

The logo for BioPharmaSpec features the company name in a blue and green sans-serif font. To the right of the text is a stylized graphic consisting of three curved lines in green and blue, with three small circular dots at their ends, suggesting a molecular or orbital structure.

BioPharmaSpec

A background image of a laboratory setting. A person in a white lab coat and purple nitrile gloves is holding a pipette. In the foreground, a pipette tip is positioned over a multi-well plate. The background is softly blurred, showing laboratory equipment like test tubes and racks.

**STRUCTURE FUNCTION
CHARACTERIZATION
OF BIOLOGICS**

customTM
BIOLOGICS

INDEX

IgG1 Antibodies

- Structural Analyses..... 4
- Adalimumab..... 5
- Bevacizumab..... 6
- Golimumab..... 7
- Infliximab..... 8
- Omalizumab..... 9
- Rituximab..... 10
- Tocilizumab..... 11
- Trastuzumab..... 12
- Ustekinumab..... 13

IgG2 Antibodies

- Structural Analyses..... 14
- Denosumab..... 15
- Evolocumab..... 16

INDEX

Fusion Proteins

- Aflibercept..... 17
- Etanercept..... 19

Fab Fragment

- Abciximab..... 21
- Certolizumab..... 23
- Ranibizumab..... 25

Other

- Darbepoetin..... 27
- Erythropoietin..... 29
- Filgrastim..... 31
- Peg-Filgrastim..... 33

Structural Characterization

- Primary (*de novo*) Amino Acid Sequence
- Amino Acid Composition
- Terminal Amino Acid Sequence
- Peptide Map
- Disulfide Bridges
- Carbohydrate Structure
- Higher Order Structure

Physicochemical Characterization

- Identity and Purity
- Molecular Weight
- Isoform Pattern
- Liquid Chromatographic Patterns
- Aggregation

Product Specific Technical Considerations

- Levels of Pyroglutamination
- Disulfide bridges
- Heavy chain C-terminal Lysine
- Glycosylation (in particular, levels of galactosylation and fucosylation)
- Deamidation
- Oxidation

Adalimumab binds with high affinity and specificity to human TNF. It is used to treat arthritis, psoriasis, and Crohn's disease.

Brand Name

Humira®

Target

TNF α

Functional Assays

- Antibody-Dependent Cell Cytotoxicity (ADCC) Reporter Bioassay
- Complement-Dependent Cytotoxicity (CDC) Assay
- Cell Proliferation Assay
- Annexin V Apoptosis Assay
- Caspase 3/Apoptosis Assay
- NF κ B Reporter Assay
- Binding Assays – TNF α , FcGamma, FcRn, C1q
 - ELISA
 - MSD
 - BLI
- TNF α Neutralization Assay

ADALIMUMAB

Bevacizumab directly binds vascular endothelial growth factor (VEGF) to inhibit interaction with VEGF receptors preventing angiogenesis. It is used to treat macular degeneration as well as a variety of cancers.



Brand Name

Avastin®



Target

VEGF



Functional Assays

- Antibody-Dependent Cell Cytotoxicity (ADCC) Reporter Bioassay
- Complement-Dependent Cytotoxicity (CDC) Assay
- VEGF Reporter Bioassay
- Binding Assays – ELISA, MSD, BLI
 - VEGF
 - FC-Gamma Receptor Assays
 - FcRn Assays
 - C1q
- Neutralization Assay
- Proliferation Assays

Golimumab binds to TNF α modulating a reduction in levels of inflammatory markers. Golimumab is used to treat pain and swelling associated with arthritis.

 **Brand Name**
Simponi[®]

 **Target**
TNF α

 **Functional Assays**

- Cell-based Neutralization Assays
- Binding Assays – Fc, Fab
 - ELISA
 - MSD
 - BLI
- Antibody-Dependent Cell Cytotoxicity (ADCC) Reporter Bioassay
- Complement-Dependent Cytotoxicity (CDC) Assay

GOLIMUMAB

Infliximab is a chimeric IgG1 monoclonal antibody that neutralizes the activity of TNF α by binding to the soluble and transmembrane form of TNF α thereby inhibiting receptor binding. Infliximab is used to treat chronic inflammatory diseases such as Crohn's disease, rheumatoid arthritis, and psoriasis.



