96-PP-M

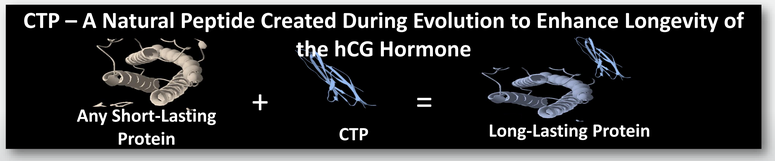
In-vitro Evaluation of Factor VIIa-CTP, a Novel Long-Acting Coagulation Factor

Gili Hart[1] , Tami Livnat* [2], Boris Shenkman[2], Ahuva Bar -Ilan[1], Lior Binder[1], Malka Hoffmann[1], Gili Kenet[2]

[1]R&D, Opko Biologics, Nes Ziona, [2]National Hemophilia Center, Tel Hashomer, Ramat Gan, Israel

Introduction

OPKO Biologics is a clinical stage public company developing bio better long acting versions of existing therapeutic proteins utilizing a technology termed CTP.



The technology involves fusion of the C terminus peptide of hCG to one or both ends of the target protein. The technology was clinically validated and proven as a safe and efficient way for increasing the half-life of several therapeutic proteins while maintaining their biological activity.
MOD-5014 is a long-acting form of recombinant Factor VIIa (rFVIIa -CTP).

The objective of this work was to characterize the FVIIa-CTP *ex-vivo* and *in-vitro* activity of MOD-5014 in comparison to rFVIIa, and to evaluate the off target effect of this product following the attachment of CTP

Methods

FVII-CTP was expressed in CHO cells, purified and activated utilizing a CTP specific purification process. In this study a comparative assessment to commercial rhFVIIa was performed

- ❖ MOD-5014 activity was compared to commercial rhFVIIa in a dose-dependent manner by:
  - Factor X activation- FVII chromogenic assay
  - Affinity to TF by SPR
  - PT and aPTT
- ❖ Off target

MOD-5014 interaction with 87 non specific receptors was evaluated and compared to the commercial rFVIIa

- ❖ Activity of the CTP Moiety of MOD-5014

The clotting activity of non-gamma carboxylated FVIIa-CTP was evaluated using the clotting assay

Conclusions

- ✓ FVIIa-CTP and rFVIIa demonstrated a comparable in –vitro activity as reflected by equivalent clotting time and EC50.
- ✓ FVIIa-CTP affinity to TF was similar to rFVIIa as assessed by SPR (Surface Plasmon Resonance).
- ✓ No unique off target effect when comparing FVIIa-CTP interaction with rFVIIa to non- specific receptors
- ✓ The fusion of CTP moiety to FVIIa does not affect the off target binding profile of MOD-5014
- ✓ Non carboxylated FVIIa-CTP had no clotting activity

Our data suggests that the attachment of CTP to FVIIa maintains comparable functionality and affinity as those of rFVIIa. These results provide a solid rational for the proposed dose range to be used in the upcoming MOD-5014 clinical study in hemophilic patients and implies that MOD-5014 has the potential of being a promising and effective long acting rhFVIIa

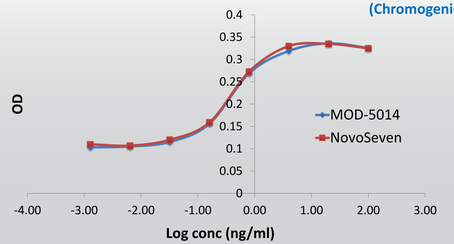
In-vitro Activity

Comparable PT & aPTT values in FVII and FVIII deficient plasma spiked with MOD-5014 and NovoSeven®

Test article	Tested concentration (µg/ml)	PT (sec)		aPTT (sec)	
		Hemophilic Plasma	FVII Deficient Plasma	Hemophilic Plasma	FVII Deficient Plasma
MOD-5014	100	8.7	8.9	22.8	21.0
	4	8.5	8.8	45.0	24.0
NovoSeven	100	8.5	8.7	21.0	21.0
	4	8.3	8.6	38.9	22.7
Control	0	11.9	No coagulation	87.1	27.5
Normal values		11-13.5 Extrinsic pathway		25-35 Intrinsic pathway	

Comparable FX activation MOD-5014 Vs. NovoSeven®

(Chromogenic Assay)



	Average EC50 (ng/ml)
MOD-5014	0.41
NovoSeven®	0.38

MOD-5014 and NovoSeven Present Comparable Binding Kinetics to rh-TF

(By SPR)

	10^5 k on (M^{-1} s^{-1})	10^{-3} k off (s^{-1})	K_d (nM)
MOD 5014	6.08/5.88	1.46/1.36	2.4/2.32
NovoSeven	9.54/11.5	1.82/1.98	1.72/1.91

Off Target

Off Target Binding

The assay package consists of 87 primary molecular targets including 13 enzyme and 74 binding assays. For each assay, the % inhibition of specific binding or activity were measured.

No off target binding effect was observed when comparing MOD-5014 and rFVIIa interactions with the majority of non-specific receptors tested.

Formulation buffer related inhibitions were observed for both MOD-5014 and rFVIIa. This was reflected by the same extent of inhibition when adding formulation buffer as control.

In one out of 87 assays, MOD-5014 and rFVIIa exhibited significant inhibition that can be related to the FVIIa portion of the molecule.

Assay	Analyte	Species	Protein conc.	% Inhibition
Peptidase CTSG (Cathepsin G)	MOD-5014	Human	0.3µM	76
	rFVIIa	Human	0.3µM	84

This data suggests that the CTP moiety of MOD-5014 does not contribute to the high inhibition and does not represent a safety concern

Evaluation of the activity of non-γ-carboxylated FVII-CTP

The measurable activity is related to the Gla domain of the protein as the non carboxylated FVII-CTP did not exhibit any measurable activity

	Clotting time (Sec)	Average activity (%)*
FVIIa-CTP	41.1	49.9
non-γ-carboxylated FVIIa-CTP	>80	No activity

*the results represent the FVII level of the tested sample and are presented as percent of activity relative to normal plasma.

This finding confirms that the CTP moiety of the long-acting FVIIa, does not contribute any measurable activity to the fused MOD-5014 molecule.