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REVIEW ARTICLE

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Escherichia coli in the production of biopharmaceuticals

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Abstract

Escherichia coli has shouldered a massive workload with the discovery of recombinant DNA technology. A new era began in the biopharmaceutical industry with the production of insulin, the first recombinant protein, in *E. coli* and its use in treating diabetes. After insulin, many biopharmaceuticals produced from *E. coli* have been approved by the US Food and Drug Administration and the European Medicines Agency to treat various human diseases. Although *E. coli* has some disadvantages, such as lack of post-translational modifications and toxicity, it is an important host with advantages such as being a well-known bacterium in recombinant protein production, cheap, simple production system, and high yield. This study examined biopharmaceuticals produced and approved in *E. coli* under the headings of peptides, hormones, enzymes, fusion proteins, antibody fragments, vaccines, and other pharmaceuticals. The topics on which these biopharmaceuticals were approved for treating human diseases, when and by which company they were produced, and their use and development in the field are included.

KEYWORDS

biopharmaceuticals, *E. coli*, recombinant DNA technology, recombinant proteins

1 | INTRODUCTION

Escherichia coli was discovered to potentially produce recombinant proteins robustly and cost-effectively with the introduction of recombinant DNA technology in the 1970s.[1] Until then, the pancreas of pigs and cows was the source of the insulin needed to treat diabetes.[2] Nonetheless, the US Food and Drug Administration (FDA) approved the first recombinant insulin produced by *E. coli* in the early 1980s, starting the next phase in the treatment of diabetes and providing a pathway for the production of more recombinant pharmaceuticals.[3] This achievement showed that even though insulin is a heterodimer that needs oxidative protein folding, it can be synthesized in *E. coli*.[4] Since then, improved recombinant pharmaceuticals such as monoclonal antibodies have started to be produced using a variety of expression hosts, including *E. coli*, yeast, filamentous fungi, insect cells, and mammalian cells.[5] Compared with other recombinant microorganisms, *E. coli* remains one of its most attractive hosts. The well-characterized genetics of this bacterium and various cloning techniques and expression systems have allowed it to be used effectively to produce a wide range of proteins.[6–8] A general method for producing recombinant biopharmaceuticals in *E. coli* is presented in Figure 1.

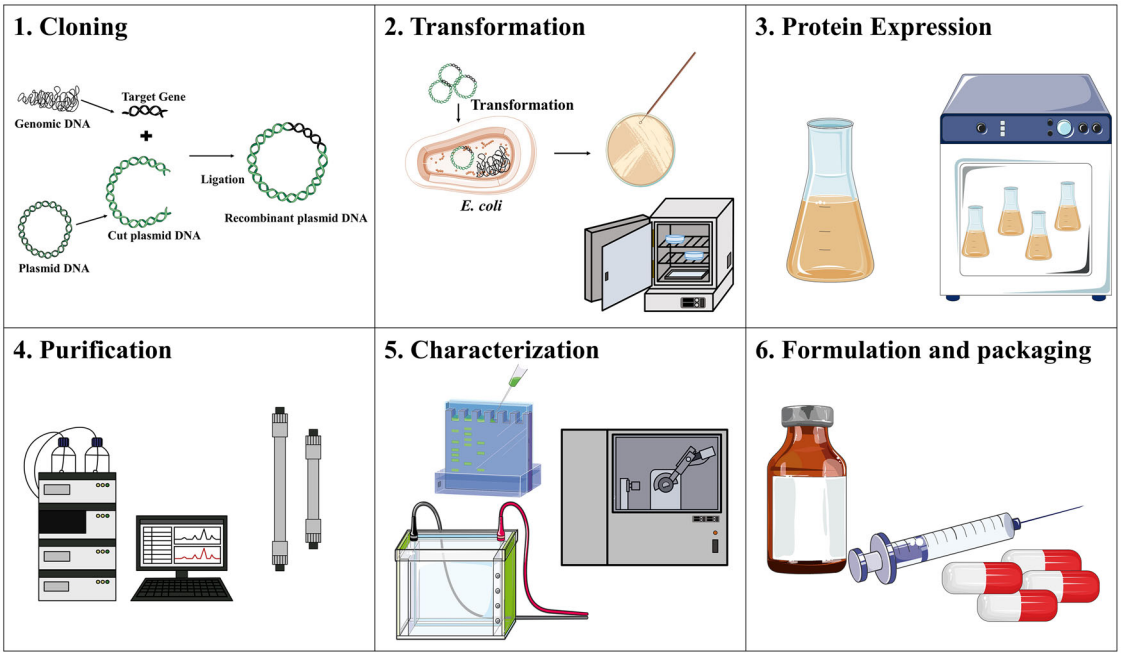

FIGURE 1 General method for producing recombinant biopharmaceuticals in *Escherichia coli*.

