

REVIEW ARTICLE

Human cell lines for biopharmaceutical manufacturing: history, status, and future perspectives

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*Biogen, Cambridge, MA, USA***Abstract**

Biotherapeutic proteins represent a mainstay of treatment for a multitude of conditions, for example, autoimmune disorders, hematologic disorders, hormonal dysregulation, cancers, infectious diseases and genetic disorders. The technologies behind their production have changed substantially since biotherapeutic proteins were first approved in the 1980s. Although most biotherapeutic proteins developed to date have been produced using the mammalian Chinese hamster ovary and murine myeloma (NS0, Sp2/0) cell lines, there has been a recent shift toward the use of human cell lines. One of the most important advantages of using human cell lines for protein production is the greater likelihood that the resulting recombinant protein will bear post-translational modifications (PTMs) that are consistent with those seen on endogenous human proteins. Although other mammalian cell lines can produce PTMs similar to human cells, they also produce non-human PTMs, such as galactose- α 1,3-galactose and *N*-glycolylneuraminic acid, which are potentially immunogenic. In addition, human cell lines are grown easily in a serum-free suspension culture, reproduce rapidly and have efficient protein production. A possible disadvantage of using human cell lines is the potential for human-specific viral contamination, although this risk can be mitigated with multiple viral inactivation or clearance steps. In addition, while human cell lines are currently widely used for biopharmaceutical research, vaccine production and production of some licensed protein therapeutics, there is a relative paucity of clinical experience with human cell lines because they have only recently begun to be used for the manufacture of proteins (compared with other types of cell lines). With additional research investment, human cell lines may be further optimized for routine commercial production of a broader range of biotherapeutic proteins.

Introduction

Protein therapeutics (including monoclonal antibodies [mAbs], peptides and recombinant proteins) represent the largest group of new products in development by the biopharmaceutical industry (Durocher & Butler, 2009; Ho & Chien, 2014).

These products are produced in a wide variety of platforms, including non-mammalian expression systems (bacterial, yeast, plant and insect) and mammalian expression systems (including human cell lines) (Ghaderi et al., 2012). Importantly, the most appropriate expression system depends on the particular protein to be expressed. Mammalian expression systems are generally the preferred platform for

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manufacturing biopharmaceuticals, as these cell lines are able to produce large, complex proteins with post-translational modifications (PTMs; most notably glycosylation) similar to those produced in humans (Durocher & Butler, 2009; Ghaderi et al., 2012; Swiech et al., 2012). Moreover, in the case of mammalian cell lines, and animal cell lines in general, most proteins can be secreted rather than requiring cell lysis to extract with subsequent protein refolding (as is the case with bacteria/prokaryotes). The most common mammalian (non-human) cell lines used for therapeutic protein production include Chinese hamster ovary (CHO) cells, baby hamster kidney (BHK21) cells and murine myeloma cells (NS0 and Sp2/0) (Estes & Melville, 2014). However, these non-human mammalian cell lines also produce PTMs that are not expressed in humans, namely galactose- α 1,3-galactose (α -gal) and *N*-glycolylneuraminic acid (NGNA). Because humans possess circulating antibodies against both of these *N*-glycans, non-human cell lines are usually screened during their production to identify clones with acceptable glycan profiles (Ghaderi et al., 2010).

Human cell lines have the ability to produce proteins most similar to those synthesized naturally in humans, which may be an advantage compared with other mammalian expression systems (Ghaderi et al., 2010). In particular, the structure,

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number and location of post-translational *N*-glycans can affect the biologic activity, protein stability, clearance rate and immunogenicity of biotherapeutic proteins (Arnold et al., 2007; Ghaderi et al., 2010; Swiech et al., 2012).

The first human cell line, HeLa, was established in 1951 from a cervical cancer (Scherer et al., 1953). Human diploid cells were developed in the 1960s for vaccine manufacturing; however, concerns for a latent oncogenic agent in these cell lines (despite a lack of suggestive phenotypic characteristics) delayed their acceptance. Currently, human diploid cells are used in the manufacture of many viral vaccines (Petricciani & Sheets, 2008). However, due to their rapid growth, high protein yield, and the investment in system optimization, animal cells remained the substrate of choice for the production of recombinant proteins and mAbs (Petricciani & Sheets, 2008).

Today, advances in technology have allowed for increased productivity with human cell lines, and there are now approved recombinant biotherapeutic products produced from the human embryonic kidney 293 (HEK293) and fibrosarcoma HT-1080 cell lines (Beck, 2009; Casademunt et al., 2012; Dumont et al., 2012; Glaesner et al., 2010; Peters et al., 2010; Wraith, 2008; Zimran et al., 2013). Additional biotherapeutic products produced in the PER.C6, HKB-11, CAP and HuH-7 human cell lines are currently being evaluated (Enjolras et al., 2012; Estes & Melville, 2014; Jones et al., 2003; Mei et al., 2006; Swiech et al., 2011, 2015). This article is a narrative review of the cell lines (with a focus on human cell lines) used for production of biotherapeutic proteins, both approved and in development.

Non-human expression systems used to manufacture biotherapeutic products

Many non-human expression systems have been utilized in the production of currently approved biotherapeutic proteins (Table 1).

