Infliximab: A Comprehensive Review of Production Processes, Therapeutic Mechanisms, and Biomanufacturing Applications

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***Abstract*- Taking advantage of its monoclonal antibody structure, infliximab has revolutionized managing autoimmune diseases, mainly Crohn's disease, rheumatoid arthritis, ankylosing spondylitis and ulcerative colitis. Binds to TNF-α in bloodstream and cell membranes, preventing this 'inflammation maker' from causing damage. By restraining cytokine signaling, causing cell death in TNF-expressing cells and influencing immune response, it shows clinical activity. It describes the methods used to make Infliximab in biomanufacturing. Companies use recombinant DNA and gene-engineered Chinese Hamster Ovary (CHO) cells or mammalian expression systems for upstream process. Monoclonal antibodies are made by growing cells in special environments. After initial steps, product is refined with chromatography methods and inactivated viruses to ensure purity, strength and safety. Quality checks at every stage prevent batch-to-batch variances and guideline deviations. It clarifies this drug's role in biosimilar development. Since Infliximab is chemically complex and significant for use, companies like Inflectra and Renflexis have used it as reference for biosimilars. Comparability studies in analysis, function and clinical areas are vital to meet FDA or EMA regulatory needs. Pharmacovigilance is key in monitoring reactions and risks after immunized products reach market. The review addresses contemporary biomanufacturing methods, including HEK293 and CHOK1 cells, continuous bioprocesses and AI and digital twins to monitor production. These improvements enhance efficiency, scalability, and cost-effectiveness while producing high-quality therapeutics.**

***Index Terms*- Infliximab, Autoimmune Diseases, Monoclonal Antibodies, Biomanufacturing Applications, Therapeutics**

I. INTRODUCTION

Treatment of seditious and autoimmune diseases has greatly improved because of monoclonal antibodies (mAbs). Infliximab was among the initial biologic drugs approved to be used in patients; it consists of human constant parts and murine variable parts [1]. The branded name for infliximab which was developed by Centocor (now Janssen Biotech), is Remicade. Its manufacture also informs the modern biopharmaceutical sector concerning advanced cell culture, purification and monitoring quality [2,10]. This drug is a chimeric monoclonal antibody that identifies and binds to tumor necrosis factor-alpha (TNF-α) found in the inflammatory process. With both human and murine parts, infliximab stops TNF-α from interacting with its receptors on cells, binding to both kinds of TNF-α found in the body [4,5] As a result, this action blocks signaling in the body that can lead to inflammation and harm to tissues [18].

It was first designed to treat autoimmune disorders like rheumatoid arthritis and Crohn's disease, but its use has grown into oncology. Findings show that TNF-α helps cancers grow by triggering inflammation, forming extra blood vessels and increasing cell multiplication [3,8] TNF-α which plays a key part in the tumor microenvironment, is blocked by infliximab, suggesting it may be effective as a cancer treatment [9]. Approved for first use in medicine, infliximab helped shape the future of biomanufacturing as a monoclonal antibody therapy. Thanks to DNA technology in mammalian cell lines, the drug's development made it easier to mass-produce biologic medicine [10]. Because infliximab worked so well, new kinds of similar drugs called biosimilars were made to give people other, less expensive choices for treatment [7,15]. This paper aims to outline infliximab, discussing its function, production, possible adverse effects and its growing significance in fighting cancer and new biological therapies [4,2] Dosage and the effects of using infliximab to treat cancer will receive specific attention, along with both its opportunities and disadvantages in oncology [9,3].

II. MOLECULAR STRUCTURE AND CHARACTERISTICS OF INFLIXIMAB

The molecular design of Infliximab begins with the immunization of mice using recombinant human tumor necrosis factor-alpha (TNF-α) is used to encourage the immune system to work properly. By fusing the B-cells that generate anti-TNF-α antibodies from mice with myeloma cells, then obtain hybridomas [16]. Then confirm that the selected hybridoma cells make high-affinity antibodies that bind to and disable TNF-α.

After spotting a useful clone, the information containing its variable heavy (VH) and light (VL) regions is taken from mRNA using reverse transcription and is further amplified and sequenced by PCR [17]. When the V regions of mice are fused with human constant regions, IgG1 for the heavy chain and kappa or lambda for the light chain, a chimeric antibody is produced. As a result, the mouse antibody parts recognize TNF-α exactly [2], yet show reduced foreignness and allow human proteins to trigger the immune response. After making the chimeric antibody genes, they are cloned into mammalian expression vectors and put into host cells such as CHO or HEK293 cells, for production. Following purification, Infliximab is analyzed using binding (ELISA or SPR) and functional tests to make sure it can attach to and block human TNF-α effectively [4]. Before starting clinical trials, more stability, pharmacokinetic and in vivo research is done to prove the item's safety and effectiveness. Because of molecular engineering, infliximab becomes a chimeric monoclonal antibody that fights autoimmune diseases by blocking the actions of TNF-α. It is an antibody with parts of the mouse and human sections worked together, aiming at targeting human TNF-α. With this design, the original recognition of the mouse antibody is kept while reducing risks to immunogenicity and improving its length of action in the body, required for using the antibody as a therapeutic treatment in multiple chronic inflammatory disorders including rheumatoid arthritis and Crohn's disease [4,2]

At the beginning, recombinant human TNF-α is immunized into mice which triggers an immune system reaction to produce antibodies targeting TNF-α. B-cells taken from a patient are fused with cancer cells to make hybridomas that make one kind of antibody [16]. Using ELISA and neutralization tests, those hybridomas producing good-quality antibodies to TNF-α are chosen. From the selected clone, you take out the DNA for the variable parts of both the heavy chain (VH) and the light chain (VL) and put them through sequencing [17]. Each variable region in the mouse is then added to human IgG1 constant regions to form a chimeric antibody. There are many reasons why the constant region of human IgG1 is selected. First, because it is human-derived, it is less likely to cause a patient's immune system to develop anti-drug antibodies. It is important since murine antibodies often cause the human body to get rid of them quickly and reduce their effect [2,22]. Secondly, both effector functions ADCC and CDC are made possible by the Fc region on human IgG1 antibodies, although they do not play a critical role in Infliximab's activity [4]. IgG1 Fc region also engages the neonatal Fc receptor (FcRn) which is important for preventing its lysosomal breakdown and keeps its life span at about 8–10 days in humans [23].