E. coli is favored for large-scale manufacturing due to its quick growth, inexpensive nutritional needs, simplicity in scaling up, and capacity to produce high-yield, high-quality pharmaceuticals.[9] Nevertheless, the application of *E. coli* has some restrictions. The production of some complicated proteins may be limited, for instance, by the incapacity to undertake specific posttranslational modifications and limits protein maturation and disulfide bond formation.[10] It is well-recognized that proper glycosylation and folding are crucial, particularly for complex proteins such as monoclonal antibodies.[11] Currently, the European Medicines Agency (EMA) and the US FDA have approved several recombinant therapeutic medicines produced from *E. coli* for several clinical purposes.[12] In this review, we discussed the critical biopharmaceuticals produced by *E. coli* within the categories of hormones, enzymes, peptides, vaccines, fusion proteins, antibody fragments, and other biopharmaceuticals.

2 | . *E. coli* IN THE PRODUCTION OF BIOPHARMACEUTICALS

2.1 | Hormones

Hormones represent an essential category in biopharmaceutical production, and *E. coli* has been instrumental in advances in this field. Human insulin (Humulin), the first recombinant biopharmaceutical derived from *E. coli* for use in the treatment of diabetes mellitus, was produced by the Eli Lilly and was approved in the United States by the US FDA in 1982.[13] Later, Lantus, a long-acting insulin glargine molecule, was produced in *E. coli* by the Sanofi-Aventis and was approved in the United States and EU in 2000.[4,14] Insulin glargine is a human insulin analog produced recombinantly and binds to insulin receptors. In later years, many insulin analogs were produced recombinantly in *E. coli*. One of these, Lyumjev (insulin lispro), rh rapid-acting insulin analog, is produced in *E. coli* by the company Eli Lilly and was approved by the FDA and EMA in 2020 for adults and children (1 year and above) with diabetes mellitus.[15,16]

Growth hormones are among the earliest biopharmaceuticals that can be produced in *E. coli*. Somatropin (Humatrope), a growth hormone, was produced recombinantly in *E. coli* for use in the treatment of growth hormone deficiency (GHD) in children by the company Eli Lilly and was approved by the FDA in 1987.[4] Sogroya (Somapacitan-beco), a long-acting growth hormone, was produced by Novo Nordisk in *E. coli* using recombinant DNA technology. Modifying the somapacitan molecule allowed it to bind reversibly to albumin in the blood. In this way, the elimination of somapacitan from the body was slowed down and its duration of action was prolonged. It was approved by the FDA in 2020 and EMA in 2021 for use in adult and pediatric patients (2.5 years and older).[17,18] In a clinical study, the use of somapacitan once a week in adults with GHD was effective and well tolerated.[19] In another phase III study, weekly use of somapacitane was shown to have similar efficacy and safety compared to daily GH in

children with GHD.[20] Skytrofa (Lonapegsomatropin-tcgd), a long-acting growth hormone, was produced by Ascendis Pharma using recombinant DNA technology in *E. coli*. In 2021, it was approved by the FDA for treating pediatric patients (1 year and older) with growth deficiency due to insufficient secretion of growth hormone.[21]

Recombinant interferon α-2b (Intron A) was produced for the treatment of genital warts, cancer, and hepatitis by Merck Sharp & Dohme and was approved by the FDA in 1986. Later, modified variants of the interferon molecule (interferon alpha-2a, alpha-2b, alpha-1a, alpha-1b) were developed for the treatment of various patients (kidney cancer, renal cell carcinoma, non-Hodgkin lymphoma, cutaneous T-cell lymphoma, chronic myelocytic leukemia, melanoma).[4,22,23] Besremi (ropeginterferon alfa-2b-njft) was produced by PharmaEssentia using recombinant DNA techniques in *E. coli* and was approved by the FDA in 2021 for the treatment of polycythemia vera (PV) (a rare blood disorder characterized by an increase in blood cells) in adults.[24] After 3 years of treatment in patients with PV, ropeginterferon alfa-2b is effective and safe, with a better response to standard treatment.[25] There are also studies on the efficacy and safety of ropeginterferon alfa-2b in pregnant women with PV. However, these studies emphasized the need for more case studies.[26,27]