Bacterial expression systems (e.g. *Escherichia coli*) possess the advantages of being straightforward to culture, with rapid cell growth and high yields. In addition, protein expression can be initiated through promoter induction by addition of lactose or the lactose analogue isopropyl- β -D-thiogalactopyranoside (IPTG; IPTG induces the promoters *lac*, *tac* and *trc*). However, such systems are unable to produce complex, mammalian-like glycosylation due to the absence of the necessary enzymatic components and the intracellular compartmentalization required (Ghaderi et al., 2012; Graumann & Premstaller, 2006). In addition, mammalian proteins produced in these systems often aggregate, forming inclusion bodies, due to the low solubility of mammalian proteins in prokaryotic cells and absence of appropriate protein chaperone systems. Proteins produced in bacterial expression systems must often be extracted from inclusion bodies and refolded. Bacterial systems are therefore generally used for production of non-glycosylated proteins, including some mAbs, hormones, cytokines and enzymes (Ghaderi et al., 2012; Graumann & Premstaller, 2006).

Similar to bacterial expression systems, yeast expression systems (e.g. *Saccharomyces cerevisiae* and *Pichia pastoris*) achieve rapid cell growth and high-protein yields with

straightforward production scalability and without the need for animal-derived growth factors (Gerngross, 2004). Yeast cell lines may also be used to produce proteins that cannot be obtained from *E. coli* due to the problems associated with folding and stereochemistry (Gerngross, 2004). The key challenge associated with yeast expression systems is their production of high mannose residues within their expressed PTMs (50–200 vs three molecules in human cells, as part of either *N*- or *O*-linked glycan structures), which may confer a short half-life and render proteins less efficacious and even immunogenic in humans (Dean, 1999; Gemmill & Trimble, 1999; Gerngross, 2004; Lam et al., 2007; Mochizuki et al., 2001). The development of yeasts that have been genetically modified to address the issue of high mannose content has been reported (Chiba et al., 1998; Gerngross, 2004; Ghaderi et al., 2012; Hamilton et al., 2003). The expression of a fully humanized sialylated glycoprotein in glycoengineered yeast constitutes a major advance in the use of yeast expression systems for biopharmaceutical manufacturing (Hamilton & Gerngross, 2007).

Plant and insect cell expression systems are able to produce proteins with complex glycosylation patterns; however, the glycan structures produced are significantly different from those produced in humans (Ghaderi et al., 2012). Plants lack many of the key glycosylated residues present in humans, most notably sialic acids. In addition, they produce α 1,3-fructose and β 1,2-xylose, which are absent in humans and may be immunogenic (Ghaderi et al., 2012). Notably, in 2012, taliglucerase alfa (ELELYSO[®]; Pfizer, New York, NY) was approved by the US Food and Drug Administration (FDA) for the treatment of type 1 Gaucher disease. This therapy is produced using genetically modified carrot plant root cells that produce the enzyme with a human compatible glycan profile (ELELYSO[™], 2014).

Insect cells infected with the viral vector baculovirus (baculovirus-insect cell expression system) can also efficiently express recombinant proteins, and these systems are mostly used for the development of virus-like particles and, subsequently, vaccines (Kost et al., 2005; Liu et al., 2013). However, although they produce *N*-glycan precursors, these are trimmed, resulting in either high mannose or paucimannose residues that do not develop further into terminal galactose and/or sialic acid residues (Kost et al., 2005). This is evidenced by the lack of either galactosyltransferase or sialyltransferase activity. As in plants, insect systems may also express the fucosylated α 1,3-linkage (Staudacher et al., 1999). However, in recent years, there have been developments in the use of transgenic insect cells, with humanized protein glycosylation mechanisms (Kost et al., 2005).

The majority of currently licensed biotherapeutic products are produced in non-human mammalian expression systems (Table 1), as these systems are able to produce PTMs that (outside of a human expression system) most closely resemble those in humans (Ghaderi et al., 2010). These expression systems are used to produce mAbs, hormones, cytokines, enzymes and clotting factors (Ghaderi et al., 2012).

The most frequently used mammalian system is the CHO cell line, which is used in the manufacture of >70% of currently approved recombinant proteins (Butler & Spearman, 2014). This cell line has demonstrated several major

Table 1. Non-human expression systems used in the production of biotherapeutics approved in the United States and Europe^{a,b}.