The two chimeric VH and VL gene segments from mouse are joined to human CH and CL gene segments and finally inserted into mammalian expression systems. Expression of these vectors is carried out in Chinese Hamster Ovary (CHO) or HEK293 cells. Following the manufacturing and purification process, the antibody is tested in many different ways such as for its strength in binding, ability to block infection, physical stability and the composition of its glycosylation. They reveal that the chimeric antibody retains a strong attachment to TNF-α with additional desirable pharmacological qualities [17,24]. The result, Infliximab, combines the high level of antigen matching from the mouse antibody with lower immunogenicity and longer life given by the human IgG1 constant region. Because of its molecular design, Infliximab lasts in the body long enough to be effective while keeping adverse immune responses low, so it is used as a long-term treatment for conditions driven by TNF-α dysregulation [4,23].

Great care was given to designing it so that it can bind well to the human TNF-α in any form, stopping it from causing inflammatory problems in people with autoimmune diseases. First, the team immunized mice with TNF-α to stimulate their immune system. The mice's immune cells specialized in making antibodies against TNF-α. High-affinity antibodies capable of binding both blood and cell-surface TNF-α were screened from B-cell and myeloma hybridomas [16,17]. The specific regions of the most effective murine antibody parts involved in antigen binding (VH and VL) were isolated and sequenced. Then, stem cell–

derived regions from these regions were joined with the human IgG1 constant parts, creating a chimeric monoclonal antibody [17,22]. As a result of chimerization, the antibody worked well in people and had the targeted TNF-α–binding function from the mouse. Molecular Weight of Infliximab is a therapeutic monoclonal antibody with an estimated molecular weight of around 149 kilodaltons (kDa). This overall weight is inclusive of its polypeptide chains
and other structural adjustments like glycosylation, which are common among antibodies of its type [30]. Isotype of this antibody is an IgG1 subclass immunoglobulin. Infliximab is chimeric, that is, it
is constructed by juxtaposing murine-
derived variable regions with human constant regions [31]. The IgG1 background ensures a long half-life and facilitates immune system functions including complement activation and Fc receptor binding, essential for its therapeutic effect [32]. Structural Domain of Infliximab has the characteristic Y-shaped structure of immunoglobulin G molecules and is composed of four protein chains: Heavy Chains (Human-derived) of Each heavy chain consists of one variable domain (VH) and three constant domains (CH1, CH2, CH3). The CH2 and CH3 domains constitute the Fc region, which plays a role in binding to immune cell receptors and activating the complement system [33]. Light Chains of both light chains consist of a single variable domain (VL) and a single constant domain (CL). The chains stabilize the antibody structure and are responsible for specific antigen recognition. Fab (Fragment antigen-binding) Regions of these areas are created by the merging of VH and VL regions and are accountable for the specific binding of infliximab to its antigen — tumor necrosis factor-alpha (TNF-α). Binding to TNF-α serves the purpose of inhibiting its action and combating inflammation [30,31].

Infliximab was engineered to attach to soluble TNF-α that leads to inflammation and also to transmembrane TNF-α involved in signaling and immune cell activation. The close binding to both forms makes it possible to fully stop TNF-α's activity. This is important because it keeps TNF-α from binding to its receptors (TNFR1 and TNFR2), stopping the body's inflammatory response [4,25]. The TNF-α antibody combination and half-life extend by the Fc region included on human IgG1 allow Infliximab to block both TNF-α forms effectively and last a long time. The reason it works well for chronic inflammatory diseases like rheumatoid arthritis, Crohn's disease and ankylosing spondylitis is because it targets two different cells [2,23].

