EP707

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 **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

Process Step	Step function
UFDF-1 and depth filtration	Concentration; buffer exchange; particles and bioburden removal
Detergent Virus inactivation	**Inactivation of lipid enveloped viruses**
AIEX chromatography	Capture; process and product related impurities removal; **Virus removal**
HIC chromatography	Process related impurities removal
UFDF-2	Concentration and buffer exchange; aggregates removal
Mixed mode chromatography	Process related impurities removal; **Virus removal**
CIEX chromatography	Process and product related impurities removal; **Virus removal**
Virus filtration	**Virus removal**
UFDF-3 and Final filtration	Concentration and buffer exchange to final formulation; bioburden reduction

- 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.

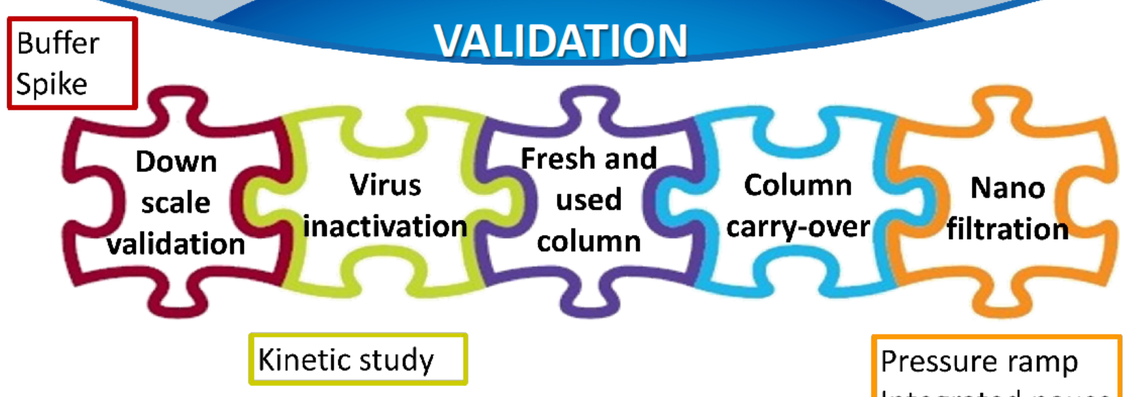
MODEL VIRUSES

- **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.

Information	X-MuLV	PRV	Reo-3	MVM
Size [nm]	80 – 110	120 – 200	60 – 80	20 – 26
Lipid envelope	yes	yes	no	no
Family	Retroviridae	Herpesviridae	Reoviridae	Parvoviridae
Genome	ssRNA	dsDNA	dsRNA	ssDNA
Resistance	low	medium	Medium	high
Rationale	Non-defective C-type retrovirus	Model for human Herpes virus	Model for Reoviridae	Model for both the human and animal parvoviruses

- 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

Step	Run	Carry-over runs: Detectable infectivity in product pool			
		MuLV	PRV	Reo-3	MVM
AIEX	Fresh	No	No	No	1/960*
	Used	No	No	No	No
MMC	Fresh	No	No	No	No
	Used	No	No	No	No
CIEX	Fresh	No	No	No	No
	Used	No	No	No	No

* 1 colony out of 960 wells

- 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**.

Step	Run	Log10 VRF			
		MuLV	PRV	Reo-3	MVM
Viral Inactivation	1	≥ 5.4	≥ 5.2	N/A	N/A
	2	≥ 4.7	≥ 5.2		
AIEX	Fresh	> 4.1	3.0	2.16	1.9
	Used	> 4.0	>3.0	0.62	1.2
MMC	Fresh	2.6	6.5	0.42	2.5
	Used	2.9	4.1	0.86	1.3
CIEX	Fresh	≥ 5.3	5.7	2.42	4.2
	Used	≥ 4.9	≥ 5.8	2.07	3.8
Nanofiltration	1				≥ 7.3
	2	≥ 6.94	≥ 7.8	≥ 7.46	≥ 7.4
	3	≥ 6.88	≥ 7.9	≥ 7.64	≥ 7.4
	4				≥ 7.2
Overall reduction	N/A	≥ 23.1	≥ 25.7	9.5	≥ 13.6

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.

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