Expression system	Biotherapeutic product	FDA approval	EMA approval
Plant cells	Enzymes		
	Taliglucerase alfa	Approved	NA
Insect cells	Vaccines		
	Cervical cancer vaccine	Approved	Approved
Bacteria	Monoclonal antibodies		
	Certolizumab pegol	Approved	Approved
	Cytokines		
	tbo-filgrastim	Approved	NA
	Romiplostim	Approved	Approved
	Enzymes		
	Asparaginase <i>Erwinia chrysanthemi</i>	Approved	NA
	Glucarpidase	Approved	NA
	Pegloticase	Approved	Approved
	Collagenase <i>Clostridium histolyticum</i>	Approved	NA
	Peptides	Approved	NA
	Metreleptin		
	Therapeutic toxins	Approved	NA
	Incobotulinumtoxin A		
	Vaccines		
Meningitis vaccine	Approved	Approved	
Pneumococcal vaccine	Approved	Approved	
Yeast	Enzymes		
	Ocriplasmin	Approved	Approved
	Peptides		
	Abiglutide	Approved	Approved
	Liraglutide	Approved	Approved
Clotting factors			
Catridecacog	Approved	Approved	
Mammalian (non-human) cell lines			
CHO	Monoclonal antibodies		
	Adalimumab	Approved	Approved
	Alemtuzumab	Approved	NA
	Bevacizumab	Approved	Approved
	Brentuximab vedotin	Approved	Approved
	Denosumab	Approved	Approved
	Golimumab	Approved	Approved
	Ibritumomab tiuxetan	Approved	Approved
	Ipilimumab	Approved	Approved
	Obinutuzumab	Approved	Approved
	Omalizumab	Approved	Approved
	Panitumumab	Approved	Approved
	Pertuzumab	Approved	Approved
	Rituximab	Approved	Approved
	Siltuximab	Approved	Approved
	Tocilizumab	Approved	Approved
	Trastuzumab	Approved	Approved
	Vedolizumab	Approved	Approved
	Ado-trastuzumabemtansine	Approved	Approved
	Ustekinumab	Approved	Approved
	Cytokines		
	Darbepoetin alfa	Approved	Approved
	Interferon beta-1a	Approved	Approved
	Epoetin alfa	Approved	Approved
	Epoetin beta	NA	NA
	Epoetin theta	NA	Approved
	Enzymes		
	Agalsidase beta	Approved	Approved
	Alglucosidase alfa	Approved	Approved
	Alteplase	Approved	Approved
	Elosulfase alfa	Approved	NA
	GalNAc 4-sulfatase	Approved	NA
	Human DNase	Approved	Approved
	Hyaluronidase	Approved	NA
	Imiglucerase	Approved	NA
	Laronidase	Approved	NA
	Tenecteplase	Approved	Approved
	Fc-fusion proteins		
	Abatacept	Approved	Approved

(continued)

Expression system	Biotherapeutic product	FDA approval	EMA approval
NS0	Aflibercept	Approved	Approved
	Alefacept	Approved	Approved
	Belatacept	Approved	Approved
	Etanercept	Approved	NA
	Rilonacept	Approved	Approved
	Ziv-aflibercept	Approved	Approved
	Hormones		
	Choriogonadotropin alfa	Approved	NA
	Follitropin alfa	Approved	Approved
	Follitropin beta	Approved	Approved
	Luteinizing hormone	Approved	Approved
	Osteogenic protein-1	Approved	Approved
	Thyrotropin alfa	Approved	Approved
	Clotting factors		
	Factor VIII	Approved	Approved
Factor IX	Approved	Approved	
Sp2/0	Monoclonal antibodies		
	Belimumab	Approved	Approved
	Natalizumab	Approved	Approved
	Ofatumumab	Approved	Approved
	Palivizumab	Approved	Approved
BHK	Monoclonal antibodies		
	Ramucirumab	Approved	NA
	Abciximab	Approved	NA
	Basiliximab	Approved	Approved
	Canakinumab	Approved	Approved
Murine C127	Cetuximab	Approved	Approved
	Infliximab	Approved	Approved
	Clotting factors		
Murine C127	Factor VIIa	Approved	Approved
	Factor VIII	Approved	Approved
Murine C127	Hormones		
	Somatropin	Approved	Approved

FDA, US Food and Drug Administration; EMA, European Medicines Agency; NA, not approved; CHO, Chinese hamster ovary; BHK, baby hamster kidney.

^aData obtained from publically available resources (October 2014); all approved products may not be included.