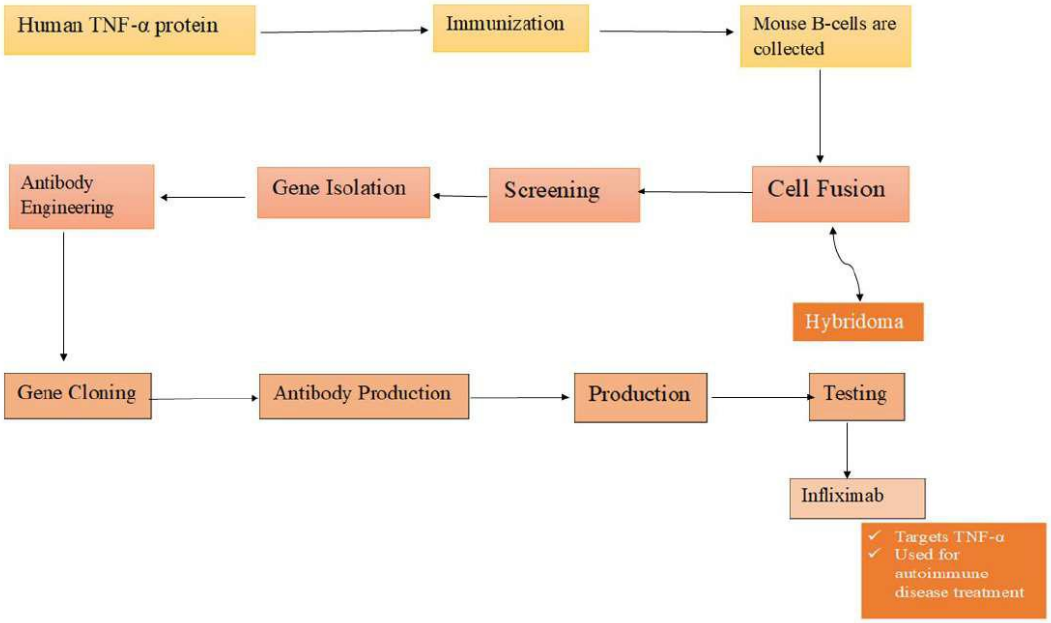

Fig. 1. Infliximab Molecular Design

Production and Biomanufacturing Processes

Cell Line Development

For therapeutic monoclonal antibodies like Infliximab, a cell line is developed that remains steady and lets the antibody be vertically produced. Many researchers pick CHO as their host cell because it grows fast, supports many post-translational alterations and is accepted for use [19,10]. At the start, scientists add the heavy chain (HC) and light chain (LC) genes into vectors for expression. Then hold well-known promoters (such as CMV and EF1α) and important markers (DHFR and GS) to help with picking the right cells and regulating transcription and translation expression. In the case of Infliximab, murine variable sections are joined to human constant regions and placed in vectors [6,26]. Afterward, researchers transfect the expression vectors into the host cells by either electroporation or chemical transfection. Even though transfection is done, cells keep being grown under the right selective conditions (methotrexate or GS-free medium) to identify the successful transfer of the gene. Then, thousands of clones are checked for antibody production, how well they remain stable and their quality. Proper folding and glycosylation are evaluated in high-producing clones and they are tested to see if they can be grown in media without serum [27].

If top-quality clones are acknowledged, these forms then become the strain in an MCB (Master Cell Bank) under GMP rules. From MCB, everything for future production is derived and all the safety and traceability come from it [28, 29]. The CHO or SP2/0 cells chosen are then multiplied in large bioreactors, where the monoclonal antibody is released into the solution and then cleaned [19,6]. Using a step-by-step approach guarantees the production of Infliximab with a strong structure, large scale and predictable effects on patients. A stable approach to moving antibody genes into host cells is to use transfection, in which the HC and LC genes are added and linked permanently to the DNA of a host cell, for example CHO cells. It is necessary for making a cell line that can be used to produce Infliximab over a long period [26,27].

This all starts when you make expression vectors to carry the coding sequences for the HC and LC of your antibody. To make sure there is plenty of the gene's product, strong viral or mammalian promoters like the cytomegalovirus (CMV) promoter are used. The vectors also have genes for selectable marks, for example, DHFR and GS, that support the differentiation of successfully transfected cells from the rest [6,10]. Delivery of the vectors into host cells is carried out by using either chemical (e.g., lipofection) or physical (e.g., electroporation) methods. The cells are then added to a medium containing a nutrient that the integrated marker gene is able to provide which only cells with the marker gene can tolerate [10,27]. With this, the genes of HC and LC are Let into the cell's genome, so the cells become richer in important factors [3,6]. With cells that are well transfected identified, researchers separate out single clones that create the desired types of antibodies in proper amounts. After creating many clones, they are tested for stability. A Master Cell Bank (MCB) is established by using the most suitable clone in a Good Manufacturing Practice (GMP) setup [28, 29].

This stable transfection-based cell line development ensures consistent, high-yield antibody production for clinical and commercial manufacturing. The clone selection is a critical step in the development of stable cell lines for therapeutic antibody production, such as Infliximab. After successful transfection of host cells (commonly CHO or SP2/0 cells) with vectors carrying the heavy and light chain genes under strong promoters (e.g., CMV), the next phase involves identifying and isolating high-producing, genetically stable clones [26,6].

Culturing transfected cells happens under selective pressure, with markers inside the expression vectors helping to do this. Often, researches use dihydrofolate reductase (DHFR) and glutamine synthetase (GS) as markers. DHFR-positive cells can only survive when cultured in a medium where hypoxanthine and thymidine are absent. GS-carrying cells grow in special media that lacks glutamine and only survive and multiply when they express the GS gene [3,6]. They successfully separate transfected cells and then enhance the production of antibodies by methotrexate if choosing the DHFR system or methionine sulfoximine for the GS system [6].

Before cloning can be done, a small group of alive cells must be created using events like limiting dilution or FACS. Clones are tested separately and their productivity is found by measuring antibodies secreted from the cells, usually using ELISA or immunoassays [89]. The characteristics of clone cell growth, protein quality, proper assembly and glycosylation are all examined. Only the best clones

which show good expression, steady genes and the required product quality, are selected for increased production. After being expanded, the clones are kept in a Master Cell Bank (MCB) made under GMP conditions and used to produce huge amounts of antibodies. Because of this careful approach, therapeutic protein production is reliable, scalable and legal [28,29].

Upstream processing

Initially, the immune cell clone that makes the highest amount of antibody is grown in a bioreactor to produce large-scale antibody. The first step is to inoculate a seed culture from the MCB, grow it through various scales and finally move it into a production bioreactor. CHO and SP2/0 are typical host cells that are grown in fed-batch or perfusion culture [19].

During a fed-batch process, essential nutrients are slowly given to the culture, but without removing any of the old medium. The technique supports making more products and allows for crowded cells over a fixed period [91]. Alternatively, perfusion allows constant infusion of new medium and eliminates what is thrown out, helping the organisms stay at their maximum levels and yield the same quality products for a longer duration [6].

In every system, it is important to tightly control bioreactor conditions to permit proper growth of cells and antibody production. Parameters for pH (6.8–7.2), DO, temperature (36–37°C) and nutrients (glucose and amino acids) are continuously checked and adjusted automatically by the environment. Homogeneity and oxygen transfer are kept with the use of agitation and gas sparging [90].

In this phase, small samples are taken to measure the condition of the cells, level of metabolites and antibody titers. As soon as the culture reaches peak production, the antibody is immediately harvested from the cell-free supernatant that remains. By using this upstream process, the production of antibodies needed for treatment is always of high quality and quantity [19].

Downstream Processing

Mostly, scientists work on producing cells that create the wanted antibody. Yet, when the bioreactor culture has been harvested, several preparatory purification processes must come before the final formulation. Even though these steps are considered part of early downstream processing, they help change the crude harvest into a safe and purified therapeutic product [5].

The liquid collected from cell culture, with the antibody mixed within, is first clarified to remove the cells and debris, mostly using centrifugation and depth filtration methods. Following clarification, the antibody mixture is purified further through chromatographic steps intended to pull out HCPs, DNA and aggregates that come from the process [5].

