An evaluation of the cGMP manufacturing process economics and high-throughput characterisation of Targeted Secretion Inhibitors

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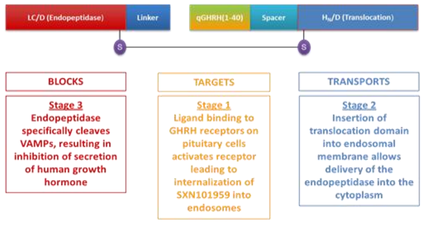
Introduction

Targeted Secretion Inhibitors (TSIs) are a novel class of recombinant biotherapeutics, using protein engineering to re-target Botulinum neurotoxins for treatment of diseases with secretion disorders. SXN101959 has been successfully manufactured to cGMP standards and is an example of the emerging recombinant TSI manufacturing platform. SXN101959 is a multi-domain, multi-functional recombinant protein expressed within *Escherichia coli*, composed of a light chain (LC/D) endopeptidase domain and a heavy chain (H_N/D) domain with a growth hormone releasing hormone (GHRH) targeting peptide ligand (Figure 1).

Figure 1. Schematic of SXN101959 structure and function

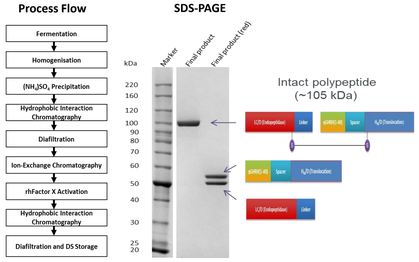


LC/D	Light chain endopeptidase domain of Botulinum neurotoxin serotype D
GHRH	Growth hormone releasing hormone
H_N/D	N-terminal domain of the Botulinum neurotoxin serotype D heavy chain
VAMP	Vesicle-associated membrane protein

The Engineering and Physical Sciences Research Council (EPSRC) within the UK has established a Centre for Innovative Manufacturing in Emergent Macromolecular Therapies at University College London (Figure 2). The Centre provides an international lead in delivering biopharmaceutical manufacturing innovations for next generation therapies, which has included an evaluation of the manufacture and characterisation of SXN101959.

Figure 2. EPSRC centre for innovative manufacturing in emergent macromolecular therapies

Figure 3. SXN101959 drug substance manufacturing process



Under non-reducing conditions, SXN101959 (~105 kDa) maintains the interchain disulphide bond. Under reducing and denaturing conditions, SXN101959 is converted into the free light chain (~50 kDa) and the heavy chain with qGHRH ligand (~55 kDa). Each of these moieties has a specific electrophoretic migration pattern.

Methods

SXN101959 is expressed as a soluble single polypeptide chain and is subsequently purified by selective precipitation, column chromatography and diafiltration, to greater than 95% purity. During the purification process, the protein is activated by the addition of recombinant human Factor X, which cleaves at a specific recognition site, producing the di-chain protein that is joined by a disulphide bond (Figure 3). Although SXN101959 is based on Botulinum neurotoxin, it is engineered on a backbone without the Hc neuronal targeting domain, allowing manufacturing at Biosafety Category 1.

GMP Manufacturing of SXN101959

GMP manufacturing of SXN101959 drug substance (DS) was performed by SynCo Bio Partners (Amsterdam, The Netherlands) at 200 Litre scale, followed by drug product (DP) manufacturing. Purified SXN101959 DS was stored in polypropylene bottles and a proportion converted into DP in 2R vials at a concentration of 1 mg/mL. 12 months of GMP-compliant stability data and shelf-life were established for DS at -70°C and for DP at 2 to 8°C.

Results

Here we describe an assessment of the manufacturing economics and manufacturability for SXN101959, as well as the development of biophysical characterisation tools that rapidly assess aggregation propensity and facilitate formulation development.

Manufacturing process economics

Process and economic data from the cGMP manufacturing of SXN101959 were collated and combined into a spreadsheet-based mathematical model, to determine the key cost factors and facilitate future improvements to reduce manufacturing costs.

- Figure 4 (A) shows the distribution of material costs per batch by unit operation. The initial chromatography step is the most expensive; 62% of the overall costs being associated with resin costs. Upstream (fermentation) is the next most expensive step, comprising media and single-use bag costs.
- Figure 4 (B) illustrates how the total process cost of goods (COG) was affected by variations both in product potency and titre. Increasing product titre at high and low product potencies has a lesser effect on the total COG compared to decreasing it.

Figure 4. Process economics modelling of a novel Targeted Secretion Inhibitor manufacturing platform

(A) Material costs per batch by unit operation

(B) Surface plot of related cost trends

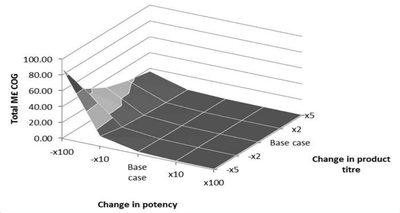
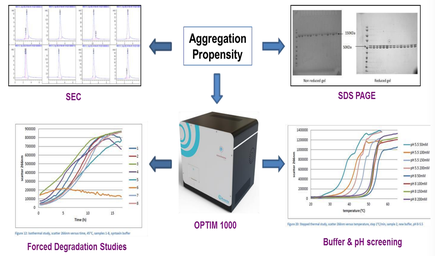


Figure 5. Rapid evaluation of molecule aggregation propensity using high-throughput stress tests



Biophysical characterisation

The aggregation properties of SXN101959 were studied by Size Exclusion High Performance Liquid Chromatography (SE-HPLC), Static Light Scattering (SLS), Intrinsic Fluorescence and monitoring binding of fluorescent dyes. These studies were performed in various buffers.

- SE-HPLC provided an initial detection of soluble aggregates and degradation products during storage, SDS-PAGE being used to determine the identity and purity of SXN101959.
- Isothermal studies, monitored by SLS at 266nm at 45°C, indicated that all the samples aggregated, except sample 6.
- Stepped thermal stability studies of the DS, monitored by light scattering indicates that 200mM NaCl provides more stability than lower salt concentrations and pH 5.5 provides less stability than pH 7 or pH 8. A DS formulation of pH7 and 200mM NaCl is therefore proposed.

Conclusions

- Targeted Secretion Inhibitors are a novel class of recombinant biotherapeutics, developed by re-targeting Botulinum neurotoxins for the treatment of diseases with secretion disorders.
- Targeted Secretion Inhibitors, such as SXN101959, can be produced by cGMP compliant manufacture for supply to toxicology or clinical studies.
- A spreadsheet-based mathematical model has been developed to identify key costs in the manufacturing process, and to estimate the effects of changes in manufacturing on the costs of goods.
- A suite of biophysical characterisation decisional tools have been developed to rapidly assess aggregation propensity and facilitate formulation development.

Keywords

Botulinum neurotoxin (BoNT); Targeted Secretion Inhibitor (TSI); SXN101959; Manufacture; Process development, Product characterisation.

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