Signal Peptide Optimization: Effect On Recombinant Monoclonal IgG Productivity, Product Quality And Antigen-Binding Affinity

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Abstract
A signal peptide is a 20-30 amino acid (aa) peptide present at the N-terminus of secretory proteins. Signal peptides are known to have a strong impact on both the efficiency of protein secretion and correct processing at the N-terminus. N-terminal heterogeneity can have an impact on both the product quality and bioactivity of the biopharmaceutical product.

In this study, we evaluated two signal peptide sequences for a model recombinant humanized IgG. Productivity was evaluated at the transient transfection and stable (bulk and minipool) stage using FACS analysis. N-terminal cleavage was evaluated using peptide mapping and intact mass analysis. N-terminal heterogeneity was observed with one of the signal peptides resulting in a major species with D mass 159 added to the N-terminus of the Light Chain. No effect on either the product quality or the antigen binding affinity of the purified product was seen. Site-directed mutagenesis was successfully used to re-engineer the signal peptide to prevent this mis-cleavage. However, this resulted in a decrease in the productivity of the recombinant IgG without affecting the product quality or bioactivity. The results underscore the importance of evaluating more signal peptide designs with a balanced optimization strategy to ensure correct N-terminal cleavage and high productivity.

Background and Methods

MATERIALS AND METHODS

Cell Culture and Fed Batch Assay
CHOZN GS – hi cell lines were transfected and single cell clones generated expressing the model humanized monoclonal antibody. Two different vector backbones: CLE 305 and CLE 306 were evaluated. Fed batch assays were carried out in duplicate 50 ml TPP® bioreactor tubes and replenished with glucose and glutamine or glucose alone on days 4 and 7. Cultures were maintained in a 5% CO2 incubator. CHOZN GS CHO CD Fusion (Sigma-Aldrich, Cat No: 94531).

Surface Antigen Staining of Secreted IgG and Analysis by FACS
Transient or stably transfected cells were stained using a PE conjugated Fab'2 fragment Donkey Anti-human IgG (Jackson ImmunoResearch Laboratories Inc #709-116-149). Washed two times with PBS and analyzed on FACS

Site Directed Mutagenesis
Primers including B labor pair delution corresponding to the two amino acids at the C-terminus of the Signal Peptide (Threonine and Glycine) on the Light Chain were designed and synthesised. Site-Directed Mutagenesis of CLE 306 vector was performed using the QuikChange II XL Kit, (Agilent, Cat#20521-1) per manufacturer’s protocol. The new vector was called CLE 323. CLE 306 was also re engineered germline signal peptide on the Light Chain. The new vector was called CLE 320.

Results

Expression Confirmed at Transient and Stable Pools

Expression Vector

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<tr>
<th>Expression Vector</th>
<th>CLE 305</th>
<th>CLE 306</th>
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<tr>
<td>Transient transfection</td>
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<td>Stable Pools –Gin Selection</td>
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N-terminal heterogeneity of Light Chain

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<th>CLE 320</th>
<th>CLE 323</th>
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<td>HC peaks</td>
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Engineering of Signal Peptide for Correct Processing

![Signal peptide sequence](image)

- Site-directed mutagenesis was used to delete two C-terminus as predicted from the Signal Peptide sequence on the LC. Signal P and SIGCleave software predicted correct processing of the engineered signal peptide.

Conclusions

- Signal peptide on the Light Chain of CLE 305 and CLE 306 resulted in both transient and stable expression of the model IgG.
- This version resulted in mis-cleavage of the signal peptide sequence resulting in:
  - A major peak with D mass 159
  - This mass corresponds to the mass of two amino acids Threonine + Glycine preceding the cleavage site
  - No effect on product quality was observed

Acknowledgements

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![Image](image)