Usually, the first major step in intermediate purification is ion-exchange chromatography which divides proteins based on how negatively or positively charged they are. Under different pH and buffer states, CEX and AEX are employed to extract or polish the produced antibody [92,34].

Hydrophobic interaction chromatography (HIC) is the next option. The process divides proteins according to how much they like water and it helps eliminate misfolded or aggregated ones that might have the same charge but different surface features [37,36].

A major security policy is applying virus removal filtration which uses nanofilters (usually 20 to 50 nm) to physically catch any viral impurities. It is important for the final reaction to follow regulatory standards to ensure viral safety [35,36].

The goal of polishing in downstream processing is to create a purified, identical and unaggregated end product. The primary methods of ion-exchange chromatography (IEX) and hydrophobic interaction chromatography (HIC) are followed by further polishing with size exclusion chromatography (SEC) [37,38].

SEC is another term for gel filtration chromatography and means molecules are separated depending on their size and shape. The solution of antibodies is now filtered through a column containing porous gel. Small molecules reside in the pore network of the column and thus move slowly, whereas larger aggregates are not able to enter and often come out sooner [39].

Antibody aggregates that may appear during production or storage are the main focus of SEC in polishing. These aggregates are not wanted because they may influence how well, safely and effectively the therapeutic product works. SEC ensures that most of the antibodies in the final product are monomeric and well-folded, by separating monomers from dimers, trimers and similar aggregates [37,38].

Besides its main aim, SEC provides better uniformity in the antibodies, ensuring each batch meets high quality standards. After going through SEC, an antibody is more generally concentrated

and migrated into a suitable buffer for formulation next, before being sterilized and loaded into vials [38].

Quality control and Characterization

During the quality control (QC) process in monoclonal antibody manufacture, such as Infliximab, a number of analytical methods are used to evaluate purity and potency. These assays verify that the final product conforms to regulatory requirements for safety, effectiveness, and reliability.

SDS-PAGE (Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis): This method is employed to analyze the purity and molecular weight of the antibody. SDS-PAGE differentiates proteins by size, enabling detection of impurities, aggregates, or broken-down forms of the antibody. Single band correlating to the expected molecular weight of the antibody (usually ~150 kDa for IgG1) shows high purity, but multiple bands may indicate possible impurities or breakdown products [42,43].

ELISA (Enzyme-Linked Immunosorbent Assay): ELISA is employed to test the potency of the antibody by determining its function of binding to human TNF-α or target receptor. The product with high potency will exhibit intense binding to TNF-α, reflecting the functional efficacy of the antibody. ELISA can further be utilized to determine the concentration of the antibody and confirm that the product has reached the specified therapeutic dose [40,41].

HPLC (High-Performance Liquid Chromatography): HPLC is used for purity testing and to determine the monomeric integrity of the antibody. Through monitoring of retention time and peak profiles, HPLC can differentiate between monomeric and aggregated species of the antibody [5]. Reversed-phase HPLC is also routinely used to determine the charge heterogeneity and glycosylation profile in order to ensure the antibody is folded and glycosylated correctly [24].

One of the safety issues most critical with therapeutic antibodies is their ability to generate immune responses in patients, such as the development of anti-drug antibodies (ADAs). These can neutralize the therapeutic antibody's effect and cause loss of efficacy, changed pharmacokinetics, or even anaphylactic allergic reactions [45]. To evaluate this risk, ADA assays are routinely conducted in the quality control process.

The main goal of an ADA assay is to identify whether the patient's serum contains antibodies that bind and recognize the therapeutic antibody (Infliximab) and how much they are present. These assays would often be carried out by methods such as enzyme-linked immunosorbent assay (ELISA) or radioimmunoassay (RIA) [46]. The test encompasses incubating patient serum with the therapeutic antibody, with subsequent detection of any interaction with the antibody's antigen-binding site (variable regions) or Fc region. A stepwise approach is frequently adopted, involving screening followed by confirmatory tests to differentiate among various forms of ADAs (e.g., neutralizing vs. non-neutralizing) [44].

Detection of ADAs early in the production or clinical trial phase ensures that the monoclonal antibody is safe and reduces the risk of immunogenicity when infused into patients. Regulatory bodies such as the FDA mandate extensive immunogenicity testing during the preclinical and clinical development of therapeutic antibodies [44 ,45].

Glycosylation is a key post-translational modification that affects both the activity and safety of monoclonal antibodies. The glycosylation pattern, namely the N-glycans that are present on the Fc region of the antibody, can have a profound impact on its pharmacokinetics, efficacy, and immunogenicity [24,47]. For example, the presence of specific glycan structures, for example, bisecting GlcNAc or fucosylation, can increase or decrease the interaction of the antibody with Fc receptors or the neonatal Fc receptor (FcRn), impacting its half-life and effector mechanisms such as antibody-dependent cellular cytotoxicity (ADCC) [49].

Mass spectrometry (MS) is an effective tool used in quality control to examine the glycosylation profile. It starts with the enzymatic cleavage of N-glycans from the monoclonal antibody using enzymes such as PNGase F. Subsequent analysis of these glycan structures using high-resolution mass spectrometry provides the composition, structure, and heterogeneity [48]. The mass spectrometer measures the molecular mass of the removed N-glycans, which correlates directly with their individual sugar components as well as their glycosidic linkages.

The glycosylation profile is thoroughly scrutinized to see that it conforms to the specified requirements for the therapeutic monoclonal antibody. Any variation in the desired pattern can result in variations in efficacy or immunogenicity, since changed glycosylation can cause an immune response or influence the way the antibody binds

with immune cells or other target molecules. In addition [24,47], mass spectrometry is capable of detecting glycan heterogeneity, which is an occurrence of variations in glycosylation pattern among individual molecules of an antibody. This is significant in ensuring batch-to-batch consistency during production [48,50].

Mass spectrometric characterization of N-glycans also confirms that the therapeutic antibody possesses the proper pharmacological characteristics. For example, sialylation (the attachment of sialic acid to the glycan chain) can increase the anti-inflammatory activity of the antibody, so it is especially useful for therapeutic applications against autoimmune diseases [49,51].

