Robust Fed-Batch Manufacturing Process of Long Acting Factor VIIa (MOD-5014) in CHO Cells

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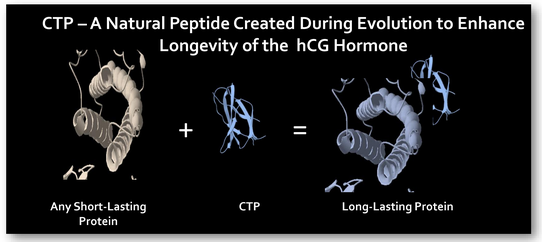
INTRODUCTION AND OBJECTIVE

- **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.
- One of the unmet needs in the coagulation field is to improve the relevant pharmacokinetic parameters. CTP was utilized to generate a long-acting human factor VIIa (FVIIa) (MOD-5014) that is produced in a CHO stable cell line, and is being developed to support a bi-weekly injection for on demand and prophylactic treatment of hemophilia A and B patients.
- **Objective:** develop a fed batch upstream process by recombinant DNA technology using CHO cells in a chemically defined medium, followed by a reproducible and scalable downstream process purifying the highly glycosylated and highly-gamma carboxylated MOD-5014.



METHODS

- The cDNA of MOD-5014 was transfected into CHO cells and stable clones were generated by limiting dilution steps. Highest producing clones were amplified and final clone was selected for further development.
- Comprehensive process development program included media and feed screening followed by process parameters optimization.
- The downstream process was developed to purify and activate FVIIa-CTP with the highest content of gamma carboxylation and effectively remove process and product relates impurities.
- The quality of MOD- 5014 was tested by applying various analytical methods, including:
  - O-glycans and sialic acid content
  - Oxidative related forms
  - Potency by STA-CLOT
  - % of Gla domainless
  - % of non-activated FVII

Production Procedure

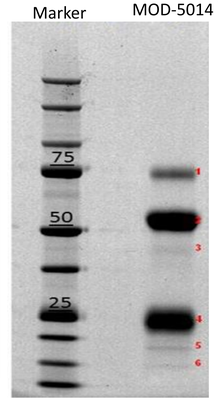
- **Upstream** :
  - Stable clone of CHO cells expressing MOD-5014 is inoculated into 1000 or 2000 L bioreactors, in serum free chemically defined media, and grown in a fed-batch approach, supplemented with vitamin K.
- **Downstream** :
  - Purification process is based on 4 chromatographic columns. The protein is purified on affinity chromatography, mixed mode chromatography, hydrophobic interaction step, and activated on an anion exchange column. the process also contain a virus-inactivation and Nano filtration steps.
  - The downstream process is capturing and purifying the highly-gamma carboxylated, highly glycosylated MOD-5014. The process has high capacity for removal of process related impurities and results in high product quality.

MOD-5014 Production Scheme
Vial Thaw
↓
Seed train
↓
Production BR
↓
Purification
↓
Virus reduction
↓
Activation
↓
Formulation

Quality Attributes

- Highly sialylated and highly Gamma-carboxylated drug substance is obtained at the end of the production process.
- Reduced SDS-PAGE analysis shows the following protein bands:
  75kDa- non activated form (1)
  55kDa- heavy chain-CTP (2)
  25kDa- light chain (4)
  LMW forms (3, 5 and 6).

Method	CMO-1		CMO-2	
	ER	GMP1	GMP1	GMP2
Potency (U/mg)	15,563	16,720	22,478	23,608
Non activated FVII	2.7	2.4	2.6	3.0
Oxidized forms (%)	4.0	4.9	2.9	3.9
Gla domainless (%)	5.4	5.5	0.6	0.6
Sialic acid (mol/mol)	17.1	17.1	18.4	17.2
O-Glycans (mol/mol)	12.3	13.2	13.2	12.5



CONCLUSIONS

- A reproducible fed-batch manufacturing process was developed for the production of highly glycosylated long acting FVIIa-CTP (MOD-5014).
- High content levels of O-Glycans and Sialic Acid and low levels of non-activated FVII were obtained.
- Further development is on going to optimize MOD-5014 quality attributes.

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Hemophilia - clinical
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