Manufacturing of Complex Biobetters: Half-life
Extended Fusion Proteins and Bispecific Molecules
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Introduction
Rentschler Biotechnology is currently manufacturing a huge variety of fusion proteins and bispecific molecules. Several of them belong to the class of biobetters with engineered properties to extend the plasma half life. If they are lacking a common affinity motif, no platform process for simple host cell protein removal exists. Some are sensitive to low pH treatments, thus requiring alternative virus inactivation steps. Particularly complex molecules suffer from product related impurities such as aggregates or mispairing.

Concepts for half life (t1/2) extensions

Size and recycling
- Albumin
- IgG (Fc)
- Transferrin
- FcRn Binder

Hydrodynamic radius
- Repetitive peptides XTEN or PAS
- Glycosylated peptides CTP or NNT/S

Aggregate formation
- Elastin like peptides
- Gelatine like peptides

Other concepts
- Receptor-ligand fusions
- Latency peptide fusions

Conclusions
Modifying glycosylation is an important tool to generate biobetters:
- It increases hydrodynamic radius of proteins thus improving renal clearance
- Half life extension is achieved both through N- or O-glycosylation
- Glycosylation sites can be introduced into the molecule by mutagenesis or through peptide fusion at either N- or C-terminus
- Existing glyco-patterns can be improved through cultivation conditions
- Selection of glyco-isomers during downstream processing always require the presence of an anion exchanger step

Study 1: t1/2 improved variants of FVIIa

F7F: O-Glycosylation sites fused to heavy chain
F7G: N-Glycosylation sites within LC and HC

Effects: 5x better half life than native FVIIa

Manufacturing challenges:
- Homogeneity of drug substance
- Protease activation and auto-cleavage
- Removal of product related impurities
- Virus inactivation due to low pH sensitivity

DSP unit operations

Common:
- Low expression needs concentration by UF/DF
- Virus inactivation by Triton due to pH sensitivity
- Hydroxyapatite to remove impurities
- CTP or NNT/S

Study 2: Improved variant of Belatacept (CTLA4-Fc fusion)

Original: binds CD80 & CD86, but prefers CD80
Variant NYF: maintains CD80 binding but improved CD86 binding through mutagenesis

Effects: NYF has increased immunosuppressive potency (improved efficacy and safety?)
Manufacturing challenges:
- Improvement of titer and glycosylation in USP

USP: New cultivation and feed strategy

Effects on product quantity
- Shortened process time by 1 day
- Comparable cell concentration and viability
- Doubled protein titer

Effect on product quality

Human
Hamster (CHO)
Mouse (NSO)

GMP 1-4
GMP 5-9

Harvest

Subheading

Figure

Graph

Table

Diagram