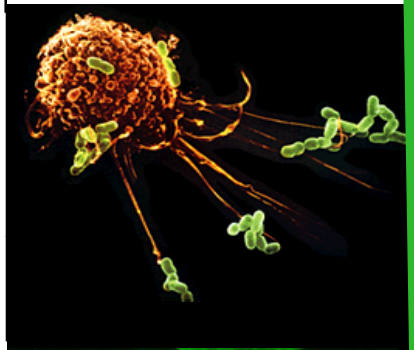
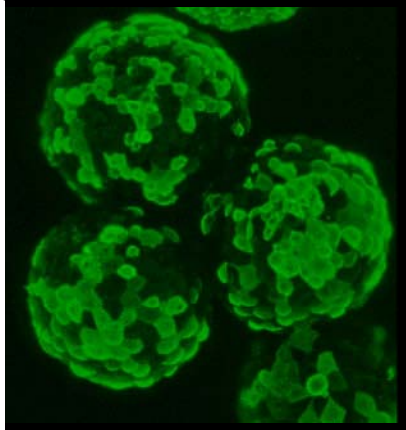
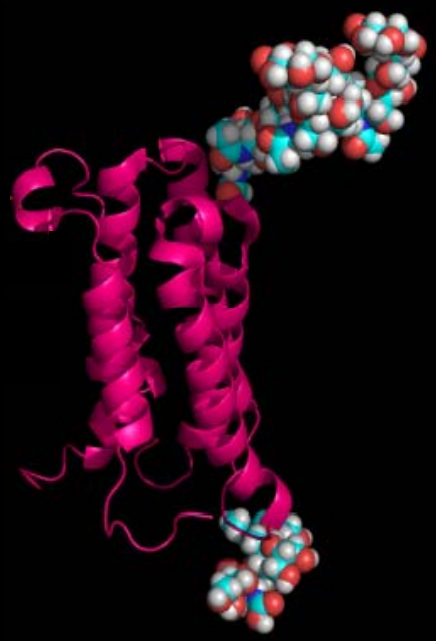
Alios BioPharma





Harnessing Innate Immunity to Cure Disease

Development of Novel Hyperglycosylated Type I IFNs: A Strategy to Improve PK Without Loss of Biological Potency



Lawrence M Blatt, PhD

Clinical Needs for Novel Interferons

» **Chronic Hepatitis C**

- Currently less than 50% of HCV genotype 1 patients can achieve a SVR following Peg-IFN + Ribavirin Therapy
- STAT-C Promising But Some Patients May Not Benefit From 1 Direct Antiviral + Peg-IFN + Ribavirin

» **HBV**

- Goal of Therapy is Sero-conversion in HBeAg positive patients

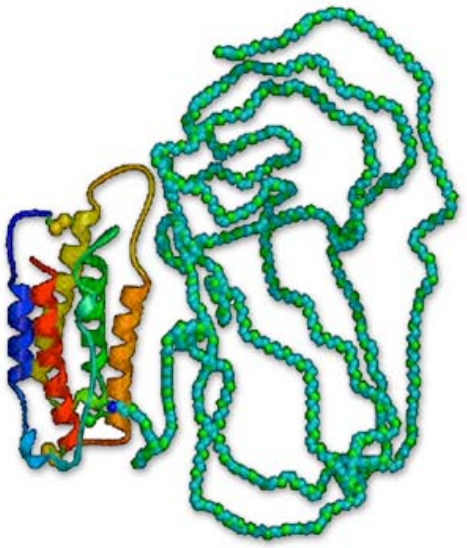
Non-Responders to Triple Rx May Require Novel IFN

