Practical application of analytical tools for characterization of an impurity-related particle formation mechanism

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Protein aggregation measurement in biotherapeutics

Maryland Center for Excellence in Regulatory Science and Innovation (MCERSI) and the Bio- & Nano-Technology Center of the University of Maryland.

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Manufacturing of monoclonal antibodies (mAbs)

Cell culture

Purification
Formulation
Fill-Finish

Drug Product
Unusually high levels of particles were observed for mAb-1

Particles formed over a period of weeks at 40°C and months at 2-8°C
The particles contained mAb-1 and its fragments

- Particles have a proteinaceous appearance
- No heavy elements were detected in the particles
- FTIR showed bands typical of near-native or native mAbs (β-sheet, turns)
- Particles contained mAb heavy chain (HC), light chain (LC) and fragments
- Elemental impurities not detected by ICP-MS
- No bioburden was detected
Stability and purity issues were observed for early mAb-1 lots

<table>
<thead>
<tr>
<th>Lot</th>
<th>Process/Scale</th>
<th>HCP Level (ng/mg)</th>
<th>Rate at 40°C (% per month) HPSEC Purity Loss</th>
<th>RP-HPLC Fragmentation</th>
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<td>A</td>
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<tr>
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High HCP levels

High and variable fragmentation rates at 40°C

Formation of delayed-onset particles
Could high HCP levels be linked to particle formation and fragmentation?

**A residual host cell protease?**

- Proteome analysis has identified > 6000 chinese hamster ovary (CHO) HCPs (Baycin-Hizal et al., 2012)

- Some HCPs can bind to the mAb making them harder to remove during the purification process (Valente et al. 2015)


Trace residual levels of an aspartyl protease was the cause of particle formation

Protease activity assay

Sub-visible particle formation

Soluble fragmentation

- Aspartyl protease inhibitor reduced protease activity, particle formation, and fragmentation rate (other inhibitors did not have same effect)
- Inhibitor only slightly decreased soluble fragment levels
- Mass spec detected multiple c-terminal heavy chain fragments in insoluble particles (these same fragments were not detected in soluble form by RP-HPLC)
With affinity enrichment, the aspartyl protease was identified as cathepsin D

- Cathepsin D is a 48 kDa glycosylated aspartyl protease active at < pH 6
- Active site located in hydrophobic cleft; preferentially cleaves between two hydrophobic amino acid residues under slightly acidic or acidic conditions (Sun et al. 2013)
- Spiking this purified cathepsin D into mAb-1 caused particle formation
Trace residual levels of CHO cathepsin D caused particle formation in the final mAb-1 product.

The fix: process was optimized to remove HCPs

Process optimization focused on removal of HCPs was successful in eliminating particle formation in the final mAb-1 product.

‘Caprylate wash’ – developed by David Gruber and Richard Turner and applied to mAb-1 by Christopher Afdahl and Kristin Jusino

Process 1b lots were confirmed to be free of any detectable protease activity

In addition, the optimized process 1b lots did not form delayed-onset particles (12 weeks at 40°C and 12 months at 5°C)
Why did mAb-1 have this problem?

*Does it bind cathepsin D?*

SPR of immobilized CHO cathepsin D was used to detect its binding to a panel of mAbs

Bee at al. Identification of an IgG CDR sequence contributing to co-purification of the host cell protease cathepsin D. Submitted, under review

The Fab region of mAb-1 was involved in binding
An ‘LYY’ motif was a unique match for the 2 mAbs (out of 13 tested) that bound cathepsin D

Potential cathepsin D binding sequences were those that were a unique match to both, but only, mAb-1 and mAb-6.
Mutation of ‘LYY’ to ‘AAA’ eliminated binding to cathepsin D, but unfortunately also eliminated target binding.

Mutation confirmed that the LYY motif in the HC CDR2 was involved in weak binding to CHO cathepsin D.
Summary

• Particles formed in mAb-1 were found to contain mAb-1 and its HC fragments using microscopy, SEM-EDX, FTIR microscopy, and mass spectrometry.

• The presence of trace amounts of an aspartyl protease, cathepsin D, was the cause of particle formation.

• Optimization of the purification process was able to reduce the HCP levels resulting in a stable product.

• Further studies identified an ‘LYY’ motif in mAb-1 that could bind to cathepsin D, resulting in its trace co-purification.
Acknowledgments

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Jeffrey Gill – Fab and Fc generation
Li Peng – design and making of AAA mutant
Richard L. Remmele Jr. and Mariana Dimitrova – hypothesis generation and expt. design
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