^bReferences: (ABSEAMED[®], 2012; ACTEMRA[®], 2013; ACTILYSE[®], 2014; ACTIVASE[®], 2012; ADCETRIS[®], 2013; ADVATE[®], 2014; ALDURAZYME[®], 2008; ARANESP[®], 2006; ARCALYST[™], 2008; ARZERRA[®], 2014; AVASTIN[®], 2010; AVONEX[®], 2007; BENEFIX[®], 2012; BENLYSTA[®], 2011; CAMPATH[®], 2014; CATHFLO[®] ACTIVASE[®], 2010; CERAZYME[®], 2010; CERVARIX[®], 2012; CIMZIA[®], 2013; CIMZIA[®], 2014; CYRAMZA[®], 2014; ELELYSO[™], 2014; ENBREL[®], 2010; ENTYVIO[®], 2014a,b; EPERZAN[™], 2014; Epoetin alfa HEXAL[®], 2012; EPORATIO[®], 2009; ERBITUX[®], 2009; ERWINAZE[®], 2011; EYLEA[®], 2012; EYLEA[®], 2013; FABRAZYME[®], 2006; FERTAVID[®], 2009; FOLLISTIM[®], 2011; GAZYVA[™], 2014; GAZYVARO[®], 2014; Ghaderi et al., 2012; GONAL-F[®], 2010; GRANIX[™], 2014; HELIXATE[®] NexGen, 2010; HERCEPTIN[®], 2010; HUMIRA[®], 2008; HYLENEX[®], 2012; ILARIS[®], 2014; JETREA[®], 2012; JETREA[®], 2013; KADCYLA[®], 2013, 2014; KOGENATE[®] Bayer, 2010; KRYSTEXXA[®], 2013; LUMIZYME[®], 2010; LUVERIS[®], 2005; MABTHERA[®], 2008; MENVEO[®], 2010; METALYSE[®], 2006; MYALEPT[™], 2014; MYOZYME[®], 2011; NAGLAZYME[®], 2005; NOVOSEVEN[®], 2006; NOVOTHIRTEEN[®], 2012; NPLATE[®], 2009; NULOJIX[®], 2011; OBIZUR[™], 2014; Office of Device Evaluation and Center for Devices and Radiological Health, 2001; OPGENRA[®], 2014; ORENCIA[®], 2012; OVIDREL[®], 2014; OVITRELLE[®], 2006; PERJETA[®], 2013a,b; PREVNAR[®], 2009; PROCRT[®], 2000; PROLIA[®], 2010; PULMOZYME[®], 2010; RAXIBACUMAB[™], 2014; REBIF[®], 2008; REFACTO AF[®], 2014; REMICADE[®], 2009; REOPRO[®], 2013; RITUXAN[®], 2014; ROACTEMRA[®], 2013; SAIZEN[®], 1987; SEROSTIM[®], 1987; SIMPONI[®], 2009; SIMULECT[®], 2008; Somatropin Biopartners, 2013; STELARA[®], 2013; Swiech et al., 2012; SYLVANT[®], 2015; SYLVANT[™], 2014; SYNAGIS[®], 2009; TANZEUM[™], 2014; tbo-filgrastim, 2012; THYROGEN[®], 2010; TNKASE[®], 2011; TRETEN[®], 2014; TYSABRI[®], 2011; US Food and Drug Administration, 2010, 2011, 2012, 2013, 2014; Vectibix[®], 2014; Victoza[®], 2009; VIMIZIM[®], 2014a,b; VORAXAZE[®], 2012; XEOMIN[®], 2014; XIAFLEX[®], 2014; XOLAIR[®], 2010; XYNTHA[®], 2011; YERVOY[®], 2011; ZALTRAP[®], 2013a,b; ZEVALIN[®], 2009).

advantages. First, CHO cells are able to grow in suspension culture (which enables large-scale production; other cell lines, such as insect cells, also have this ability) and serum-free chemically defined media (enabling reproducibility across different batches of cultures with a better safety profile than in media that contain human- or animal-derived proteins) (Kim et al., 2012; Lai et al., 2013; Rossi et al., 2012). Historically, CHO cells allowed gene amplification, resulting in a higher recombinant protein yield (up to the gram per liter range for

some proteins) and specific productivity, which was previously an issue in other mammalian cell lines (Carlage et al., 2012; Kim et al., 2012; Yang et al., 2014a,b). Other advances, such as the creation of stronger expression units and advanced hosts, better selection strategies (e.g. through technologic advances in screening for high-productivity clones) and targeting the transgene to transcriptional hotspots (site-specific integration of transgenes), also contribute to the high protein yields attained from these cells (Kim et al.,

2012). In addition, this expression system is highly tolerant to changes in pH, oxygen level, pressure or temperature during manufacturing (Ghaderi et al., 2012; Lai et al., 2013). Furthermore, due to the long period of time that this cell line has been used, there is a degree of familiarity with the CHO platform within development and manufacturing organizations, regulatory agencies, and suppliers (e.g. cell culture media suppliers), which could potentially decrease overall timelines. This familiarity may also be beneficial when assessing contaminant profiles (e.g. host cell proteins), which may be better characterized for CHO cells compared with newer cell lines.

The first recombinant biotherapeutic protein produced in CHO cells was tissue plasminogen activator, approved in 1986 (Kim et al., 2012). Therefore, the safety profile of CHO cells has been established for more than 20 years (Butler & Spearman, 2014; Kim et al., 2012). CHO cells have been shown to have reduced susceptibility to certain viral infections compared with other mammalian cell lines (e.g. the BHK cell line), and routine screening systems for adventitious agents are effective in detecting cell line infections (Berting et al., 2010). This reduced susceptibility may be due to the fact that many viral entry genes are not expressed in CHO cells (Xu et al., 2011). Further, there is perceived species barrier protection with the use of hamster-derived cells, reducing the potential risk of transfer of contaminating adventitious agents to humans (Berting et al., 2010; Swiech et al., 2012). However, many viruses have the ability to cross the species barrier and may still pose a risk (Pauwels et al., 2007).

Perhaps the most important advantage of CHO cells is that they are able to produce proteins with complex bioactive PTMs that are similar to those produced in humans. However, CHO cells are unable to produce some types of human glycosylation (CHO cells lack α [2-6] sialyltransferase α [1-3/4] fucosyltransferases) and they produce glycans that are not expressed in humans, namely α -gal and NGNA (Bosques et al., 2010; Dietmair et al., 2012; Ghaderi et al., 2012). Circulating antibodies against both of these *N*-glycans are present in humans, which may lead to increased immunogenicity and altered pharmacokinetics of these products when used in humans (Ghaderi et al., 2010; Padler-Karavani et al., 2008). Additional screening in CHO cells is required in order to isolate clones lacking the α -gal and NGNA glycans. This screening may result in otherwise productive clones needing to be discarded (Ghaderi et al., 2010). However, the attachment of non-human glycans may not be a concern for therapeutic proteins that do not require glycosylation, which illustrates the importance of considering the specific product molecule when choosing an appropriate cell line for production of a protein.