The Pegylation model for improving PK

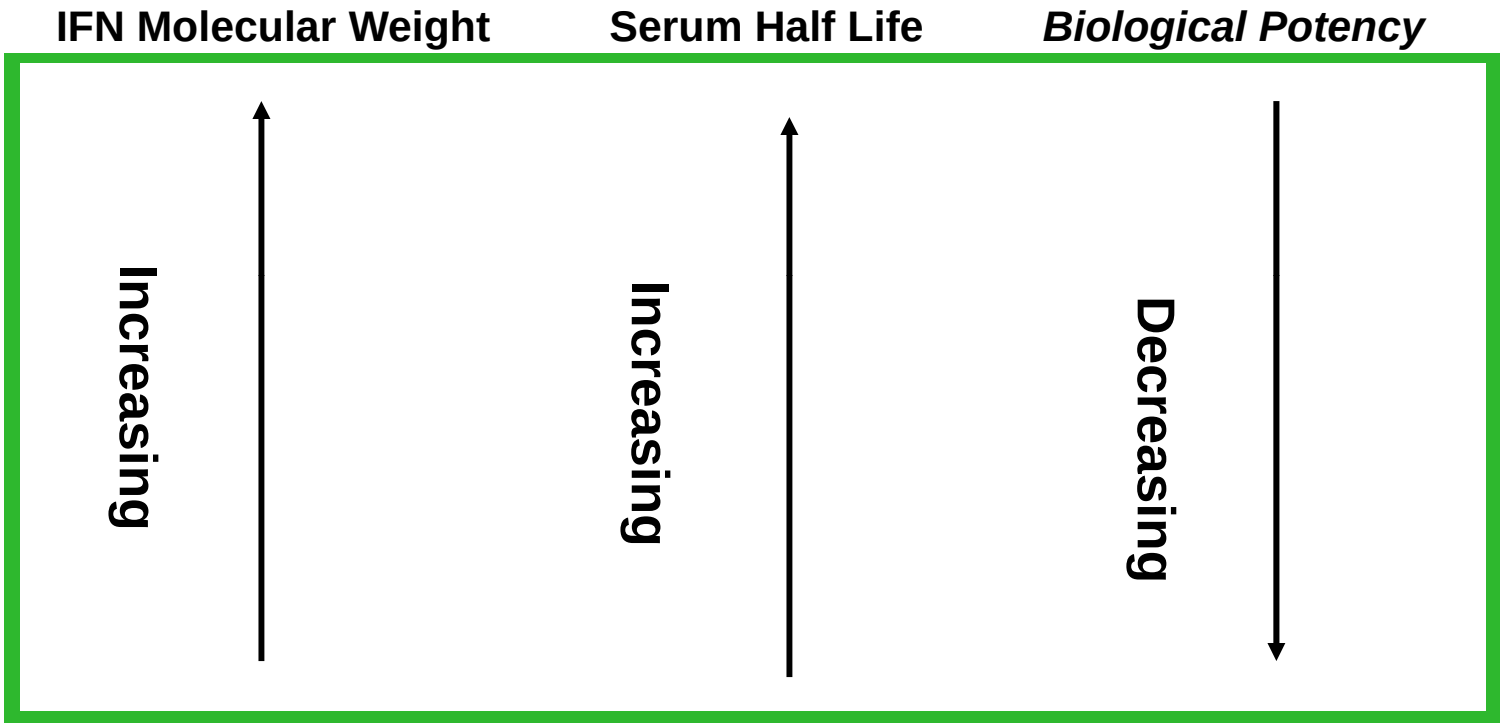
IFN Molecular Weight **Serum Half Life** ***Biological Potency***

