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## **Infliximab: Biological response modifier, chimeric IgG1 therapeutic recombinant monoclonal antibody**

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### **Abstract**

Infliximab is a chimeric IgG1 $\kappa$  monoclonal antibody (composed of human constant and murine variable regions) specific for human tumor necrosis factor- $\alpha$  (TNF $\alpha$ ). Infliximab is produced by a recombinant cell line cultured by continuous perfusion and is purified by a series of steps that includes measures to inactivate and remove viruses. The annual global sale of Infliximab was \$8 billion in 2013, according to industry data. This article describes the overview of infliximab including mechanism of action, indications and development genetics.

**Keywords:** Infliximab, Monoclonal Antibody, Recombinant, Quality Control.

### **1. Introduction**

Infliximab is a chimeric IgG1 $\kappa$  monoclonal antibody with an approximate molecular weight of 149,100 daltons. It is composed of human constant and murine variable regions. Infliximab binds specifically to human tumour necrosis factor alpha (TNF $\alpha$ ) with an association constant of 1010 M<sup>-1</sup> [1]. Infliximab is produced by a recombinant cell line cultured by continuous perfusion and is purified by a series of steps that includes measures to inactivate and remove viruses.

The infliximab molecule contains 1328 amino acids and consists of 2 identical H chains and 2 identical L chains which associate by non-covalent H-H and H-L interactions and covalent H-H and H-L disulfide bonds. Infliximab is a glycoprotein consisting of 5 major glycoforms, each containing 2 (1 on each H chain) asparagine-linked (N-linked) asialo-, core-fucosylated, biantennary oligosaccharide chains with terminal galactose microheterogeneity. The oligosaccharide is bound exclusively to Asn-300 in the CH2 region of both H chains.

#### **1.1 Physicochemical properties**

Infliximab drug substance is a purified, recombinant DNA-derived, chimeric human-mouse IgG monoclonal antibody (MAb) which binds to and neutralises human tumour necrosis factor  $\alpha$  (TNF $\alpha$ ) with high affinity (K<sub>a</sub>=1 X 1010 M<sup>-1</sup>) [2]. Infliximab contains murine heavy (H) and light (L) chain variable regions (V<sub>H</sub> and V<sub>L</sub>, respectively) derived from the murine anti-TNF $\alpha$  MAb, A2, and genomic DNA-derived human H and L chain constant regions (C<sub>H</sub> and C<sub>L</sub>, respectively).

### **2. Indications and Usage**

#### **2.1 Crohn's Disease**

Infliximab is indicated for reducing signs and symptoms and inducing and maintaining clinical remission in adult and pediatric patients with moderately to severely active Crohn's disease who have had an inadequate response to conventional therapy

Infliximab is indicated for reducing the number of draining enterocutaneous and rectovaginal fistulas and maintaining fistula closure in adult patients with fistulizing Crohn's disease.

#### **2.2 Ulcerative Colitis**

Infliximab is indicated for reducing signs and symptoms, inducing and maintaining clinical remission and mucosal healing, and eliminating corticosteroid use in patients with moderately to severely active ulcerative colitis who have had an inadequate response to conventional therapy [4].

#### **2.3 Rheumatoid Arthritis**

Infliximab, in combination with methotrexate, is indicated for reducing signs and symptoms, inhibiting the progression of structural damage, and improving physical function in patients

with moderately to severely active rheumatoid arthritis.

#### 2.4 Ankylosing Spondylitis

Infliximab is indicated for reducing signs and symptoms in patients with active ankylosing spondylitis.

#### 2.5 Psoriatic Arthritis

Infliximab is indicated for reducing signs and symptoms of active arthritis, inhibiting the progression of structural damage, and improving physical function in patients with psoriatic arthritis.