Forsteo (teriparatide), a human parathyroid hormone analog, was produced in *E. coli* by Eli Lilly and was approved by the EMA in 2003 for the treatment of osteoporosis.[28] In later years, many drugs biosimilar to Forsteo (Movymia, Terrosa, and Sondelbay) were produced in *E. coli*. One of these, Sondelbay (teriparatide), a human parathyroid hormone fragment, was produced by Accord Healthcare using recombinant DNA techniques in *E. coli* and was approved by the EMA for the treatment of osteoporosis in 2022.[29] Natpara (parathyroid hormone) was produced recombinantly in *E. coli* by NPS Pharmaceuticals. It was approved by the FDA in 2015 to control calcium deficiency (hypocalcemia) in patients with hypoparathyroidism.[30] Clinical studies with recombinant parathyroid hormone therapy lasting 5 years[31] and 12 months[32] showed that it improved biochemical parameters in patients with hypoparathyroidism and also confirmed the efficacy and safety of the treatment.[31,32] Additionally, Natpar, a recombinant human parathyroid hormone, was produced in *E. coli* by Takeda Pharmaceuticals and was approved by the EMA in 2017 for treating hypoparathyroidism.[33] Hormones produced using recombinant DNA technology in *E. coli* and approved by the FDA and EMA are described in Table 1.

Recent studies are aimed at investigating the production of new hormone analogs and modifications in *E. coli*. Clinical studies are underway for ultrafast-acting insulin analogs such as VIAject/Linjeta, Biodel insulin (BIOD-531), BioChaperone Lispro, and LY900014. These formulations outperform conventional insulins such as Humalog, NovoLog, and Fiasp regarding speed of action and glucose control. Another strategy is to combine insulin analogs with recombinant human hyaluronidase. Insulin–hyaluronidase combinations were found to cause faster onset and lower postprandial hyperglycemia in healthy volunteers and type 1 diabetic patients. Companies are also undertaking tests on novel insulins for type 2 diabetes. For example, Eli Lilly is testing LY3209590, a designed insulin linked to an antibody Fc domain for a long-acting basal profile, compared with glargine insulins.[34]

2.2 | Enzymes

Enzymes, which play an important role in various biological processes, are another group of therapeutic proteins that can be produced in *E. coli*. Pegloticase "recombinant human urate oxidase" (Krystexxa) was approved in the United States in 2010. Pegloticase was produced recombinantly in *E. coli* to treat chronic gout in adult patients by Savient Pharmaceuticals.[4] Furthermore, a recent study in patients with uncontrolled gout showed that combining pegloticase with methotrexate (MTX) induced a higher treatment response and lower immunogenicity.[35]

Voraxaze (glucarpidase), a carboxypeptidase enzyme, was produced by BTG International to reduce the amount of plasma MTX in patients with impaired renal function. Glucarpidase converts MTX to glutamate and 2,4-diamino-N(10)-methylpteroic acid. The glucarpidase produced recombinantly in *E. coli* was approved for medical use by the FDA in 2012 and EMA in 2022.[36–38]

Calaspargase pegol (Asparlas), an asparaginase enzyme, was produced by the Servier Pharmaceuticals in *E. coli* to treat acute lymphoblastic leukemia (ALL) patients aged 1 month to 21 years. Calaspargase pegol, produced using recombinant DNA technology, was approved by the FDA in 2018.[39] In a study of ALL patients, calaspargase pegol had more extended asparaginase activity than pegaspargase, an asparaginase enzyme naturally derived from *E. coli*.[40]

Pegvaliase-pqpz (Palynziq) was produced in *E. coli* by the Biomarin. In 2018, this enzyme, which reduces the amount of phenylalanine in the blood, was approved by the FDA for use in adults with phenylketonuria.[41] In a study, case experiences of patients with phenylketonuria using pegvaliase were presented.[42] In addition, a clinical trial was recently conducted in patients treated with pegvaliase on treatment course, treatment discontinuation, and dose adjustment.[43]

Revcovi (Elapegademase-lvlr) was produced recombinantly in *E. coli* by the Leadiant Biosciences. This enzyme

TABLE 1 Approved recombinant hormones produced in *Escherichia coli* and their mechanism of action.

Trade name	Ingredient	Therapeutic indication	Mechanism of action
Humulin	Human insulin	Diabetes mellitus	It binds to insulin receptors
Lyumjev	Insulin lispro, rapid-acting insulin	Diabetes mellitus	It binds to insulin receptors
Humatrope	Somatropin	Growth hormone deficiency	It binds to the human GHR, which is expressed by relevant cells in the liver and cartilage
Sogroya	Somapacitan-beco, a long-acting growth hormone	Growth hormone deficiency	It binds to the GHR
Skytrofa	Lonapegsomatropin-tcgd, a long-acting growth hormone	Growth hormone deficiency	It binds to the GHR
Intron A	Interferon α-2b	Genital warts, cancer, hepatitis B and C infections	It binds to type I interferon receptors (IFNAR1 and IFNAR2c) that activate Jak tyrosine kinases (Jak1 and Tyk2)
Besremi	Ropeginterferon alfa-2b-njft	Polycythemia vera	It is known to bind to the interferon-alpha/beta receptor and activate downstream JAK/STAT signaling
Forsteo	Teriparatide, a human parathyroid hormone analog	Osteoporosis	It binds to PTH type 1 receptors expressed on various cells (osteoblasts, osteocytes, and renal tubular cells)
Sondelbay	Teriparatide	Osteoporosis	It has a similar mechanism of action to Forsteo
Natpara	Parathyroid hormone	Hypocalcemia	It increases the amount of serum calcium by ensuring calcium flow from bones, kidneys, and intestines
Natpar	Parathyroid hormone	Hypoparathyroidism	A recombinant human parathyroid hormone