Increasing

Increasing

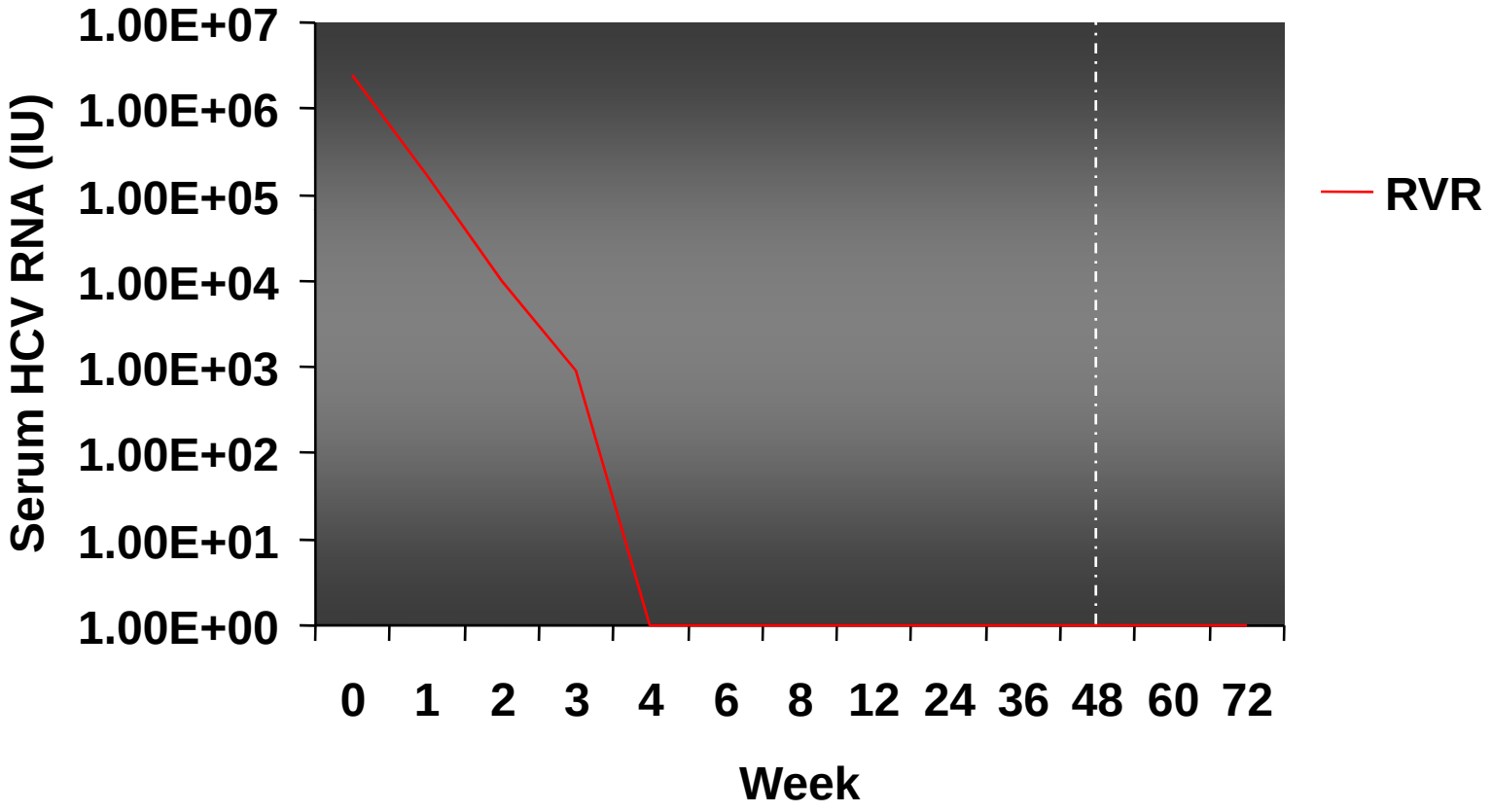


The Pegylation model for improving PK



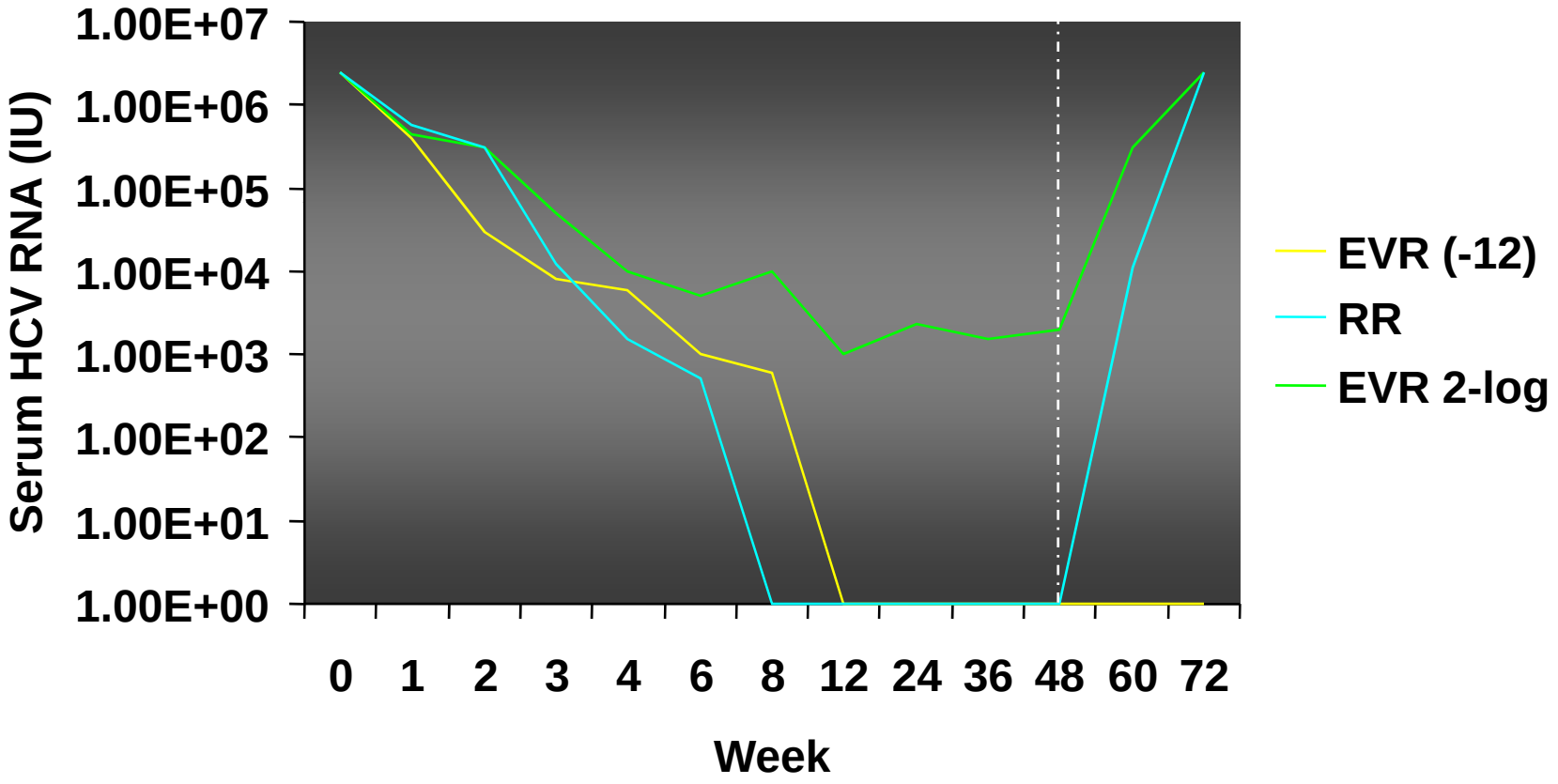
IFN with improved PK but undiminished potency may have advantage