Brand Name

Remicade[®]



Target

TNF α



Functional Assays

- Cell Proliferation Assays
- TNF α Neutralization Assay
- Antibody-Dependent Cell Cytotoxicity (ADCC) Reporter Bioassay
- Complement-dependent Cytotoxicity (CDC) Assays
- Binding Assays– TNF α , Fc-Receptors, C1q, FcRn
 - ELISA
 - MSD
 - BLI

Omalizumab is a rDNA-derived humanized IgG1 monoclonal antibody that specifically binds to free human IgE in the blood and interstitial fluid and to membrane-bound IgE on B lymphocytes. Omalizumab is used to treat severe allergic asthma.



Brand Name

Xolair®



Target

IgE Fc domain region



Functional Assays

- ELISA
- Mediator-Release Assay
- Receptor Binding Assay
- Affinity Binding Assay

OMALIZUMAB

Rituximab binds to the cell surface protein CD20 thereby inducing apoptosis of CD20+ cells. It is used to treat autoimmune diseases and certain types of cancer.



Brand Name

Rituxan[®]



Target

CD20



Functional Assays

- Antibody-Dependent Cell Cytotoxicity (ADCC) Reporter Bioassay
- Complement-Dependent Cytotoxicity (CDC) Assay
- Binding Assays – CD20, Fc-Receptors, C1q
 - ELISA
 - MSD
 - BLI

Tocilizumab binds soluble and membrane bound interleukin-6 receptors, hindering IL-6 from exerting its pro-inflammatory effects. It is used for the treatment of rheumatoid arthritis and systemic juvenile idiopathic arthritis.



Brand Name

Actemra®



Target

IL-6



Functional Assays

- IL-6R Binding Assays
 - ELISA
 - MSD
 - BLI
- Neutralization Assays
 - Classical/Trans Signaling IL-6R

TOCILIZUMAB

Trastuzumab is a humanized monoclonal antibody that targets the extracellular domain of Her2 and is used in combination with chemotherapy to treat breast cancer.



Brand Name

Herceptin®



Target

HER2 (Human Epidermal growth factor Receptor 2)



Functional Assays

- Antibody-Dependent Cell Cytotoxicity (ADCC) Reporter Bioassay
- Complement-Dependent Cytotoxicity (CDC) Assay
- ELISA
- Affinity Binding Assay
- Proliferation Inhibition Assay

Ustekinumab binds to the p-40 subunit of IL-12 and IL-23 inhibiting receptor binding, thereby blocking the T1 and T17 inflammatory pathways. Ustekinumab is used to treat psoriasis and psoriatic arthritis.

 **Brand Name**
Stelara®

 **Target**
IL-12 and IL-23

 **Functional Assays**

- Binding Assays – IL-12 IL-23, C1q
 - ELISA
 - MSD
 - BLI
- IL-12 Neutralization Assay

USTEKINUMAB

Structural Characterization

- Primary (*de novo*) Amino Acid Sequence
- Amino Acid Composition
- Terminal Amino Acid Sequence
- Peptide Map
- Disulfide Bridges
- Carbohydrate Structure
- Higher Order Structure

Physicochemical Characterization

- Identity and Purity
- Molecular Weight
- Isoform Pattern
- Liquid Chromatographic Patterns
- Aggregation

Product Specific Technical Considerations

- Levels of Pyroglutamination
- Disulfide bridges and analysis of the various disulfide bridged isoforms
- Heavy chain C-terminal Lysine
- Glycosylation (in particular, levels of galactosylation and fucosylation)
- Deamidation
- Oxidation

Denosumab is designed to target RANKL (RANK ligand), a protein that acts as the primary signal to promote bone removal/resorption, preventing it from activating its receptor. It is used to treat those with osteoporosis at high risk for fractures, bone loss due to certain medications, and in those with bone metastases.