Abbreviation: GHR, growth hormone receptor; PTH, parathyroid hormone; JAK/STAT, janus kinase/signal transducer and activator of transcription.

was approved by the FDA in 2018 to treat adenosine deaminase severe combined immunodeficiency (ADA-SCID) in adult and pediatric patients.[44] Clinical studies with ADA-SCID patients have shown the efficacy and safety of elapegademase therapy[45] and the use of Revcovi to effectively treat ADA-SCID.[46] Enzymes produced using recombinant DNA technology in *E. coli* approved by the FDA and EMA are presented in Table 2.

In recent years, enzymes produced in *E. coli* have been evaluated in preclinical research to develop new enzyme candidates for various clinical applications, aiming to enhance the effectiveness of existing treatments and minimize side effects.[47]

2.3 | Peptides

Peptides constitute a fundamental category within biopharmaceuticals, encompassing a wide range of therapeutic agents with important clinical applications. This section focuses on peptides that can be produced in *E. coli* are focused on. Natrecor (nesiritide), a recombinant human natriuretic peptide, was produced in *E. coli* by Johnson & Johnson/Scios. In 2001, it was approved by the FDA for the treatment of patients with acute decompensated heart failure.[48]

The FDA approved Increlex (mecasermin), an insulin-like growth factor-1 (IGF-1) produced by recombinant DNA technology in *E. coli*, in 2005 for the treatment of growth failure in children with severe primary IGF-1 deficiency or growth hormone gene deletion. Clinical studies have shown that mecasermin effectively promotes linear growth in these patients.[49]

Fortical (calcitonin), a hormone used to treat osteoporosis and other bone conditions, is also produced in *E. coli*. This peptide, produced by Unigene Laboratories, was approved by the FDA in 2005. It has been shown to effectively reduce bone resorption and increase bone mineral density in patients with osteoporosis.[50]

Gattex (teduglutide), manufactured by NPS Pharmaceuticals, was approved by the FDA in 2012 for the treatment of short bowel syndrome. Studies have shown that teduglutide treatment significantly improves intestinal absorption and reduces the need for parenteral nutrition in patients with short bowel syndrome.[51]

Voxzogo (Vosoritide), a natriuretic peptide, produced by Biomarin Pharma in *E. coli*. In 2021, it was approved by the FDA and EMA for the treatment of achondroplasia in pediatric patients.[52,53] In a phase III study in children (5 and <18 years old) with achondroplasia, findings were presented that 52 weeks of vosoritide treatment is an important treatment option.[54] Subsequently, vosoritide treatment was reported to be safe and sustained growth-promoting in children with achondroplasia.[55]

TABLE 2 Approved recombinant enzymes produced in *Escherichia coli* and their mechanism of action.

Trade name	Ingredient	Therapeutic indication	Mechanism of action
Krystexxa	Pegloticase	Chronic gout	It converts uric acid into allantoin, an inert and highly water-soluble metabolite
Voraxaze	Glucarpidase	Kidney dysfunction	It converts methotrexate to glutamate and 2,4-diamino-N(10)-methylpteroic acid
Asparlas	Calaspargase pegol	Acute lymphoblastic leukemia	It catalyzes the conversion of the amino acid L-asparagine into aspartic acid and ammonia
Palynziq	Pegvaliase	Phenylketonuria	It reduces blood phenylalanine concentrations by converting phenylalanine to ammonia and trans-cinnamic acid
Revcovi	Elapegademase-lvlr	Adenosine deaminase severe combined immunodeficiency	It eliminates deoxyadenosine (a metabolite formed when DNA is fragmented) by converting it into deoxyinosine

Oxervate (cenegermin), a nerve growth factor, was produced by Dompé U.S. using recombinant DNA techniques in *E. coli*. It was approved by the EMA in 2017 and the FDA in 2018 for treating neurotrophic keratitis (an eye condition).[56,57] A study reported that cenegermin treatment effectively improved visual acuity, corneal epithelium, and corneal sensation in limbal stem cell deficiency and neurotrophic keratopathy patients, but more comprehensive studies are needed.[58] However, another study reported that topical cenegermin treatment may cause drug precipitation on both the lens and ocular surface when used with a bandage contact lens.[59] Another peptide produced in *E. coli* is Idefirix (imlifidase). It is a drug used to prevent the body from rejecting a transplanted kidney, was produced by Hansa Biopharma using recombinant DNA techniques in *E. coli* and was approved for use by the EMA in 2020.[60] Studies have reported that imlifidase treatment may be an effective and promising approach to desensitize kidney transplant recipients, but more clinical data are needed regarding its use and application.[61,62] Peptides produced using recombinant DNA technology in *E. coli* approved by the FDA and EMA are given in Table 3.