Patterns of Response to Peg-IFN +Ribavirin



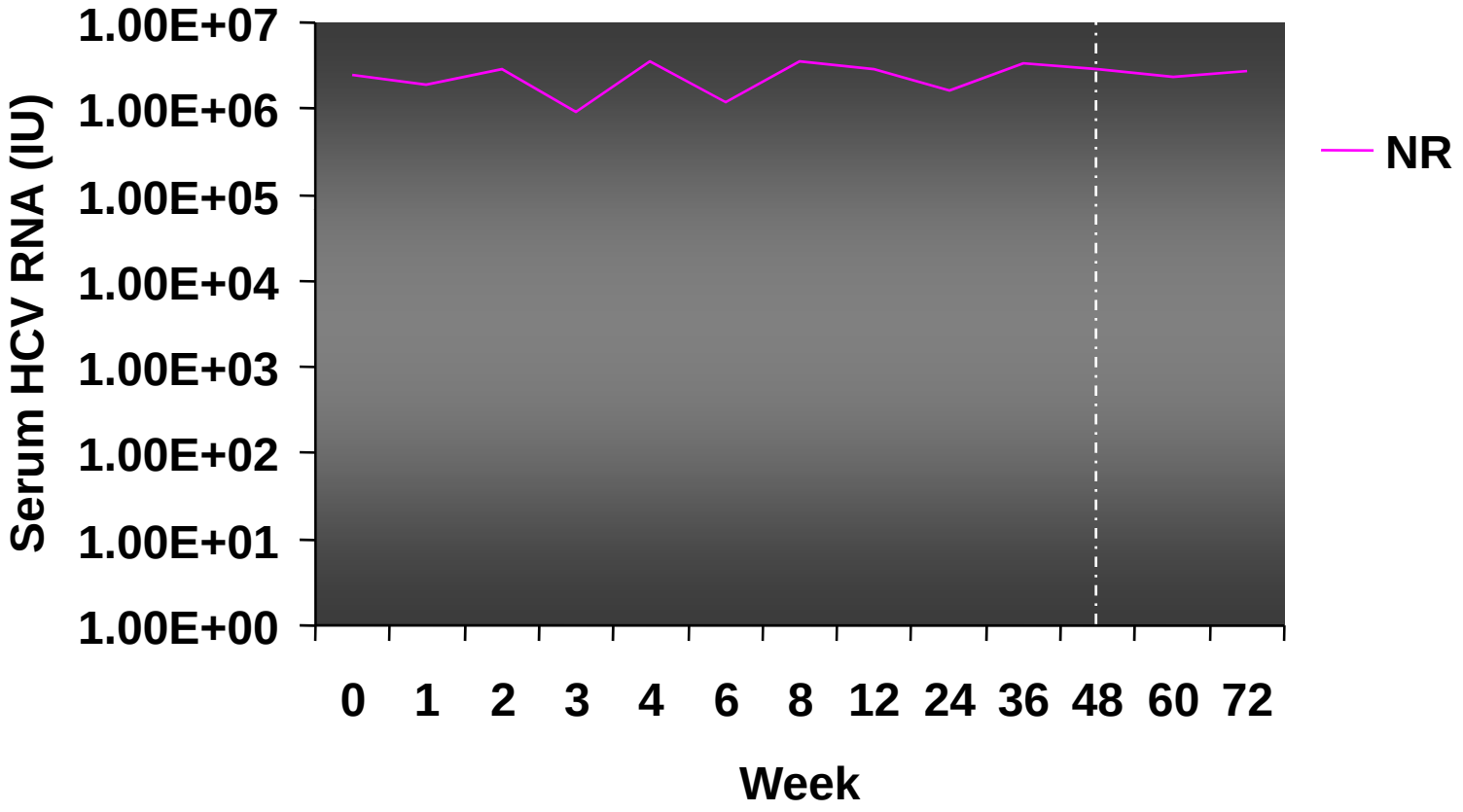
Adapted from Manns et al and Fried et al

Patterns of Response to Peg-IFN +Ribavirin: Patients Who May Benefit From STAT-C Triple Rx



Adapted from Manns et al and Fried et al

Patterns of Response to Peg-IFN +Ribavirin: Recent Data Suggests Null-Responders Will Not Benefit From STAT-C Triple Rx