Brand Name

Prolia®



Target

RANKL



Functional Assays

- RANK IκB Neutralization Assay
- RANKL Binding Assay
 - ELISA
- Receptor Binding Assay

DENOSUMAB

Evolocumab is a fully human monoclonal antibody that inhibits proprotein convertase subtilisin/kexin type 9 (PCSK9) a protein that targets LDL receptors for degradation thereby reducing the liver's ability to remove LDL-C from the blood. It is used to treat hyperlipidemia.



Brand Name

Repatha®



Target

PCSK9



Functional Assays

- ELISA
- Binding Assays
- Receptor Binding Assays

Structural Characterization

- Primary (*de novo*) Amino Acid Sequence
- Amino Acid Composition
- Terminal Amino Acid Sequence
- Peptide Map
- Disulfide Bridges
- Carbohydrate Structure
- Higher Order Structure

Physicochemical Characterization

- Identity and Purity
- Molecular Weight
- Isoform Pattern
- Liquid Chromatographic Patterns
- Aggregation

Product Specific Technical Considerations

- Disulfide bridges
- C-terminal Lysine
- Glycosylation (in particular levels of galactosylation, fucosylation and sialylation, and glycosylation site profiling and occupancy assessment)
- Deamidation
- Oxidation

Aflibercept binds to circulating VEGFs and acts like a "VEGF trap" thereby inhibiting the activity of subtypes VEGF-A and VEGF-B, as well as to placental growth factor (PGF), inhibiting the growth of new blood vessels. Aflibercept is an anti-angiogenic treatment that inhibits both VEGF-A and PGF 1-4. It is used to treat macular degeneration and macular edema (Eylea) as well as metastatic colorectal cancer (Zaltrap).



Brand Name

Eylea[®] and Zaltrap[®]



Target

VEGF



Functional Assays

- ELISA
 - Anti-Human FC
 - FcRn Binding Assay
- Neutralization Assays
- Receptor Binding Assays
- VEGF Binding Assay

Structural Characterization

- Primary (*de novo*) Amino Acid Sequence
- Amino Acid Composition
- Terminal Amino Acid Sequence
- Peptide Map
- Disulfide Bridges
- Carbohydrate Structure
- Higher Order Structure

Physicochemical Characterization

- Identity and Purity
- Molecular Weight
- Isoform Pattern
- Liquid Chromatographic Patterns
- Aggregation

Product Specific Technical Considerations

- Disulfide bridges (particularly in the TNF α region)
- C-terminal Lysine
- Glycosylation (in particular, levels of galactosylation, fucosylation and sialylation, N-glycosylation site profiling and occupancy and O-glycosylation structure and site occupancy).
- Deamidation
- Oxidation

Etanercept is a TNF inhibitor that binds to the TNF receptor to the constant end of the IgG1 antibody. It is used to treat ailments such as rheumatoid arthritis and plaque psoriasis.



Brand Name

Enbrel®



Target

TNF α



Functional Assays

- Caspase 3/Apoptosis Assay
- NF κ B Reporter Assay
- TNF α Neutralization Assay
- Antibody-Dependent Cell Cytotoxicity (ADCC) Reporter Bioassay
- Complement-Dependent Cytotoxicity (CDC) Assay
- Proliferation Assays
- Binding Assays
 - ELISA
 - BLI

Structural Characterization

- Primary (*de novo*) Amino Acid Sequence
- Amino Acid Composition
- Terminal Amino Acid Sequence
- Peptide Map
- Disulfide Bridges
- Higher Order Structure

Physicochemical Characterization

- Identity and Purity
- Molecular Weight
- Isoform Pattern
- Liquid Chromatographic Patterns
- Aggregation

Product Specific Technical Considerations

- Levels of Pyroglutamination
- Disulfide bridges
- Deamidation
- Oxidation

Abciximab binds to the GPIIb/IIIa receptors blocking the binding of fibrinogen. This platelet aggregation inhibitor is used during and after coronary artery procedures to prevent platelets from sticking together and causing blood clots.