The production of biopharmaceutical peptides in *E. coli* is beneficial for producing long or complex peptides that may be difficult to synthesize chemically. Peptide-based biopharmaceuticals produced in *E. coli* include innovative preclinical approaches to target various diseases such as cardiovascular, metabolic, infection, cancer and autoimmune.[51]

2.4 | Vaccines

The development and production of vaccines represent a critical frontier in the field of biopharmaceuticals, protecting public health and preventing a wide range of infectious diseases. In this context, *E. coli* is used as a versatile and valuable tool to produce vaccines. Hecolin (Innovax) was the first virus-like particle-based vaccine against hepatitis E virus produced in *E. coli* bacteria and was licensed by the China Food and Drug Administration in 2011. In China, phase III studies in adults aged 16–65 reported that Hecolin has effectively prevented hepatitis E infection.[63,64] Human papillomavirus (HPV) L1 VLPs vaccine (Cecolin; Innovax) was produced in *E. coli* bacteria and was approved for market in China. In a phase III study with the HPV vaccine, it was effective against genital lesions and persistent infections.[65]

The Strangvac vaccine, containing *Streptococcus equi* CCE, mEq84, and IdeE antigens, was produced recombinantly in *E. coli* by Intervacc against *S. equi* bacteria that caused strangles in horses and was approved by the EMA in 2021. One study reported that vaccination with Strang-

TABLE 3 Approved recombinant peptides produced in *Escherichia coli* and their mechanism of action.

Trade name	Ingredient	Therapeutic indication	Mechanism of action
Voxzogo	Vosoritide	Achondroplasia	It binds to NPR-B and inhibits the MAPK/ERK pathway through RAF-1 inhibition. As a result, it suppresses excessive activation of the FGFR3 signaling pathway and promotes bone growth
Oxervate	Cenegermin	Neurotrophic keratitis (an eye condition)	It binds to nerve growth factor receptors (high affinity TrkA and low affinity p75NTR) found in the eye
Idefirix	İmlifidase	Preventing rejection of a transplanted kidney	It breaks down immunoglobulin G antibodies and allows the transplant to be successful
Increlex	Mecasermin	Growth failure (severe primary IGF-1 deficiency or growth hormone gene deletion)	It binds to IGF-1 receptors and promotes cell growth and proliferation
Fortical	Calcitonin	Osteoporosis and other bone conditions	It helps increase bone density by binding to the calcitonin receptor found in osteoclasts
Gattex	Teduglutide	Short bowel syndrome	It increases nutrient absorption by binding to glucagon-like peptide-2 receptors in intestinal cells

Abbreviation: IGF-1, insulin-like growth factor-1.

vac is safe and protects against equine strangles caused by *S. equi*.[66,67]

The Prevnar 13 vaccine for the 13 serotypes of *Streptococcus pneumoniae* that causes pneumoniae was produced by Wyeth Pharmaceuticals using *Corynebacterium* bacteria and was approved by the FDA in 2010.[68] Also, in 2016, Pfizer Inc. announced that Prevnar 13 is the only pneumococcal vaccine approved for use in people of all ages. Later, pneumococcal conjugate vaccine (protein polysaccharide) was produced recombinantly in *E. coli*, and it was shown that it could be used to prevent pneumococcal infections when compared to Prevnar13 vaccine.[68–70] Furthermore, a study was carried out to improve the yield of pneumococcal bioconjugate vaccines produced in *E. coli* and reduce endotoxin formation.[71]