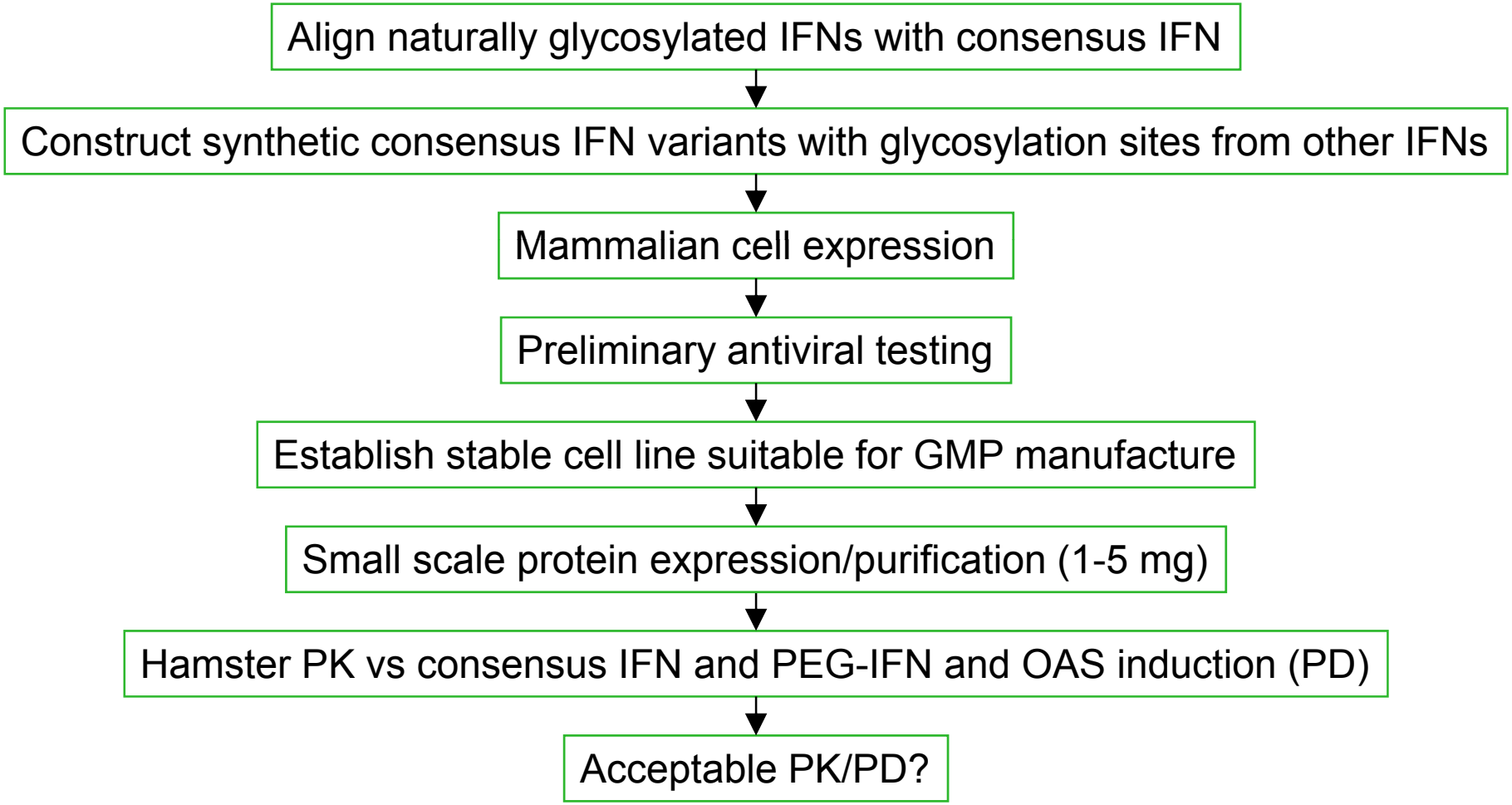


Adapted from Manns et al and Fried et al

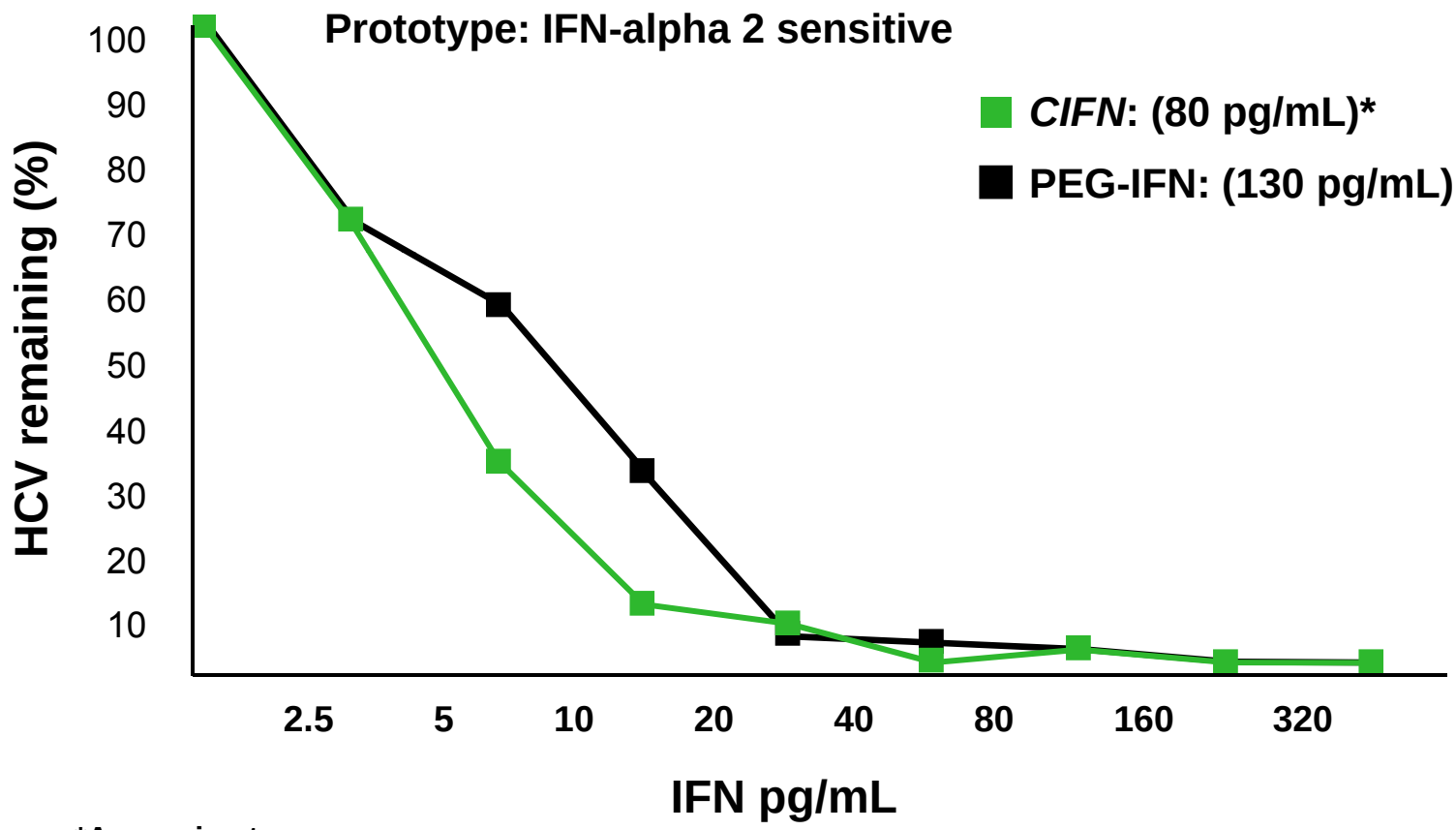
Glycoengineering concept

- **Natural Human Cytokines including several IFN's are post-transcriptionally modified with carbohydrate residues**
  - These moieties increase molecular weight and hydrodynamic radius
  - Block Proteolysis
  - Block Antigenicity
- **Introduction of Glycosylation Sites can improve a therapeutic protein's pharmacokinetic characteristics**
- **Aranesp® (darbepoetin)**
  - Hyperglycosylated Erythropoietin : Reduced dosing from TIW to QW with no loss of efficacy
  - Approved WW for the treatment of Anemia
  - Amgen (Thousand Oaks, California)

