimilar to the regulatory authorities, data has to be compiled through clinical, animal and analytical studies where the results must indicate that the biological entity reproduces the same clinical results as the parent drug. The time and costs associated with mammalian biopharmaceutical development and manufacturing of biosimilars will be a far greater investment for a pharmaceutical compared to what is required for small-molecule generics as it is estimated that the development time for a biosimilar recombinant proteins could range from 5 to 8 years compared to the 1–2 years for a generic small molecule (Lanthier et al. 2008; Grabowski et al. 2006). This is due to the complexity of large glycosylated molecule and the process development required. For example, the host cell will tend to go through the process of cell line selection and development to create a suitable host for the target bioprocess to produce the biosimilar, which can take months to years depending on the biological product. This is in addition to the development of the commercial scale manufacturing process that requires strict quality controls and process monitoring of all upstream and downstream processes till final formulation and product testing.

In 2006, the first biosimilar was approved in both the EU and the USA under the trade name Omnitrope™. This biosimilar was an *E. coli* expressed 22.1 kDa recombinant growth hormone (hGH) identical to the native protein consisting of a 191 amino acid single chain polypeptide manufactured by Sandoz for the treatment of growth hormone deficiencies (European Medicine Agency 2014). This approval was due to the regulatory framework that was established since 2005 by the EMA and established legislation in the USA (Woodcock et al. 2007; Schneider and Kalinke 2008). The EU has further build on their regulatory framework that by 2010 draft guidelines were established for Mab biosimilars leading to a final version of the guidelines completed by the EMA's Committee for Medicinal Products for Human Use (CHMP) that included IgG1 Fc-fusion protein biosimilars in the scope of MAb biosimilars (Schneider et al. 2012). The regulatory framework in the EU allowed the EMA to approve several biosimilars that are recombinant biopharmaceutical proteins produced in mammalian cell culture (see Table 23.1) beginning with a biosimilar for recombinant erythropoietin (Epoetin alfa). The first five Epoetin alfa biosimilars were approved in 2007 each marketed by Hexal AG, Sandoz GmbH, Medice Arzneimittel Pütter GmbH & Co. KG, Hospira UK Limited, and Stada Arzneimittel AG under the trade names; Epoetin alfa Hexal®, Binocrit®, Abseamed®, Retacrit®, and Silapo® respectively. This was a milestone accomplishment for a getting biosimilars of a glycosylated protein onto the market and helped set the stage for mAbs. In June 2013, Celltrion and Hospira received permission from the EMA's CHMP to market their biosimilars to Johnson & Johnson's Remicade® (infliximab) under the trade names Remsima® and Inflectra® respectively (see Table 23.1 for further details).

The impact of mammalian cell produced biologics is becoming the major contributor to pharmaceutical industry growth pipelines and the existence of a regulatory framework for biosimilars has meant further increases in mammalian cell culture capacity. BioProcess Technology Consultants, Inc. have given an interesting analysis of the global mammalian cell culture capacity with currently, the existence of one contract manufacturing organization (CMO) (Lonza), one excess capacity company acting as both product manufacturer and CMO (Boehringer Ingelheim), and 10 product companies with an installed capacity greater than 100,000 L each. These companies are; Roche, Johnson & Johnson, Amgen, Pfizer, Sanofi-Aventis, Novartis, Eli Lilly, Biogen Idec, Bristol-Myers Squibb, and Celltrion. Samsung Biologics being an additional CMO to join the list by 2017 (Ecker and Ransohoff 2014). Celltrion, Samsung BioLogics, and Innovent Biologics are examples of companies outside of the USA and Europe increasing capacity due to growing interests in mammalian cell culture biopharmaceutical manufacturing and the biosimilar market. It is predicted that by 2017 the worldwide capacity for mammalian cell culture manufacturing will be close to 4,400,000 L (this includes a perfusion factor of 5× to adjust for productivity differences between fed-batch and perfusion facilities) due to the further expansion of capacity from now till then (Ecker and Ransohoff 2014). This growth in worldwide capacity is an approximate 57 % increase in capacity since 2010.

Conclusion

As the pharmaceutical market has demonstrated over the last decade, the mammalian cell derived biologicals market has continued to thrive and drive major growth in the pharmaceutical industry. The ability to provide post translational modifications and the continued need for monoclonal antibody therapies and the rise of Fc-fusion protein therapies have given mammalian expression systems a dominant advantage over other expression systems for the next generation of engineered biopharmaceuticals. With the introduction of disposable technologies, the improvement of cell culture processes, cell line selection and development strategies giving higher titers and specific productivities, including the establishment of EU and US regulatory pathways to bring biosimilars to the market, an infusion of growth has occurred at a rate currently faster than any other pharmaceutical sector. This growth is exemplified by the increase in global manufacturing capacity, including a substantial increase in the construction of Asian GMP mammalian culture facilities over the last few years. The introduction of new players in the biopharmaceutical industry, alongside the arrival of biosimilars promising lowered healthcare expenses of animal cell derived biopharmaceuticals, allows us to make the prediction that the next decade of mammalian bioprocesses and their biological products will continue to grow at a remarkable pace in innovation and discovery providing an increased affordability of these biological medicines.

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