Trumenba (a serogroup B meningococcal vaccine), also known as MenB-FHbp, was produced by Wyeth Pharmaceuticals (a subsidiary of Pfizer) in *E. coli*. Trumenba consists of two recombinant factor H binding protein (FHbp) variants of *Neisseria meningitidis* serogroup B.[72] It was approved by the FDA in 2014 and the EMA in 2017 for use in individuals aged 10–25 years to prevent disease caused by *N. meningitidis* serogroup B.[73,74] A recent phase III study demonstrated that MenB-FHbp administered in adolescents and young adults is well tolerated, safe, and increases the protective antibody response.[75] The vaccination study conducted with Trumenba between 2018 and 2020 reported no deaths or impairment in adverse events after immunization.[76] Also, another meningococcal vaccine, Bexsero (containing fragments of *N. meningitidis* group B bacteria), also known as 4CMenB, was produced by GlaxoSmithKline in *E. coli*. Bexsero was approved by the FDA in 2015 for patients aged 10–25 years and by the EMA in 2013 for individuals 2 months and older.[77,78] In a study conducted in the United States on individuals aged 10–25 years, it was reported that the Bexsero vaccine did not pose a new safety concern as a result of the analysis of data obtained from Vaccine Adverse Event Reporting System, which included the next 4-year period after its approval, and most of the reports had non-serious side effects.[79] In light of 9 years of data, it has been reported that the use of 4CMenB does not pose significant safety problems in various age groups. However, transient fever may occur in infants after vaccination.[80] A recent study reported that using 4CMenB effectively prevented invasive meningococcal disease in children under 5 years of age.[81]

Plasmodium vivax antigens produced in *E. coli* bacteria have been shown to increase cellular and humoral immune responses in mice and may be potential vaccine candidates against *P. vivax*-based malaria.[82] In addition, one of the first animal vaccines, p45 feline leukemia virus envelope antigen (Leucogen; Virbac) was produced in *E. coli* using recombinant DNA techniques and was approved by the EMA in 2009.[83,84] Letifend (LETI Pharma), a protein Q-based vaccine from *Leishmania infantum* for the treatment of canine leishmaniasis, was produced recombinantly in *E. coli* and was approved by the EMA in 2016.[85] Recombinant vaccines produced from *E. coli* and approved for use in humans or animals are listed in Table 4.

E. coli continues to be utilized for the production of various vaccine antigens. For instance, in 1998, the FDA approved a Lyme disease vaccine that contained recombinantly produced outer surface lipoprotein OspA from *Borrelia burgdorferi*. However, this vaccine was withdrawn from the market in 2002 due to concerns regarding serious side effects. An improved version, VLA15, also produced in *E. coli*, is currently undergoing phase III clinical trials.[86]

2.5 | Fusion proteins and antibody fragments

The production of fusion proteins and antibody fragments in *E. coli* has emerged as an essential strategy in developing biopharmaceuticals and offers specific solutions for various therapeutic applications. Ranibizumab molecule (Lucentis) was produced by Genentech/Roche in *E. coli* for the treatment of age-related macular degeneration (AMD) and approved in the United States in 2006.[87] A monoclonal antibody fragment called Ranibizumab is directed against all vascular endothelial growth factor A (VEGF-A) isoforms. This binding prevents dimerization with receptors such as VEGFR1 and VEGFR2 on cell surfaces. In this way, it reduces angiogenesis, endothelial cell proliferation, and vascular leakage.[88] Ranibizumab is an important option for the treatment of diabetic macular edema and leads to improvement in proliferative diabetic retinopathy.[89] In subsequent years, Byooviz, a biosimilar to Lucentis produced in *E. coli*, was approved by the FDA and EMA in 2021 for the treatment of AMD, retinal vein occlusion following macular edema, and myopic choroidal neovascularization.[66,90] Susvimo (ranibizumab), a VEGF inhibitor, is produced by Genentech in *E. coli* and was approved by the FDA for AMD in 2021.[91]

Trastuzumab (Herceptin) monoclonal antibody was produced by Genentech/Roche in Chinese hamster ovary cells and was approved for medical use by the FDA in 1998.[92] However, studies have been carried out on the production of this molecule in *E. coli* bacteria. The human epidermal growth receptor 2 (HER2) protein, which is located on the surface of cells, has a crucial function in regulating cell growth and communication. This receptor was found to be highly expressed in approximately one-fourth of breast cancer patients. Trastuzumab (anti-HER2) targets the HER2 receptor. In this way, it has been reported that it slows the development of cancers due to HER2 signaling.[93–95]

TABLE 4 Approved recombinant vaccines produced in *Escherichia coli*.

Application type	Trade name	Ingredient	Therapeutic indication	Year of first approval
Human	Hecolin	HEV ORF2 protein	Hepatitis E infection	2012[a]
	Bexsero	Fragments of *Neisseria meningitidis* group B bacteria	İnvasive meningococcal disease	2013 (EMA)
	Trumenba	2 FHbp	*N. meningitidis* serogrup B	2014 (FDA)
	Cecolin	HPV L1 capsid protein	Genital lesions and persistent infections	2020[b]
Veterinary	Leucogen	p45 FeLV envelope antigen	Feline leukemia	2009 (EMA)
	Letifend	Protein Q	Canine leishmaniasis	2016 (EMA)
	Strangvac	*Streptococcus equi* antigens: CCE, mEq84, IdeE	Protection against equine strangles caused by *S. equi*	2021 (EMA)

Abbreviations: EMA, European Medicines Agency; FeLV, feline leukemia virus; HPV, human papillomavirus.
[a]Approved by the China Food and Drug Administration.
[b]It completed its phase III studies in 2020 and was pre-qualified by WHO in 2021.