Alios Glycoengineering Discovery Efforts:
IFN alfacon 1 as parent IFN



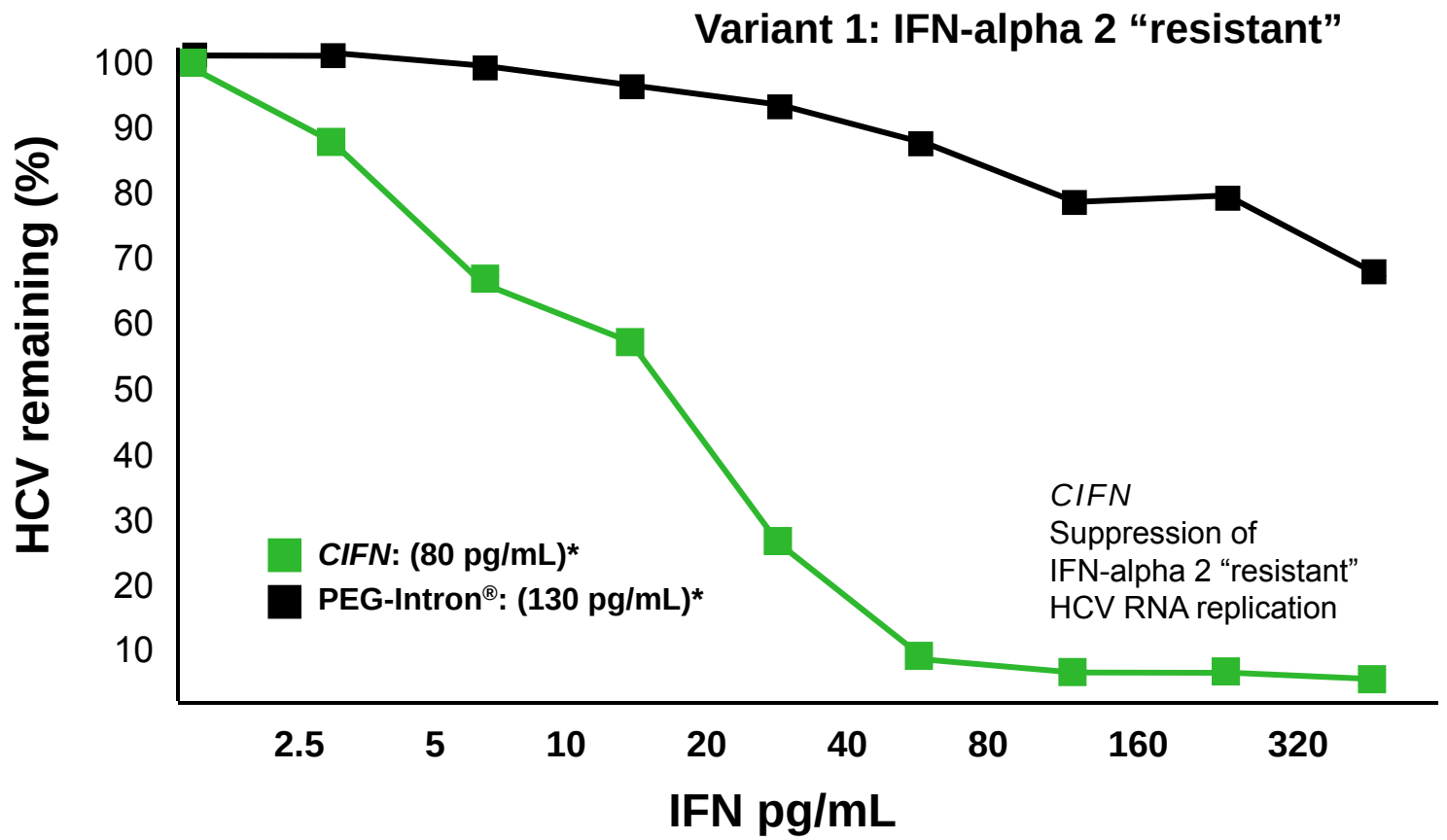
IFN Suppression of HCV RNA Replication Differs With Biological Activity



*Approximate serum max.

Erickson A, et al. Manuscript in preparation.