Other mammalian cell lines used for the production of biotherapeutic proteins include BHK-21 cells, used in the production of some coagulation factors such as factor VIII (Wurm, 2004). When murine myeloma cell lines (NS0 and Sp2/0) have been used historically, they have generally been used in the production of mAbs, for example, palivizumab and ofatumumab (Barnes et al., 2000; Butler & Spearman, 2014; Ghaderi et al., 2012). These myeloma cells were derived from immunoglobulin-producing tumor cells that no longer produced their original immunoglobulins; these cells

possess the appropriate machinery for producing and secreting these proteins (Barnes et al., 2000).

For proteins produced in all of these non-human cell lines, as well as those produced in human cell lines, potential safety concerns arise from the possibility of process-related contaminants and immunogenicity (World Health Organization, 2013). Process-related contaminants may include infectious agents (viral, bacterial, fungal, etc.) with the potential to result in host infection, nucleic acid contaminants with the potential to integrate into the host genome (theoretical), and other contaminants from the manufacturing process, such as exogenous non-human epitopes (e.g. from animal serum used during the manufacturing process) that can be incorporated into human cells and the resultant biotherapeutic protein (Ghaderi et al., 2012).

Human cell lines used to manufacture licensed products

HEK293 and HT-1080 are the two human cell lines most often used in the production of biotherapeutic proteins, which offer the advantage of producing fully human PTMs (Tables 2 and 3) (Loignon et al., 2008; Swiech et al., 2012).

HEK293 cells are easily grown in suspension serum-free culture, reproduce rapidly, are amenable to a number of transfection methods, and are highly efficient at protein production (Swiech et al., 2012; Thomas & Smart, 2005).

HEK293-H (Berkner, 1993) and 293-F (Vink et al., 2014) cell lines are clonal isolates of the HEK293 cell line that were selected for fast growth in serum-free medium, superior transfection efficiency, and a high level of protein production (Gibco, 2014). Subclone 293-H also has improved adherence to monolayer culture (when serum-supplemented media are used) compared with other cell lines. Other modified HEK293 cells include the HEK293-T cell line and HEK293-EBNA1 cells. The HEK293-T (293-T) cell line expresses the simian virus 40 large T antigen and is capable of expressing high titers of viral gene vectors for use in gene therapy (Yamaguchi et al., 2003). HEK293-T cells are often used for the production of retroviral vectors (Yamaguchi et al., 2003). HEK293-EBNA1 cells stably express the Epstein-Barr virus EBNA-1 gene, controlled by the cytomegalovirus promoter and demonstrate a greater growth rate and maximal cell density relative to parental HEK293 cells (Schlaeger & Christensen, 1999).

HEK293 cells have been widely used to produce research-grade proteins for many years and, more recently, five therapeutic agents produced in HEK293 cells have been approved by the FDA or the European Medicines Agency (EMA) for therapeutic use. These agents are drotrecogin alfa (XIGRIS[®]; Eli Lilly Corporation, Indianapolis, IN), recombinant factor IX Fc fusion protein (rFIXFc; Biogen, Cambridge, MA), recombinant factor VIII Fc fusion protein (rFVIII₂Fc; Biogen, Cambridge, MA), human cell line recombinant factor VIII (human-cl rhFVIII; NUWIQ[®]; Octapharma, Lachen, Switzerland) and dulaglutide (TRULICITY[®]; Eli Lilly, Indianapolis, IN).

Drotrecogin alfa is a recombinant activated protein C that was approved by the FDA in 2001 and by the EMA in 2002 for the treatment of patients with severe sepsis. HEK293 cells

Table 2. Human cells lines and their therapeutic protein products^{a,b}.

Cell line	Product	Indication	FDA approval status	EMA approval status
HEK293	Drotrecogin alfa	Severe septicemia/septic shock	Approved 2001; withdrawn 2011	Approved 2002; withdrawn 2011
	rFVIII Fc	Hemophilia A	Approved 2014	Submitted 2014
	rFIX Fc	Hemophilia B	Approved 2014	NA
	Dulaglutide	Type 2 diabetes	Approved 2014	Submitted 2014
	Human-cl rhFVIII	Hemophilia A	Submitted to the FDA	Approved 2014
HT-1080	Agalsidase alfa	Fabry disease	NA	Approved 2001
	Epoetin delta	Anemia secondary to chronic renal failure	NA	Approved 2002; withdrawn 2009 (Europe)
	Idursulfase	Hunter syndrome	Approved 2006	Approved 2007
PER.C6	Velaglucerase alfa	Type 1 Gaucher disease	Approved 2010	Approved 2010
	CL184	Rabies virus infection	Submitted to the FDA	NA
	MOR103	Rheumatoid arthritis, multiple sclerosis	Phase 1 clinical development	Phase 1 clinical development

FDA, US Food and Drug Administration; EMA, European Medicines Agency; HEK, human embryonic kidney; NA, not approved; rFVIII Fc, recombinant factor VIII Fc fusion protein; rFIX Fc, recombinant factor IX Fc fusion protein; rhFVIII, recombinant human factor VIII.