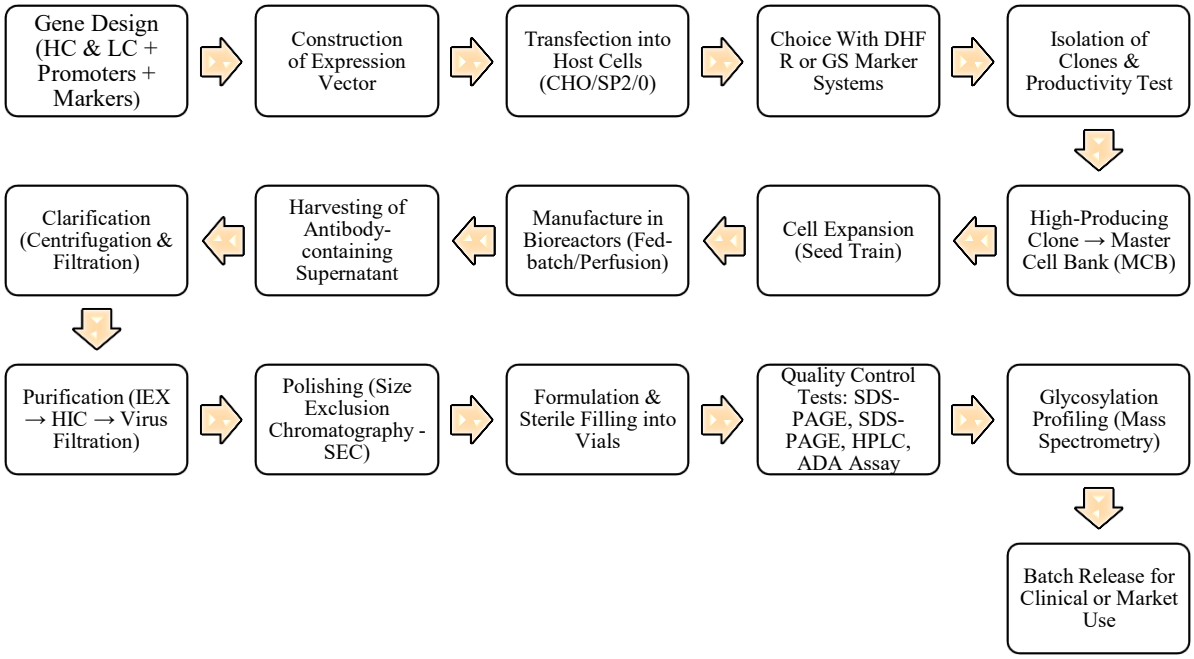

Fig. 2. Infliximab Production Process

Mechanism of action and Pharmacodynamics

Infliximab helps suppress rheumatoid arthritis, Crohn's disease and ankylosing spondylitis by targeting TNF-α, a cytokine involved in inflammation of the body [4,5]. It takes part in inflammation by turning on immune cells, stimulating other pro-inflammatory cytokines and leading to tissue destructive effects [54].

Infliximab binds directly to TNF-α in the form that is found in the bloodstream. Infliximab stops TNF-α from bonding to TNFR1 and TNFR2 on the walls of cells because it is connected to the TNF-α molecule [52]. NF-κB is activated by the inflammatory signals released by these receptors which also encourage more inflammatory mediators to be made [55].

Infliximab acts by neutralizing TNF-α which prevents this signaling pathway from causing the problems linked to chronic inflammation and damaged tissues. By joining TNF-α, Infliximab helps the immune system to remove these cytokines more quickly from the body [5].

The medication reduces inflammation, causes less harm to body tissues and helps patients with autoimmune diseases recover better. Reducing TNF-α activity with Infliximab improves painful joints, swelling, bowel inflammation and the ability of patients to live normally [18,53].

In addition to binding to TNF-α that travels in blood, infliximab also joins with the TNF-α forms that are on the surfaces of immune cells like macrophages and T-cells. The cell-to-cell communication that magnifies inflammation depends on transmembrane TNF-α [56]. When Infliximab connects to this variant of TNF-α, it blocks TNF-α from activating similar receptors which keeps pro-inflammatory pathways such as NF-κB, from operating in the target cells [4,55].

In addition to neutralizing TNF-α signals, the attachment of Infliximab to membrane TNF-α causes antibody-dependent cellular cytotoxicity (ADCC). As a result, the Fc portion of Infliximab interacts with Fc receptors on immune cells named natural killer (NK) cells and macrophages [5,57].

Once recognized, immune cells will kill the cells forming TNF-α, leading to the removal of inflammatory cells from any damaged tissue.

Furthermore, Infliximab uses the Fc region to help the complement system which plays a role in killing harmful pathogens and unhealthy cells. Due to this trigger, complements fix onto surface proteins of TNF-α-expressing cells, resulting in their destruction and decreasing inflammation [4,52].

Infliximab is an intravenous monoclonal antibody that gains access to the bloodstream directly and quickly [93]. In the bloodstream, it undergoes a two-stage process of elimination, in which it initially distributes into bod y fluid sand tissues, then slowly is eliminated from the body.

Its volume in the body is comparatively small (about 3–6 liters), which suggests it does not penetrate very deeply beyond the blood and extracellular spaces [30]. The rate of clearance from the body is different in different individuals but is usually between 10 to 15 milliliters per hour.

The drug has a long duration of activity in the body, with a half-life of about 7 to 12 days, and this favors dosing at intervals of several weeks [4]. These include body weight, the formation of anti-drug antibodies, and the use of immunosuppressive drugs at the same time. These factors can affect how long infliximab will be in the body and how well it works [31].

Since infliximab is a big protein, it does not survive the gastrointestinal environment and is not absorbed from the gastrointestinal tract. Thus, it has to be administered via vein,

which guarantees that 100% of the dose ends up in the bloodstream. This leads to 100% bioavailability upon infusion [93].

