Robust Viral Clearance Capacity of CTP-Modified Long-Acting Growth Hormone (MOD-4023) Downstream Production Process

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INTRODUCTION AND OBJECTIVE

- **OPKO Biologics** is a clinical-stage public company developing long-acting therapeutic proteins utilizing CTP technology. The technology involves fusion of the C-terminus peptide of human chorionic gonadotropin (hCG), a highly O-glycosylated peptide, to the target protein.
- CTP was utilized to generate a long-acting human growth hormone (hGH) (MOD-4023) that is produced in a CHO stable cell line, and supports a once-weekly injection in growth hormone-deficient patients.
- The purification process consists of 4 chromatographic steps, UFDF steps, a viral inactivation and a viral filtration steps.
- **Objective:** Validate effective inactivation and/or removal of viruses during the downstream process as a part of the demonstration of the safety of pharmaceutical products derived from biological sources. The study was designed to support MAA/BLA.

PRODUCTION PROCESS

<table>
<thead>
<tr>
<th>Process Step</th>
<th>Step Function</th>
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<tbody>
<tr>
<td>UFDF-1 and depth filtration</td>
<td>Concentration; buffer exchange; particles and bioburden removal</td>
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<tr>
<td>Detergent Virus Inactivation</td>
<td>Inactivation of lipid enveloped viruses</td>
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<tr>
<td>AIRX chromatography</td>
<td>Capture; process and product related impurities removal; Virus removal</td>
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<tr>
<td>HIC chromatography</td>
<td>Process related impurities removal; Virus removal</td>
</tr>
<tr>
<td>UFDF-2</td>
<td>Concentration and buffer exchange; aggregates removal</td>
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<tr>
<td>Mixed mode chromatography</td>
<td>Process related impurities removal; Virus removal</td>
</tr>
<tr>
<td>OPK chromatography</td>
<td>Process and product related impurities removal; Virus removal</td>
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<tr>
<td>Virus filtration</td>
<td>Virus removal</td>
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<tr>
<td>UFDF-3 and Final filtration</td>
<td>Concentration and buffer exchange to final formulation; bioburden reduction</td>
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- MOD-4023 is purified by a multistep process.
- The purification process was designed to present a robust and efficient purification of MOD-4023 while removing process and product related impurities.
- Steps that were evaluated for viral clearance are underlined. HIC chromatography was not included based on results of previous study.
- Theoretical viral particles per dose were calculated by TEM analysis of harvest media to be <6.9 log10, and the overall reduction of the MuLV demonstrated in this study should be at least 12.9 log to show good safety margins.

MODEL VIRUSES

- Four viruses that vary in their biophysical properties and structural features were tested for the chromatographic and filtration step.
- Two enveloped viruses were used for the virus inactivation step.
- The design covers the variation in viral resistance to physical and chemical agents or treatments, and aligned with the ICH guidelines Q5A.

VIRAL CLEARANCE VALIDATION

The study design consists of 5 main components:
- **Scaled down models for columns and nanofilter were validated (including buffer spiking)**
- **Viral inactivation kinetic study** (1 minute to 14 hours)
- **Columns viral clearance capacities** were evaluated using fresh columns and columns at the end of lifetime.
- **Carry-over runs** were performed to analyze cleaning effectiveness.
- **Nano filtration process** was challenged with both maximal pressure as a worst case, integrated pause and pressure drop, and a pressure ramp.

RESULTS

- MOD-4023 purification process provides a robust clearance capacity of ≥23.1 log for enveloped viruses and 9.5 and ≥13.6 log for non-enveloped viruses.
- Low or no infectivity was detected in all carry-over runs suggesting a powerful cleaning in place.

CONCLUSIONS

- **Valid scale-down models** for the chromatographic and filtration steps were successfully established for each process operation.
- MOD-4023 purification process provides a robust and highly efficient viral removal capacity.
- A safety margin of ≥ 16.3 log10 was calculated for X-MuLV as model virus. For each other virus type, at least two orthogonal steps were identified which contributes substantially to virus reduction or inactivation.