Brand Name

ReoPro®



Target

CD41



Functional Assays

- Binding Assays
- Receptor Binding Assays

Structural Characterization

- Primary (*de novo*) Amino Acid Sequence
- Amino Acid Composition
- Terminal Amino Acid Sequence
- Peptide Map
- Disulfide Bridges
- Higher Order Structure

Physicochemical Characterization

- Identity and Purity
- Molecular Weight
- Isoform Pattern
- Liquid Chromatographic Patterns
- Aggregation

Product Specific Technical Considerations

- Levels of Pyroglutamination
- PEGylation site assessment
- Disulfide bridges
- Deamidation
- Oxidation

CERTOLIZUMAB

Certolizumab neutralizes membrane-associated and soluble TNF α resulting in a dose-dependent inhibition of LPS-induced TNF α and IL-1 β production in human monocytes. Certolizumab treats inflammatory conditions such as rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis and Crohn's disease.



Brand Name

Cimzia[®]



Target

TNF α



Functional Assays

- ELISA
- Neutralization Assays
- Binding Affinity Assays

Structural Characterization

- Primary (*de novo*) Amino Acid Sequence
- Amino Acid Composition
- Terminal Amino Acid Sequence
- Peptide Map
- Disulfide Bridges
- Higher Order Structure

Physicochemical Characterization

- Identity and Purity
- Molecular Weight
- Isoform Pattern
- Liquid Chromatographic Patterns
- Aggregation

Product Specific Technical Considerations

- Levels of Pyroglutamination
- Disulfide bridges
- Deamidation
- Oxidation

RANIBIZUMAB

Ranibizumab binds to the receptor binding site on VEGF-A inhibiting the binding of VEGF molecules to their receptors on the surface of endothelial cells. Ranibizumab is used for the treatment of wet age-related macular degeneration, macular edema and diabetic retinopathy.



Brand Name

Lucentis®



Target

VEGF-A (Vascular Endothelial Growth Factor)



Functional Assays

- VEGF Neutralization Assay
- VEGF Reporter Bioassay
- Binding Assays

Structural Characterization

- Primary (*de novo*) Amino Acid Sequence
- Amino Acid Composition
- Terminal Amino Acid Sequence
- Peptide Map
- Disulfide Bridges
- Carbohydrate Structure
- Higher Order Structure

Physicochemical Characterization

- Identity and Purity
- Molecular Weight
- Isoform Pattern
- Liquid Chromatographic Patterns
- Aggregation

Product Specific Technical Considerations

- The analysis of glycosylation is important when analyzing DPO. As requested in the ICH guidelines (Topic Q6B), the carbohydrate content (neutral sugars, amino sugars and sialic acids) should be determined. In addition, the structure of the carbohydrate chains, the oligosaccharide pattern (antennary profile), the glycosylation site(s) and occupancy should be analyzed.
- DPO has five N-glycosylation sites at Asn24, Asn30, Asn38, Asn83 and Asn86 and one O-linked site at Ser126.
- Glycan structures should be characterized and particular attention should be paid to their degree of sialylation, the glycans present at each of the sites and the degree of glycosylation at each site (glycosylation site occupancy).

Darbepoetin (DPO) alfa binds to the erythropoietin receptor on erythroid progenitor cells, stimulating red blood cell production and differentiation. It is used for the treatment of anemia due to chronic kidney disease.