III. THERAPEUTIC APPLICATIONS

Autoimmune and Inflammatory Diseases

Through the neutralization of TNF-α, Infliximab decreases inflammation, inhibits tissue damage, and alleviates symptom severity in a number of chronic inflammatory conditions. These therapeutic effects have been proven in practice and clinical trials in a range of autoimmune disorders:

1. Crohn's Disease: Infliximab is very effective in the treatment of Crohn's disease, an inflammatory bowel disease. It decreases inflammation in the gut, induces healing of mucosa, and enhances clinical symptoms like abdominal pain, diarrhoea, and weight loss [53]. It is very effective in patients with moderate to severe disease and can cause remission, diminishing the requirement for surgery [61].

2. Rheumatoid Arthritis: In rheumatoid arthritis (RA), Infliximab decreases joint swelling and prevents the damage to joints. Physical function and quality of life are improved due to decreased pain, swelling, and stiffness. Infliximab can be added to methotrexate to increase its effectiveness in controlling disease activity [18,60].

3. Psoriatic Arthritis: In psoriatic arthritis (PsA), a condition that includes joint inflammation and skin psoriasis, Infliximab decreases joint swelling, enhances skin lesions, and offers substantial pain and disability relief. It maintains control over the inflammatory process and inhibits further joint damage [58].

4. Ankylosing Spondylitis: In ankylosing spondylitis (AS), a spinal disease, Infliximab decreases inflammation in spinal joints, resulting in relief of back pain, enhanced mobility, and prevention of disease progression. It has been demonstrated to postpone spinal fusion, a feature of advanced disease, and thereby enhance long-term functional outcome [59].

Off-label and Investigational Uses

Infliximab, which was initially formulated to treat autoimmune conditions such as Crohn's disease and rheumatoid arthritis, has also been investigated for application in other inflammatory diseases in which the standard therap y might not be sufficient. Off-label or investigational uses include sarcoidosis, uveitis, and Behçet's

disease, particularly in those who are unresponsive to conventional therapy [94,95].

1. Sarcoidosis: Sarcoidosis is an illness where there are bunches of immune cells,

or granulomas, which develop in various areas of the body, most often in the lungs and lymph nodes. When symptoms are severe or persistent, infliximab can be used.

Infliximab inhibits the action of TNF-α, a molecule that is thought to play a role in the development and persistence of granulomas. Some patients with nerve or lung involvement have also improved when they were given inflixim ab after not responding to steroids or immune suppressants [94]. Even though it is not formally approved for sarcoidosis, physicians might employ it

under certain circumstances, particularly when other treatment modalities are not effective.

2. Uveitis:

Uveitis is inflammation within the eye, which may result in blurred vision, pain, or even irreversible damage if not properly treated. It frequently develops in individuals suffering from autoimmune disorders. Infliximab has been researched for its potential to decrease eye inflammationin non-responsive patients to standard treatments such as s teroids. By binding TNFα, infliximab controls immune mediated eye issues and possibly decreases the risk of vision loss [96,97]. Although not officially licensed for this condition, occasionally it is used in severe or persistent cases under specialist direction.

3. Behçet's Disease: This is a rare illness that results in widespread inflammation of blood vessels and tissues of the body. Symptoms can involve ulcers, eye inflammation, joint aches, and even brain or vascular complications. Infliximab is likely to help with Behçet's disease when conventional treatments like colchicine or corticosteroids are insufficient. It calms the overactive immune system, particularly in severe manifestatio ns of the disease involving the eyes or nervous system [95]. Its application in Behçet's remains investigational but has been successful in treating patient outcomes in drug-resistant cases.