^aData obtained from publically available resources (October 2014); all approved products may not be included.

^bReferences: (ALPROLIX[®], 2014; Bakker et al., 2005; Behrens et al., 2014; Casademunt et al., 2012; DYNEPO[®], 2007; ELAPRASE[®], 2012, 2013; ELOCTATE[®], 2014; European Medicines Agency and Committee for Medicinal Products for Human Use, 2014; Glaesner et al., 2010; Octapharma, 2014; REPLAGAL[®], 2006; TRULICITY[™], 2014; VPRIV[®], 2010a,b; XIGRIS[®], 2008).

Table 3. Comparison of human cell lines with other expression systems in the production of therapeutic proteins.

Advantages	Disadvantages
<ul style="list-style-type: none"> • Absence of potentially immunogenic PTMs due to human-compatible glycosylation • Easily grown in suspension serum-free culture • Achieve rapid reproduction • Amenable to a number of transfection methods 	<ul style="list-style-type: none"> • Clinical experience is not as extensive as for other cell lines, although experience is growing • Potential susceptibility to human viral contamination

were chosen by the manufacturer for production of drotrecogin alfa because its activity required two PTMs, propeptide cleavage and γ -carboxylation of its glutamic acid residues, which CHO cells cannot produce with adequate efficiency (Berkner, 1993; Durocher & Butler, 2009). The product was approved (Bernard et al., 2001), but was later voluntarily withdrawn from the market by its manufacturer (Eli Lilly) in 2011 following the randomized placebo-controlled Prospective Recombinant Human Activated Protein C Worldwide Evaluation in Severe Sepsis and Septic Shock (PROWESS-SHOCK) trial, which demonstrated no mortality benefit with drotrecogin alfa compared with placebo for patients experiencing septic shock (Green et al., 2012; Ranieri et al., 2012).

rFVIII Fc and rFIX Fc are recombinant fusion proteins that were approved by the FDA in 2014 for the control and prevention of bleeding episodes, perioperative management and routine prophylaxis to prevent or reduce the frequency of bleeding episodes in people with hemophilia A and B, respectively (ALPROLIX[®], 2014; ELOCTATE[®], 2014; Mahlangu et al., 2014; Powell et al., 2013). They are also approved in Canada, Australia and Japan. rFVIII Fc consists of B domain-deleted recombinant factor VIII genetically fused to the Fc portion of immunoglobulin G1 (IgG1) and is produced in HEK293-H cells (Dumont et al., 2012; ELOCTATE[®], 2014; Peters et al., 2013). The rFVIII Fc fed-batch culture process is robust at scales of 200, 2000 and

15 000 liters, with the potential for a second-generation process to achieve even higher cell densities, on the order of 3.5×10^7 vc/ml (Huang et al., 2014). rFIX Fc was also produced using HEK293-H cells, and consists of the factor IX sequence covalently linked to the Fc domain of human IgG1 (ALPROLIX[®], 2014; Durocher & Butler, 2009; McCue et al., 2014; Peters et al., 2010). An essential PTM for FIX activity is γ -carboxylation of the first 12 glutamic acid residues in the Gla domain by vitamin K-dependent γ -glutamyl carboxylase. This modification facilitates binding of FIX to phospholipid membranes. HEK293 cells have been reported to have a greater capacity for γ -carboxylation than CHO cells (Berkner, 1993). Furthermore, FVIII contains six potential tyrosine sulfation sites, which are vital for FVIII functionality and binding to von Willebrand factor. FVIII expressed from human cell lines has been reported to be fully sulfated (Kannicht et al., 2013; Peters et al., 2013).

The use of a human cell line for replacement coagulation factors, such as rFVIII Fc and rFIX Fc, may result in reduced immunogenicity relative to non-human mammalian cell lines, as α -gal and NGNA glycan moieties are absent from these manufactured protein products (Bosques et al., 2010; McCue et al., 2014, 2015; Noguchi et al., 1995). However, it should be noted that several recombinant clotting factor products produced in non-human mammalian cell lines have been used successfully for many years. The development of inhibitors (neutralizing antibodies) against replacement clotting factors

occurs in ~30% of people with severe hemophilia A and 5% of those with severe hemophilia B. The causative *F8* or *F9* gene mutation plays a pivotal role in inhibitor development in hemophilia A and B, respectively, with large or complete deletions, non-sense mutations or inversions (e.g. intron 22 inversion in the *F8* gene) being the most commonly associated mutations (Franchini & Mannucci, 2011). The impact of PTMs on inhibitor development is unknown, and will need further research. Importantly, none of the previously treated people with hemophilia in the phase 1/2a or phase 3 clinical studies developed inhibitors to the rFVIII_{Fc} and rFIX_{Fc} fusion products (Mahlangu et al., 2014; Powell et al., 2012, 2013; Shapiro et al., 2012).