Certolizumab pegol (Cimzia) monoclonal antibody fragment was produced in *E. coli* bacteria by UCB Pharma to treat Crohn's disease and rheumatoid arthritis (RA). It is also known as a tumor necrosis factor (TNF) inhibitor. This Fab (antigen binding fragment) molecule, which is produced in soluble form was approved by the FDA in 2008.[96]

Romiplostim (Nplate) fusion protein was produced by Amgen in *E. coli* bacteria for use in treating immune thrombocytopenic purpura disease. This protein, which is produced in the form of inclusion bodies in the cytoplasm, was approved by the FDA in 2008 and EMA in 2009.[97,98]

Moxetumomab pasudotox (Lumoxiti) fusion protein produced by AstraZeneca was approved by the FDA in 2018. This recombinant protein, which specifically binds to the CD22 receptor on the surface of B cells, was produced in *E. coli* to treat hairy cell leukemia.[99,100]

Caplacizumab (Cablivi) antibody produced by the Ablynx/Sanofi for use in the treatment of acquired thrombotic thrombocytopenic purpura was approved by the FDA in 2019. Produced extracellularly and in soluble form in *E. coli*, this monoclonal antibody targets von Willebrand factor.[101–103] The EMA also approved it in 2018 for use in children (over 12 years of age) and adults.[104]

Brolucizumab (Beovu) is a monoclonal single-chain variable fragment produced by Novartis for use in the treatment of AMD. This molecule binds to the VEGF receptor. This monoclonal antibody fragment produced using recombinant DNA techniques in *E. coli* bacteria was approved by the FDA in 2019.[105] Another monoclonal antibody fragment is Bentracimab (PB2452).[106] Bentracimab reverses the activity of ticagrelor (Brilinta), an anti-thrombotic drug. Bentracimab has been shown to be effective and safe in a study of healthy volunteers.[107,108] Bentracimab's phase III Rapid and Sustained Reversal of Ticagrelor—Intervention Trial clinical trials are ongoing.[109] In addition, with an agreement made in 2023, it was announced that SFJ Pharmaceuticals will be responsible for the ongoing clinical studies of Bentracimab.

Fusion proteins and antibody fragments produced in *E. coli* are also used in cancer treatment. Tebentafusp (Kimmtrak) fusion protein produced in *E. coli* for use in the treatment of metastatic uveal melanoma by Immunocore was approved by the FDA and EMA in 2022.[110,111] Oportuzumab monatox (Vicineum) fusion protein was produced in *E. coli* for use in bladder cancer by Sesen Bio and was developed up to phase III studies. Produced using recombinant DNA techniques, this protein in soluble form targets the epithelial cell adhesion molecule antigen, which is expressed in high amounts in most solid tumors. However, on July 18, 2022, the company announced that it had decided to stop further development of Vicineum in the United States.[112–114] Recombinant antibodies and cer-

TABLE 5 Approved recombinant antibodies and fusion proteins produced in *Escherichia coli*.

Trade name	Ingredient	Therapeutic indication	Mechanism of action
Lucentis	Ranibizumab molecule	AMD	It binds to the receptor binding domain of VEGF-A, preventing it from binding to VEGFR1 and VEGFR2 receptors.
Byooviz	A biosimilar ranibizumab molecule	AMD, retinal vein occlusion following macular edema, and myopic choroidal neovascularization	It has a similar mechanism of action to Lucentis.
Susvimo	Ranibizumab	Neovascular (wet) AMD	It targets the VEGF-A protein and blocks its action.
Herceptin	Trastuzumab	HER2-positive breast cancer and gastric cancer	It targets the HER2 receptor and slows the development of cancers that develop due to HER2 signaling.
Cimzia	Certolizumab pegol	Crohn's disease and rheumatoid arthritis	It binds to the TNF-α molecule and inhibits its activity.
Nplate	Romiplostim	Immune thrombocytopenic purpura disease	It binds to the TPO receptor, activates it and increases the number of platelets.
Lumoxiti	Moxetumomab pasudotox	HCL	It binds to the CD22 receptor on the surface of HCL cells. The toxin portion of this drug taken into the cell inhibits protein synthesis and causes cell death.
Cablivi	Caplacizumab	Acquired thrombotic thrombocytopenic purpura	It binds to the vWF and prevents platelet aggregation by inhibiting the interaction between vWF and platelets.
Beovu	Brolucizumab	AMD	It binds to VEGF-A isoforms (VEGF110, VEGF121, and VEGF165) and inhibits their biological activities.
Kimmtrak	Tebentafusp	Metastatic uveal melanoma	It causes the death of cancer cells by binding to CD3 protein (on the surface of T cells) and gp100 antigen (on the surface of melanoma cells).