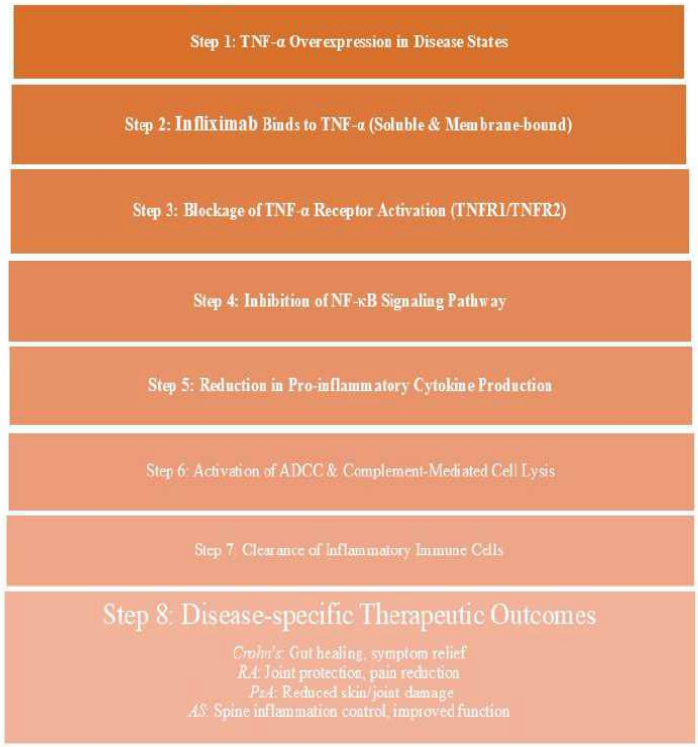

Fig. 3. Infliximab Mechanism & Effects

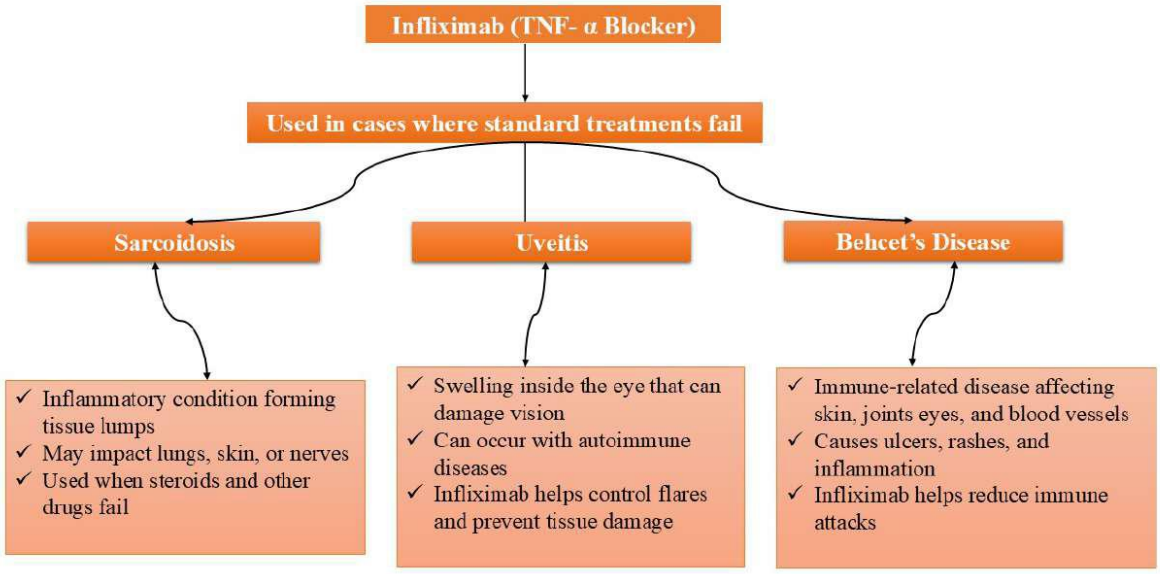

Fig. 4. Infliximab Off-label and Investigational Uses

Clinical trials and Efficacy Data

In clinical practice, treatment with Infliximab in these conditions has resulted in considerable alleviation of symptom severity and progression of disease. Through TNF-α neutralization, Infliximab stops or slows tissue damage and normalizes immune response, resulting in improved quality of life [5,64]. Reduced pain, mobility, and frequency of hospitalization for disease flare are experienced by patients. Additionally, Infliximab is proven to reduce dependence on corticosteroids, reducing the risk of side effects from long-term steroid therapy [53,62].

Lastly, Infliximab neutralizes soluble and transmembrane TNF-α, inhibits its pro-inflammatory action, and evokes immune reactions such as ADCC and CDC [63]. Its clinical benefits are seen in a number of autoimmune conditions with decreased inflammation, avoided tissue destruction, and enhanced clinical response, which greatly improves the quality of life of patients [18,59].

Applications in biomanufacturing

Because Infliximab is produced from CHO cells, there is great potential to improve the early steps of its production. How media is set up is important to promote growth and the production of antibodies. Feeding the culture helps keep the cells alive and provides high amounts of antibodies. Setting the perfusion or fed-batch system to deliver exact glucose, amino acids and nutrient amounts leads to higher cell density and more antibody production [6,67]. Bioreactor controls, including pH, oxygen, temperature and agitation, are set to achieve better and more yielding results [65].

Downstream Process Scaling and Validation: The downstream production process starts by clarifying, moving to chromatography and finishing with filters. Along with scales, the purification of antibodies with ion-exchange chromatography and size exclusion chromatography was established by using infliximab as a pattern. Making bigger batches of Infliximab is used to confirm that the benefits of downstream processing are preserved in pilot and commercial production [14].

Infliximab is studied for glycosylation engineering to find the best N-glycan profiles. Altering glycosylation of the antibody may change its power, how long it remains active in the body and how likely it is to be detected by the immune system. Analyzing glycosylation patterns in Infliximab allows scientists to figure out how to adjust glycosylation to achieve better results in therapeutic antibodies [24,68].

Biomanufacturing contributes significantly to the development of biosimilars which are like regular biologics in safety, quality and effectiveness. One important case is Infliximab, a monoclonal antibody that targets TNF-α and is used to handle rheumatoid arthritis and Crohn's disease. Its biosimilars, Inflectra from Celltrion/Pfizer and Renflexis from Samsung Bioepis/Merck, have led the industry in standards for biosimilar development [69,66].

The protein for Infliximab in both Inflectra and Renflexis is expressed using similar techniques based on growing Chinese Hamster Ovary (CHO) cells in mammalian cell culture. High purity and consistency are achieved in downstream processing by performing protein an affinity chromatography, inactivating viruses, using ion exchange and polishing the product. Showing that a biosimilar is the same as the reference product in primary structure, glycosylation, many forms of charging, agglomeration and function is considered the main requirement for approval [73,14].

Because Infliximab is complex in its structure and widely used medically, it serves as an excellent molecule for comparing biosimilars in regulatory submissions. Both the EMA and FDA want companies to perform thorough analyses of their products and then conduct clinical trials for pharmacokinetics and effectiveness. Since Inflectra and Renflexis performed well as biosimilars, their success highlights the current methods and equipment used in biopharmaceutical manufacturing and analysis for future biosimilar products. They help meet regulatory needs, keep patients secure and make biologic drugs affordable around the world [66,71,72].

Biomanufacturing relies greatly on cell line engineering which works to enhance how biologics are produced and work in the body. Because of their ability to grow well, adapt to be used in suspension culture and support human-like post-translational modifications, CHO cells are used more than any other host cells to produce recombinant proteins. Companies are now using genetic methods to adjust CHO cells to produce more of a desired protein and to give it the desired type of sugar [70,6,74].

One key application is the enhancement of antibody-dependent cellular cytotoxicity (ADCC), a critical mechanism for therapeutic monoclonal antibodies. ADCC can be improved by reducing core fucosylation of the antibody Fc region, which increases affinity for FcγRIIIa receptors on immune cells [76]. Genetic approaches such as targeted knockouts of the *FUT8* gene (encoding fucosyltransferase) using CRISPR-Cas9 have proven effective in generating low-fucose CHO cell lines. These modifications result in improved clinical efficacy of antibodies used in oncology and autoimmune diseases [75,78].

In parallel, CRISPR and RNA interference (RNAi) technologies are employed to knock down or knock out undesirable metabolic or apoptotic pathways that limit cell growth or productivity. For example, silencing genes involved in lactate accumulation or apoptosis (e.g., *BAX*, *LDHA*) enhances culture longevity and yields [67,77]. These genome editing strategies contribute to stable, high-performing cell lines capable of producing therapeutic proteins at commercial scale.