#### 2.6 Plaque Psoriasis

Infliximab is indicated for the treatment of adult patients with chronic severe (i.e., extensive and/or disabling) plaque psoriasis who are candidates for systemic therapy and when other systemic therapies are medically less appropriate. Infliximab should only be administered to patients who will be closely monitored and have regular follow-up visits with a physician

### 3. Mechanism of Action

Infliximab neutralises the biological activity of TNF $\alpha$  by binding with high affinity to the soluble and transmembrane forms of TNF $\alpha$  and inhibits binding of TNF $\alpha$  with its receptors [7-9]. Infliximab does not neutralise TNF $\beta$  (lymphotoxin  $\alpha$ ), a related cytokine that utilises the same receptors as TNF $\alpha$ . Biological activities attributed to TNF $\alpha$  include: induction of pro-inflammatory cytokines such as interleukins (IL) 1 and 6, enhancement of leukocyte migration by increasing endothelial layer permeability and expression of adhesion molecules by endothelial cells and leukocytes, activation of neutrophil and eosinophil functional activity, and induction of acute phase reactants and other liver proteins [10]. Cells expressing transmembrane TNF $\alpha$  bound by infliximab can be lysed *in vitro* by complement or effector cells [8]. Infliximab inhibits the functional activity of TNF $\alpha$  in a wide variety of *in vitro* bioassays utilising human fibroblasts, endothelial cells, neutrophils, [7] B and T lymphocytes, [11, 12] and epithelial cells. Anti-TNF $\alpha$  antibodies reduce disease activity in a cotton-top tamarin colitis model, [13] and decrease synovitis and joint erosions in a murine model of collagen-induced arthritis. Infliximab prevents disease in transgenic mice that develop polyarthritis as a result of constitutive expression of human TNF $\alpha$ , and, when administered after disease onset, facilitates eroded joints to heal.

### 4. Risk of Infections

Serious infections due to bacterial (including sepsis and pneumonia), invasive fungal, viral, and other opportunistic pathogens, have been reported in patients receiving TNF-blocking agents. Some of these infections have been fatal. Many of the serious infections in patients treated with infliximab have occurred in patients on concomitant immunosuppressive therapy that, in addition to their underlying disease, could predispose them to infections.

Infliximab should not be given to patients with a clinically important, active infection, including tuberculosis. Caution should be exercised when considering the use of infliximab in patients with a chronic infection or a history of recurrent infection. Patients should be monitored for signs and symptoms of infection while on or after treatment with infliximab. New infections should be closely monitored. If a

patient develops a serious infection, infliximab therapy should be discontinued.

Cases of histoplasmosis, coccidioidomycosis, blastomycosis, listeriosis, pneumocystosis, and tuberculosis have been observed in patients receiving infliximab. For patients who have resided in or travelled to regions where histoplasmosis, coccidioidomycosis, or blastomycosis are endemic, the benefits and risks of infliximab treatment should be carefully considered before initiation or continuation of infliximab therapy.

### 5. Adverse Drug Reaction Overview

The most common adverse drug reactions reported from both clinical trials and post-marketing reports are infections, allergic reactions and infusion-related reactions. Less common adverse drug reactions from these sources which may be serious and clinically relevant include hepatobiliary events, demyelinating disorders, and lymphoma. One of the most common reasons for discontinuation of treatment in clinical trials was infusion-related reactions (dyspnea, flushing, headache and rash). Adverse events have been reported in a higher proportion of rheumatoid arthritis patients receiving the 10 mg/kg dose than the 3 mg/kg dose, however, no differences were observed in the frequency of adverse events between the 5 mg/kg dose and the 10 mg/kg dose in patients with Crohn's disease or ulcerative colitis and between the 3 mg/kg and 5 mg/kg dose in patients with plaque psoriasis.

### 6. Immunogenicity

Patients who developed antibodies to infliximab were more likely to develop infusion-related reactions. In clinical studies using single and multiple infliximab doses ranging from 1 to 20 mg/kg, antibodies to infliximab were detected in approximately 24% of patients with any immunosuppressant therapy, and in approximately 37% of patients without immunosuppressant therapy. In rheumatoid arthritis patients who received the recommended repeated treatment dose regimens with methotrexate, approximately 8% of patients developed antibodies to infliximab. In psoriatic arthritis patients who received 5 mg/kg with and without methotrexate, antibodies occurred overall in 15% of patients (antibodies occurred in 4% of patients receiving methotrexate and in 26% of patients not receiving methotrexate at baseline). Of Crohn's disease patients who received maintenance treatment, approximately 6-13% developed antibodies to infliximab. The antibody incidence was 2-3 folds higher for patients treated episodically. Due to methodological shortcomings, a negative assay did not exclude the presence of antibodies to infliximab. Some patients who developed high titres of antibodies to infliximab had evidence of reduced efficacy. In a Phase III psoriasis study in which patients were treated with infliximab induction followed by every 8-week maintenance infusions without concomitant immunosuppressive therapy, antibodies were detected in approximately 20% of patients