Abbreviations: AMD, age-related macular degeneration; HCL, hairy cell leukemia; mCNV, myopic choroidal neovascularization; TNF-α, tumor necrosis factor-alpha; VEGF, vascular endothelial growth factor; vWF, von Willebrand factor.

tain fusion proteins produced in *E. coli* and approved for treating related diseases are listed in Table 5.

In recent years, Genentech and Sutro Biopharma have progressed clinical studies with *E. coli*-produced aglycosylated antibodies. Genentech explored the anti-MET antibody onartuzumab (OA-5D5, RG3638) in phase II and phase III studies for several advanced solid cancers; however, the phase III trials stopped due to a lack of substantial clinical effectiveness. Furthermore, Genentech's full-length IgG4 BsAb BITS7201A (RG7990) was tested in phase I research for asthma therapy and tolerated well. Sutro Biopharma's three monoclonal antibody–drug conjugates and one bispecific monoclonal antibody–drug conjugate are now in phase I studies. STRO-001 is being evaluated for multiple myeloma and B-cell malignancies; STRO-002 for FolRα-overexpressing platinum-resistant ovarian cancer and other solid tumors; ispectamab debotansine (CC-99712, BMS-986352) for multiple myeloma; and M1231 for a non-small cell lung cancer and esophageal cancers. All of these are in phase I trials.[106]

2.6 | Other biopharmaceuticals

The scope of biopharmaceuticals produced in *E. coli* extends beyond vaccines, antibodies, enzymes, fusion proteins, antibody fragments and hormones and includes various therapeutic agents with unique applications. In this section, other biopharmaceuticals produced in *E. coli* are emphasized. The filgrastim molecule (Neupogen) is a recombinant human granulocyte colony-stimulating factor produced by the Amgen of *E. coli* for the treatment of neutropenia and was approved in the United States in 1991. In 2002, the same company developed the pegylated form of the filgrastim molecule (Neulasta) for use in cancer-related infections.[4,115] In the following years, many biosimilars of Neulasta (pegfilgrastim) were produced. Stimufend (pegfilgrastim-fpgk), recently produced by the Fresenius Kabi for the treatment of neutropenia and was approved by the FDA in 2022, is one of them.[116,117]

Beromun (tasonermin), a TNF-α, was produced recombinantly in *E. coli* for soft sarcoma treatment by the Boehringer Ingelheim and was approved by the EMA in 1999.[4]

Anakinra molecule (Kineret), an interleukin-1 receptor antagonist, was produced recombinantly in *E. coli* by Swedish Orphan Biovitrum for use in the treatment of RA and was approved in the United States in 2001.[118]

Meterleptin (Myalept), a leptin analog, was produced by Aegerion Pharmaceuticals recombinantly for *E. coli*. It was approved by the FDA in 2014 for the treatment of leptin deficiency complications in patients with acquired or congenital generalized lipodystrophy.[39] Although lipodystrophy is a rare disease and limited treatment options have limited research on the effects of meterleptin, a study has shown that it significantly improved the quality of life of people who received meterleptin treatment.[36]

Tagraxofusp-erzs (Elzonris), a cytokine, was produced by Stemline Therapeutics in *E. coli* using recombinant DNA technology. It was approved by the FDA in 2018 and EMA in 2021 for use in treating blastic plasmacytoid dendritic cell neoplasms in pediatric and adult patients.[119]

3 | CONCLUSIONS

E. coli has advantages such as good knowledge of its genetic structure, easy application of cloning techniques, rapid production, simple nutrient medium requirement, and ease of production on a large scale. However, it has some limitations (post-translational modifications and restrictions on disulfide bond formation) for producing complex proteins. Nevertheless, *E. coli* remains an attractive host for the production of biopharmaceuticals.

This review examined biopharmaceuticals produced in *E. coli* that the FDA and EMA approved under the headings of hormones, enzymes, vaccines, fusion proteins, and antibody fragments. Biopharmaceuticals produced and approved in *E. coli* until today, starting from insulin, are discussed chronologically. Information about which diseases these biopharmaceuticals are used to treat is included. Additionally, ongoing studies in relevant field for the treatment of some diseases are mentioned.

As a result, *E. coli* remains a versatile and critical host for many biopharmaceuticals that have been and will be produced to treat various diseases. In the future, with studies and developments in biotechnology, limitations in *E. coli* recombinant protein production can be eliminated and its effectiveness can be increased. Thus, biopharmaceuticals produced from *E. coli* may have a strong potential for treating diseases and an essential share in the pharmaceutical industry.

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