Human-cl rhFVIII (NUWIQ[®]), an additional factor VIII replacement product for the management of hemophilia A, is being produced in the HEK293-F cell line. Like HEK293-H cells, HEK293-F cells are a derivation of HEK293 cells that have been pre-adapted for growth in serum-free culture medium (Casademunt et al., 2012). Human-cl rhFVIII has been approved by the EMA and submitted to the FDA for approval (Octapharma, 2014); this product has been shown to exhibit a similar glycosylation profile to human plasma-derived factor VIII, without α -gal and NGNA (Kannicht et al., 2013).

Glucagon-1-like peptide (GLP-1) Fc fusion protein (dulaglutide) has been approved by the FDA for the treatment of type 2 diabetes mellitus, and is produced using HEK293-EBNA cells (Glaesner et al., 2010; TRULICITY[™], 2014). Large clinical trials have demonstrated its superiority over the dipeptidyl peptidase-4 inhibitor antagonist exenatide and its non-inferiority to liraglutide (a GLP-1 agonist), when added on to oral diabetic agents (Dungan et al., 2014; Wysham et al., 2014).

Another human cell line, HT-1080, was produced from a fibrosarcoma with an epithelial-like phenotype (Swiech et al., 2012). With the use of gene activation technology (in which the endogenous DNA promoter is replaced with a more potent type), four approved therapeutic proteins have been produced by Shire (Swiech et al., 2012).

1) Epoetin delta (DYNEPO[®]) was approved by the EMA in 2002 for the treatment of anemia secondary to chronic renal failure (DYNEPO[®], 2007; ELAPRASE[®], 2013; REPLAGAL[®], 2006; Swiech et al., 2012; VPRIV[®], 2013). However, this has been voluntarily withdrawn by the manufacturer for commercial reasons.

2) Iduronate-2-sulfatase (idursulfase; ELAPRASE[®]) is licensed as enzyme replacement therapy (EMA in 2007 and FDA in 2006) for the treatment of Hunter syndrome (mucopolysaccharidosis II), an X-linked lysosomal storage disorder (ELAPRASE[®], 2013).

3) Agalsidase alfa (REPLAGAL[®]; Shire Human Genetic Therapies, Danderyd, Sweden) was approved by the EMA in 2001 for the treatment of Fabry disease (REPLAGAL[®], 2010). Compared with agalsidase beta (FABRAZYME[®]; Genzyme Therapeutics, Cambridge, MA), which is produced using CHO cells for a similar indication (FABRAZYME[®], 2010, 2014), agalsidase alfa has shown similar enzyme kinetics. However, agalsidase alfa demonstrates a lesser uptake into fibroblasts from patients with Fabry disease and also lower concentrations in the kidney, heart and spleen of

mice (Lee et al., 2003). A single clinical study has compared the two products; this showed no significant differences for all efficacy outcomes, and there were no differences for the development of antibodies (Vedder et al., 2007).

4) The fourth agent produced in HT-1080 cells, velaglycerase alfa (VPRIV[®]; Shire Human Genetic Therapies, Lexington, MA), was approved in 2010 (FDA and EMA) for the treatment of type 1 Gaucher disease (DYNEPO[®], 2007; ELAPRASE[®], 2013; REPLAGAL[®], 2006; Swiech et al., 2012; VPRIV[®], 2013). Velaglycerase alfa has been compared with two similar products: imiglucerase, produced using CHO cells, and taliglucerase alfa, produced using carrot cells (Ben Turkia et al., 2013; Tekoah et al., 2013).

These products have diverse glycan profiles and the studies have generally shown comparable uptake into macrophages, *in vitro* enzymatic activity, stability, organ distribution and efficacy (Ben Turkia et al., 2013; Tekoah et al., 2013). However, neutralizing antibodies to imiglucerase were noted in 24% of patients, which had an impact on enzyme activity. It was noted that various factors, such as the production cell line and glycosylation, may be responsible for the difference in immunogenicity, however, the specificity of the anti-imiglucerase antibodies was not stated (Ben Turkia et al., 2013).

Notably, studies that evaluated epoetin delta produced in HT-1080 cells demonstrated differences in glycosylation compared with erythropoietin produced in CHO cells, including a lack of NGNA in the proteins (Butler & Spearman, 2014; Llop et al., 2008; Shahrokh et al., 2011). However, there were additional overlapping isoforms present in endogenous human erythropoietin isolated from urine and serum relative to epoetin delta that could not be accounted for by sialic residues alone.

Human cell lines used in the expression of proteins in clinical and preclinical development

Human cell lines have been extensively utilized for the production of products that are currently in clinical development. In addition, human cell lines are a frequently used expression system for biomedical research due to their production of human PTMs and high productivity. As productivity may vary across clonal isolates, it is important to screen for those clones with the highest yield of the therapeutic protein (Berkner, 1993).