### 7. Control of starting materials

The specifications for release cover all tests commonly applied to purified protein solutions. Specifications for: endotoxin, bioburden, pH, protein concentration, infliximab charge heterogeneity, bioactivity, purity by SDS-PAGE, reduced and non-reduced, purity by GF-HPLC and identity by GFHPLC must be met for release of the active substance

[5]. During the evaluation process, a number of questions were raised regarding specifications and routine testing. Most of these have been adequately solved

## 8. Development genetics

The infliximab monoclonal antibody is expressed using chimeric antibody genes consisting of the variable region sequences cloned from the murine anti-TNF $\alpha$  hybridoma A2, and human antibody constant region sequences supplied by the plasmid expression vectors. Generation of the murine anti-TNF  $\alpha$  hybridoma was performed according to standard procedures by immunisation of BALB/c mice with purified recombinant human TNF $\alpha$ . The anti-TNF $\alpha$  hybridoma cell line A2 was established after subcloning of cells yielding the highest specific activity, and a cell clone found to be 98% homogenous with regard to expression of IgG1 was selected for further processing [6]. A description is given of the different steps starting from the preparation of the infliximab genomic libraries used for isolation of the light and heavy chain variable regions, and ending at the isolation of the two expression vectors carrying infliximab chimeric light chain or heavy chain genes. The procedures used for transfection and isolation of the clone producer cell clone included steps where the heavy and light chain vector constructs were linearised and transfected into Sp2/0 cells by electroporation. The subclone was selected and the high antibody producing subpopulation was enriched using serial cell sorting by flow cytometry. The highest-producing cell clone was used to create the research cell bank from which the master cell bank was established.

### 8.1 Cell bank system

The studies reported on the safety of the producer cell line have been performed in accordance with the EU guideline "Note for guidance of production and quality control of monoclonal antibodies". The MCB has been characterised for the absence of microbial and viral contaminants as well as inherent characteristics of the cell line as karyotype, isoenzyme patterns, authenticity, clonality and stability. Each master working cell bank (MWCB) is prepared from a single vial of the MCB. The routine analyses performed on new MWCB's are found acceptable and include tests for viability, mycoplasma, sterility, MAP, murine thymic agent, in vitro and in vivo tests for adventitious virus, bovine virus, karyology, isoenzymes, as well as the stable production and the identity of the secreted antibody. In view of the tests applied for qualification of each new MWCB, omission of stability studies on frozen cell banks is acceptable.

### 8.2 Fermentation and harvesting

Infliximab is a recombinant antibody produced and secreted from mouse myeloma cells (SP2/0 cells). The antibody is manufactured by continuous perfusion cell culture. Collected harvests are clarified by filtration before further purification.

### 8.3 Purification

The different steps of the purification process include affinity and anion chromatography, as well as two robust virus removal steps that are capable of removing adventitious agents and other contaminants. The documentation of the purification process contains a satisfactory presentation of the conditions applied during each step. All materials and equipment used in the different steps are specified. Intermediates of the process are 0.2  $\mu$ m filtered before

storage.

## 8.4 Control tests on the finished product

For the finished product, release tests and specifications are provided. Release tests include tests of samples at the end of fermentation and sterility testing of final bulk. The final lyophilised product is tested for sterility, endotoxin, appearance, residual moisture and reconstitution time. After reconstitution the product is tested for colour, visible particles and turbidity. Protein content, pH, uniformity, identity and immunore activity are determined. SDS-PAGE, gel filtration (GF) -HPLC and isoelectric focussing (IEF) are performed.

## 9. Conclusions

The development and introduction of recombinant monoclonal antibodies creates several opportunities and challenges. Physicians, Biopharmaceutical companies, regulatory agencies and health authorities should collaborate closely to ensure equal efficacy and safety of recombinant monoclonal antibodies that will allow the accessibility of these powerful agents to a broader number of patients in less privileged areas of our planet.

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