ICH Q6B Area: Structural characterisation

Glycan characterisation	
Analysis	**Technique**
***N*- and *O*- glycan characterisation**	*N*-glycans released enzymatically, *O*-glycans released by chemical treatment. Fluorescent labelling of released glycans and analysis by UPLC-FLD (Waters™ Acquity™-FLD) and LC-MS (Thermo Scientific™ Vanquish™-Q Exactive Plus™) or CE LIF (Beckman Coulter™ PA800 plus™). Linkage confirmation by exoglycosidase digestion.
Sialic acid quantitation	DMB labelling of hydrolysed sialic acid and analysis by UPLC-FLD. Quantitation using DMB labelled standards.
Sialic acid linkage relative quantitation	Derivatisation of sialic acids by DMT-MM and analysis by LC-MS.
Site occupancy	Comparison of glycosylated and deglycosylated sample peptide maps by LC-MS.

Protein characterisation	
Analysis	**Technique**
Amino acid sequence	Peptide mapping by LC-MS and bioinformatic analysis against provided protein sequence
N- and C- terminal sequencing	Confirmation of N- and C- terminal amino acids by peptide mapping (detection of blocked N- terminus pyroglutamate/pyroglutamic acid)
	Top down intact mass for orthogonal confirmation
Amino acid composition	Derivatisation and quantitation with AccQ.Tag™ Ultra by UPLC-UV/FLR
Disulfide bonds	Comparison of reduced and non-reduced peptide mapping by LC-MS
Free Thiols	Determination of free thiols using DNTB

ICH Q6B Area: Physicochemical properties

Analysis	Technique
Intact protein molecular weight	Molecular weight determination by RP-LC-MS
	Native MS
Isoform pattern	Profiling of isoforms by various techniques: cIEF peptide mapping and UPLC: IEX, HIC, RP, SEC
Determination of extinction coefficient	Amino acid analysis combined with UV 280nm dilution series

ICH Q6B Area: Process and product related impurities

Analysis	Technique
Aggregate analysis	Determination of aggregates and fragments by SEC, AUC and LC-MS
Molecular variants	Relative quantitation of deamidation, oxidation and other PTM's by peptide mapping LC-MS
	Charge variant analysis by IEX-UPLC and CIEF
	Oxidation by HIC and RP-UPLC
Host Cell Proteins (HCP)	Absolute quantitation of HCP by LC-MS and ELISA
Residual protein A	qPCR using ProteinSEQ™
Host Cell DNA	qPCR using resDNASEQ™
PPG and PEG analysis	Detection of free PPG and PEG by UPLC-CAD
Extractables and Leachables	Detection and quantitation of extracted and leached compounds by ICP-MS, GC-MS and LC-MS.

ICH Q6B Area: Immunochemical Properties

Analysis	Technique
Target antigen binding	Kinetics and affinity/epitope mapping/thermodynamic profiling by SPR (GE Healthcare™ - Biacore T100™)
Effector binding	Measurement of FcγR/FcRn/C1q binding by SPR

ICH Q6B Area: Biological Activity

Analysis	Technique
Cell proliferation	Measurement of thymidine analogue (BruD) incorporation by ELISA (Biotek®-Synergy™ H1) DNA-based measurement of cell cycle (Go/G1, S and G2/M) induction/inhibition using FCM MTT assay with colorimetric readout
Cell death (Autophagy/Apoptosis)	Extrinsic (e.g. Fas) and intrinsic (e.g. caspase activation) apoptosis measurement by FCM (BD™ - FACSMelody™) and ELISA Autophagic flux monitoring by FCM and ELISA
Antibody effector function (ADCC/ADCP)	Mechanism of Action (MOA)-based human FcγRIIIa/FcγRIIa reporter bioassays using luciferase-based detection
Enzyme activity	Kinase/phosphatase/protease activity measurement by FCM and ELISA

Bespoke Analytical Development and Consultancy

Analysis	Technique
Feasibility/pre-validation, verification and qualification	As per client request
Troubleshooting of existing client methods	As per client request
Replication of methods for IP litigation	As per client request
Consultancy	As per client request