Brand Name

Aranesp[®]



Target

Erythropoietin receptor



Functional Assays

- Neutralizing Antibody Assay
- Cell Proliferation Assays
- Receptor Binding Affinity Assays

Structural Characterization

- Primary (*de novo*) Amino Acid Sequence
- Amino Acid Composition
- Terminal Amino Acid Sequence
- Peptide Map
- Disulfide Bridges
- Carbohydrate Structure
- Higher Order Structure

Physicochemical Characterization

- Identity and Purity
- Molecular Weight
- Isoform Pattern
- Liquid Chromatographic Patterns
- Aggregation

Product Specific Technical Considerations

- The analysis of glycosylation is important when analyzing EPO. As requested in the ICH guidelines (Topic Q6B), the carbohydrate content (neutral sugars, amino sugars and sialic acids) should be determined. In addition, the structure of the carbohydrate chains, the oligosaccharide pattern (antennary profile), the glycosylation site(s) and occupancy should be analyzed.
- EPO has three N-glycosylation sites at Asn24, Asn38 and Asn83 and one O-linked site at Ser126.
- Glycan structures should be characterized and particular attention should be paid to their degree of sialylation, the glycans present at each of the sites and the degree of glycosylation at each site (glycosylation site occupancy).

Erythropoietin binds to the EPO receptor on the red cell progenitor surface and activates the JAK2 signaling cascade initiating the STAT5, PIK3 and Ras MAPK pathways. It is used as a treatment for anemia.



Target

Red blood cell production



Functional Assays

- ELISA
- Proliferation Assays
- Immunoassays
- Receptor Binding Assays

Structural Characterization

- Primary (*de novo*) Amino Acid Sequence
- Amino Acid Composition
- Terminal Amino Acid Sequence
- Peptide Map
- Disulfide Bridges
- Higher Order Structure

Physicochemical Characterization

- Identity and Purity
- Molecular Weight
- Isoform Pattern
- Liquid Chromatographic Patterns
- Aggregation

Product Specific Technical Considerations

- N-terminal heterogeneity
- Disulfide bridges
- Deamidation
- Oxidation

Filgrastim is a human granulocyte colony-stimulating factor (G-CSF) that binds to cell surface receptors on hematopoietic cells within the bone marrow accelerating neutrophil progenitor proliferation and differentiation. Filgrastim is used to treat neutropenia associated with HIV/AIDS, chemotherapy, and bone marrow transplantation.



Brand Name

Neupogen®



Target

Manufactured recombinant DNA granulocyte-colony stimulating factor



Functional Assays

- Cell Proliferation Assay
- Receptor Binding Assays
- ELISA
- Neutralization Assays

Structural Characterization

- Primary (*de novo*) Amino Acid Sequence
- Amino Acid Composition
- Terminal Amino Acid Sequence
- Peptide Map
- Disulfide Bridges
- Higher Order Structure

Physicochemical Characterization

- Identity and Purity
- Molecular Weight
- Isoform Pattern
- Liquid Chromatographic Patterns
- Aggregation

Product Specific Technical Considerations

- PEGylation site assessment
- N-terminal heterogeneity
- Disulfide bridges
- Deamidation
- Oxidation

Peg-Filgrastim is a PEGylated form of the recombinant human GCSF analog filgrastim that stimulates the production of neutrophils. It is used to stimulate bone marrow to produce neutrophils to fight infection in chemotherapy patients.



Brand Name

Neulasta®



Target

Recombinant protein that binds to granulocyte-colony stimulating factor receptor



Functional Assays

- Cell proliferation Assay
- Receptor Binding Assays
- ELISA
- Neutralization Assays

BioPharmaSpec Ltd

Suite 3.1, Lido Medical Centre

St. Saviour, Jersey

JE2 7LA, UK

Tel: +44 (0)1534 483493

Fax: +44 (0)1534 483494

BioPharmaSpec Inc

363 Phoenixville Pike

Malvern, PA 19355

USA

Tel: +1 610-640-5866

Fax: +1 610-640-5773

CONTACT US

Custom Biologics

115 Skyway Avenue

Toronto, Ontario

M9W 4Z4. Canada

Tel: 416-479-3773

Email: info@custombiologics.com