Biosimilars and Market Landscape

Development and approval of biosimilars are regulated through stringent regulatory guidelines offered by bodies such as the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA). The two bodies require extensive analytical and functional characterization to establish biosimilarity to the reference biologic [71,82]. This involves characterizing structural properties, post-translational modifications, bioactivity, pharmacokinetics, and clinical efficacy. The "totality of evidence" approach is the cornerstone of biosimilar approval, so that there is no clinically meaningful difference from the originator product [7].

Pharmacovigilance takes precedence post-approval, particularly due to the risk of immunogenicity in the case of biologics. Even minor differences in manufacturing or storage conditions have the potential to alter protein conformation or glycosylation and trigger immune stimulation [79]. Strong safety monitoring, risk management plans, and traceability are therefore part of regulatory compliance and patient safety for use as a biosimilar [15].

Commercially, exclusivity and patent expirations for blockbusters such as Infliximab, Rituximab, and Trastuzumab have driven a global biosimilar boom. This has revolutionized market dynamics, injecting competition and price cutting as well as improved accessibility [81]. The commercial landscape is, however, complex, with pricing dictated by local regulatory environments, physician adoption, payer incentives, and manufacturing capacity [80].

Future Perspectives and Challenges

Despite significant advances in the creation of biosimilars, there remain certain areas that have to be addressed, and the most critical of these is immunogenicity. Small variations in glycosylation or folding of proteins during production can lead to immune responses in patients, potentially compromising safety and therapeutic effect [84]. To prevent this, there is a growing trend towards the development of fully human monoclonal antibodies, which are less likely to induce immunogenic

responses compared to chimeric or humanized counterparts [17].

Emerging biomanufacturing strategies will tackle quality as well as efficiency. New host cell lines such as CHOK1 (an enhanced Chinese Hamster Ovary variant) and HEK293 (Human Embryonic Kidney cells) are being studied for their better protein expression profiles, improved post-translational modification capacity, and potential for lower variability [6,83]. Continuous biomanufacturing—whereby production processes are operated continuously and not in batches—is also being investigated for its potential of improved scalability, cost savings, and product consistency [85].

Looking ahead, a convergence of advanced digital technologies will transform biosimilar production. Use of digital twins—virtual copies of actual bioprocesses—combined with artificial intelligence (AI)–based analytics is able to optimize process control, predict deviations, and enhance reproducibility [86]. These technologies enable real-time monitoring and adaptive control, offering higher-consistency product quality from batch to batch.

IV. CONCLUSION

Infliximab, a chimeric monoclonal antibody targeting tumor necrosis factor-alpha (TNF-α), has revolutionized the treatment of autoimmune disorders such as rheumatoid arthritis, Crohn's disease, and ulcerative colitis [4]. Beyond its clinical success, Infliximab has emerged as a Beck, A., Reichert, J. M. (2011) [50]. Cornerstone in the field of biologics manufacturing, particularly for biosimilar development. It not only enhanced the therapeutic outcomes but also served as a model system that integrates multiple disciplines—cell biology, biochemical engineering, and regulatory science—into one framework that defines today's biopharmaceutical production.

Production of Infliximab and its biosimilars, such as Inflectra and Renflexis, is a prime example of the complexity and potential of mammalian cell culture systems, especially Chinese Hamster Ovary (CHO) cells [19]. These cells are precisely engineered for optimal expression, correct folding, and correct post-translational modification, most importantly glycosylation, which has a direct influence on the drug's efficacy and immunogenicity [87].

The downstream processing—comprising a number of steps of purification including protein A chromatography, virus filtration, and ion-exchange polishing—echoes the accuracy that is required to deliver high-quality and regulatory compliance criteria. These bioprocesses must not only deliver high-titer product but consistency, safety, and functionality across batches as well [14].

Infliximab has been at the fore in shaping regulatory expectations, most notably in relation to biosimilar development. Bodies like the FDA and EMA have formalized robust guidelines around its comparability tests, leading to a precedent on analytical characterization, clinical trial planning, and post-marketing surveillance [71]. Pharmacovigilance has become part and parcel of the same owing to the intrinsic risk of immunogenicity associated with monoclonal antibodies. Through rigorous testing of biosimilars against Infliximab, regulatory science has progressed, paving the way for global confidence in interchangeability and therapeutic equivalence of biosimilars [7].

Despite issues such as immunogenicity and manufacturing costs, innovation continues. Full human monoclonal antibodies and novel cell lines like HEK293 and CHOK1 are being designed to improve expression quality and reduce unwanted immune responses [10,83]. Sustainable biomanufacturing is also gaining momentum as a next-generation strategy to improve scalability, reduce variability, and reduce manufacturing costs [85]. These process enhancements are not just cost-cutting but also about improving quality product consistency—an all-pervasive mandate for regulatory approval and patient safety.

In the future, biomanufacturing will be transformed by digitalization. Leveraging the use of digital twins—digital replicas in real time of the bioprocess—and artificial intelligence (AI) for process control can significantly enhance reproducibility, predictive maintenance, and adaptive optimization [13]. These technologies allow for dynamic real-time tweaks in production, real-time assessment, and better decision-making, reducing the likelihood of batch failure and regulatory compliance. They also support Quality by Design (QbD) principles, allowing more process understanding and control [88].

In short, Infliximab's legacy extends beyond its designation as a therapeutic molecule. It has established the benchmark by which complex biologics may be produced, manufactured, and regulated. Its history of development has been a basis on which all biosimilars are developed across

the world. As biomanufacturing continues to progress with advancing technology and scientific know-how, the future looks bright for cheaper, more available, and safer biologic therapy—benefiting patients and healthcare systems around the world [50,7].

Ethics statement

There is no study involving human or animal subjects in this review paper.

Credit authorship contribution statement

Ahiya Mariya Jose: Writing – review & editing, Writing – original draft, Data curation, Conceptualization.

Ramgopal Dhakar: Supervision, Formal analysis, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no conflict of interest.

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