The PER.C6 cell line was created from human embryonic retinal cells, immortalized via transfection with the adenovirus E1 gene (Havenga et al., 2008). This system was originally developed for the production of human adenovirus vectors for use in vaccine development and gene therapy (Butler & Spearman, 2014). An investment was made in this cell line in order to develop a human expression system, and now an advantage of PER.C6 is its ability to produce a high level of protein when used in the production of human IgG (Jones et al., 2003). However, this does not require amplification of the incorporated gene (Jones et al., 2003). Currently, a variety of products utilizing the PER.C6 cell line are in phase 1 or 2 clinical trials (Durocher & Butler, 2009), including the MOR103 mAb, a human IgG antibody against granulocyte macrophage colony-stimulating factor, and

CL184, an antibody against the rabies virus (Nagarajan et al., 2014).

MOR103 is in clinical development for the treatment of rheumatoid arthritis and multiple sclerosis. In a phase 1b/2a, randomized, placebo-controlled study, MOR103 was active in patients with moderately severe rheumatoid arthritis; a small number of patients developed anti-MOR103 antibodies (Behrens et al., 2014). CL184 is a combination of two mAbs, human IgG1(λ) and human IgG1(κ) (Bakker et al., 2005). In a phase 1 clinical study, it demonstrated a favorable safety profile and rapid development of rabies virus neutralizing activity, while there was no evidence to suggest the development of human anti-human antibodies (Bakker et al., 2008). CL184 has been granted FDA fast-track approval status.

Two additional cell lines are utilized by products currently in preclinical development. The CAP cell line is derived from human amniocytes obtained through amniocentesis; these cells are immortalized through an adenovirus type 5 E1 gene (Schiedner et al., 2008; Swiech et al., 2011). In addition to the ability to produce human PTMs, the primary advantage of this cell is the potential for high protein yields (Schiedner et al., 2008).

The HKB-11 cell line was created through polyethylene glycol fusion of HEK293-S and a human B-cell line (modified Burkitt lymphoma cells) (Cho et al., 2003; Durocher & Butler, 2009; Picanco-Castro et al., 2013). The advantages of this cell line include high-level protein production without the formation of aggregates, which can be a problem in other human cell lines (Picanco-Castro et al., 2013). Notably, HKB-11 has demonstrated increased expression of human FVIII compared with expression in HEK293 and BHK21 (Mei et al., 2006). Similar to other human cell lines, it has been shown to produce human glycosylation patterns including α (2,3) and α (2,6) sialic acid linkages (Picanco-Castro et al., 2013). HKB-11 has been used to produce a recombinant factor VIII protein and tissue factor (Cho et al., 2003).

A more recently developed cell line, HuH-7, originates from a human hepatocellular carcinoma (Enjolras et al., 2012). A recent study has shown that the HuH-7-CD4 clone is capable of producing recombinant human factor IX with a human glycosylation profile. PTM profiles (e.g. glycosylation, sialylation, phosphorylation and sulfation) were similar to plasma-derived and recombinant factor IX (rFIX), and were improved relative to rFIX produced in CHO cells (Enjolras et al., 2012). More recently, the HuH-7 cell line has been used to produce mutant forms of rFIX that have improved binding affinity for activated FVIII, and also demonstrated enhanced clotting activity in mice (Perot et al., 2015).

Perceptions of risks versus benefits of using human cell lines

The human-specific glycosylation pattern of the PTMs produced by human cell lines offer several advantages compared with those produced in animal cell lines. Although other mammalian cells can produce similar PTMs to human cells, most also produce α -gal and NGNA, PTMs

that are not present in the structure of human proteins (Ghaderi et al., 2012). Patterns of post-translational glycosylation are known to affect protein yield, bioactivity, and clearance (Ghaderi et al., 2010). In addition, antibodies to NGNA have been widely reported to occur in humans (Chung et al., 2008; Ghaderi et al., 2012). One study utilizing an NGNA knockout mouse model demonstrated increased immunogenicity of cetuximab due to anti-NGNA antibodies (Ghaderi et al., 2010). In addition, in patients receiving the mAb cetuximab for the treatment of colorectal or head and neck cancers, the majority of severe hypersensitivity reactions observed in clinical trials were associated with pre-existing IgE antibodies against α -gal (Chung et al., 2008; Ghaderi et al., 2012). Such antibodies may alter the efficacy or immunogenicity of proteins with the presence of non-human glycan structures. Thus, human cell lines can serve as a valuable niche expression system for biotherapeutic proteins that require human PTMs. A theoretical concern with the use of human cell lines is an increased risk of transfer of human adventitious agents, given the lack of a species barrier (Swiech et al., 2012). However, current manufacturing technologies, typically inclusive of multiple viral inactivation or clearance steps, such as nanofiltration, have largely mitigated this concern and may provide more effective viral clearance than has been observed in CHO cells (Kelley et al., 2010; McCue et al., 2014, 2015).

Future perspectives

Production of biotherapeutic proteins in human cell lines is expanding, with several products currently approved for clinical use and others in clinical development in different therapeutic areas. Advantages of human expression systems include achieving equal productivity to other mammalian cell lines and the production of proteins that lack potentially immunogenic, non-human PTMs (most notably α -gal and NGNA). In the future, with additional research investments and a continuation of the technologic advances that have already led to improvements in the use of human cell lines for protein manufacture, human cell lines will be further optimized, more sophisticated product collection strategies will be developed, and these cell lines may become one of the preferred platforms for protein biotherapeutic production.

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Declaration of interest

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