In Vitro Model of Nonresponse to PEG-IFN Alpha 2



***Approximate serum max.**

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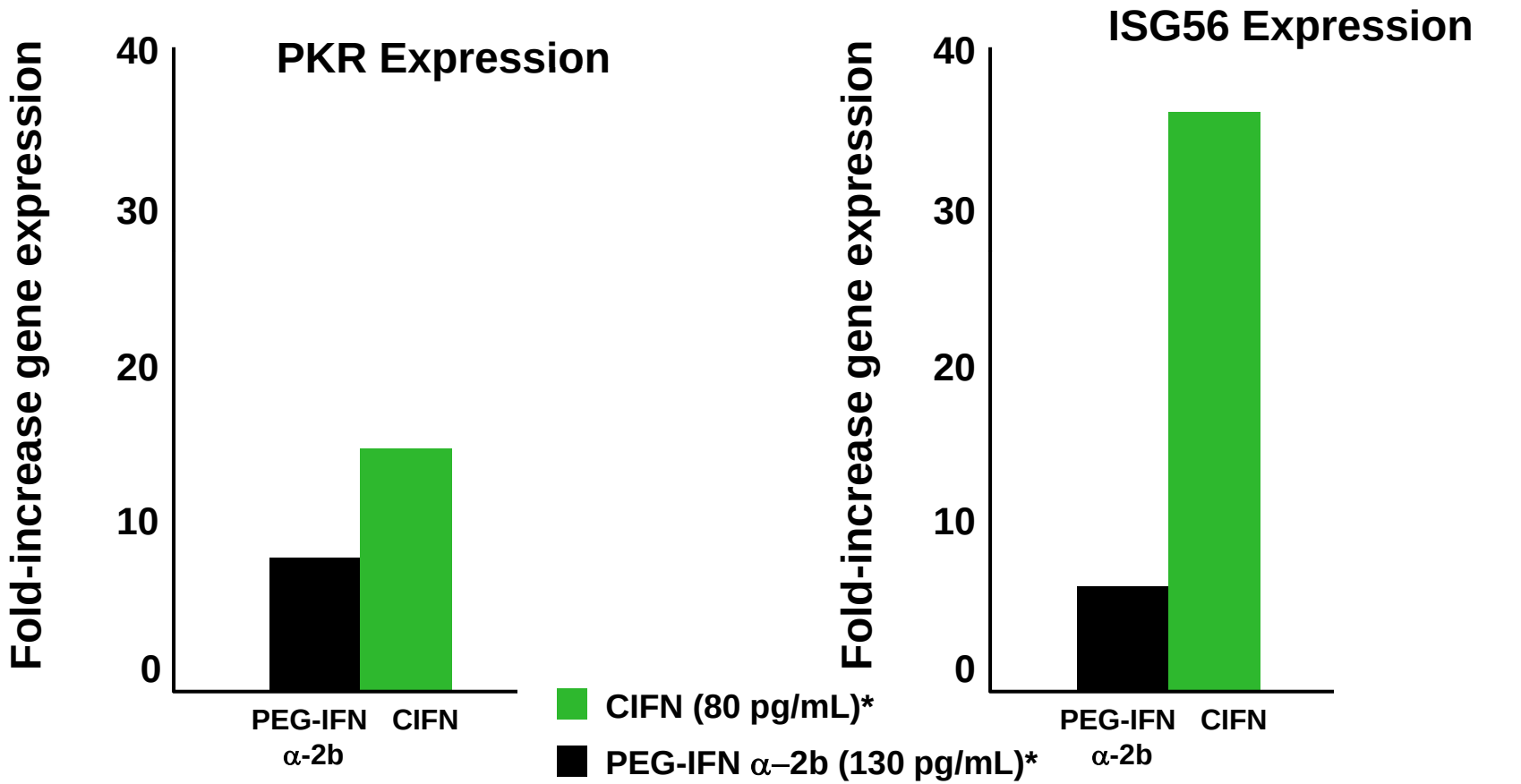
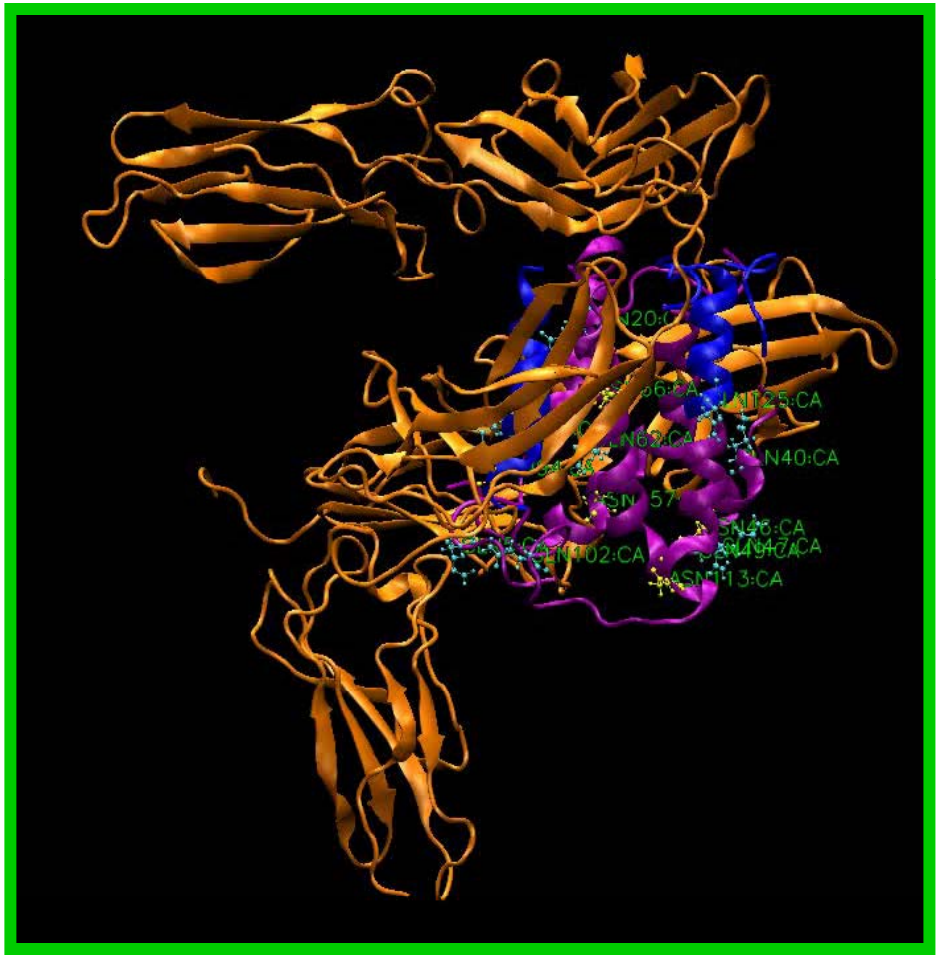
Comparison of ISG Expression by IFN α-2b or CIFN



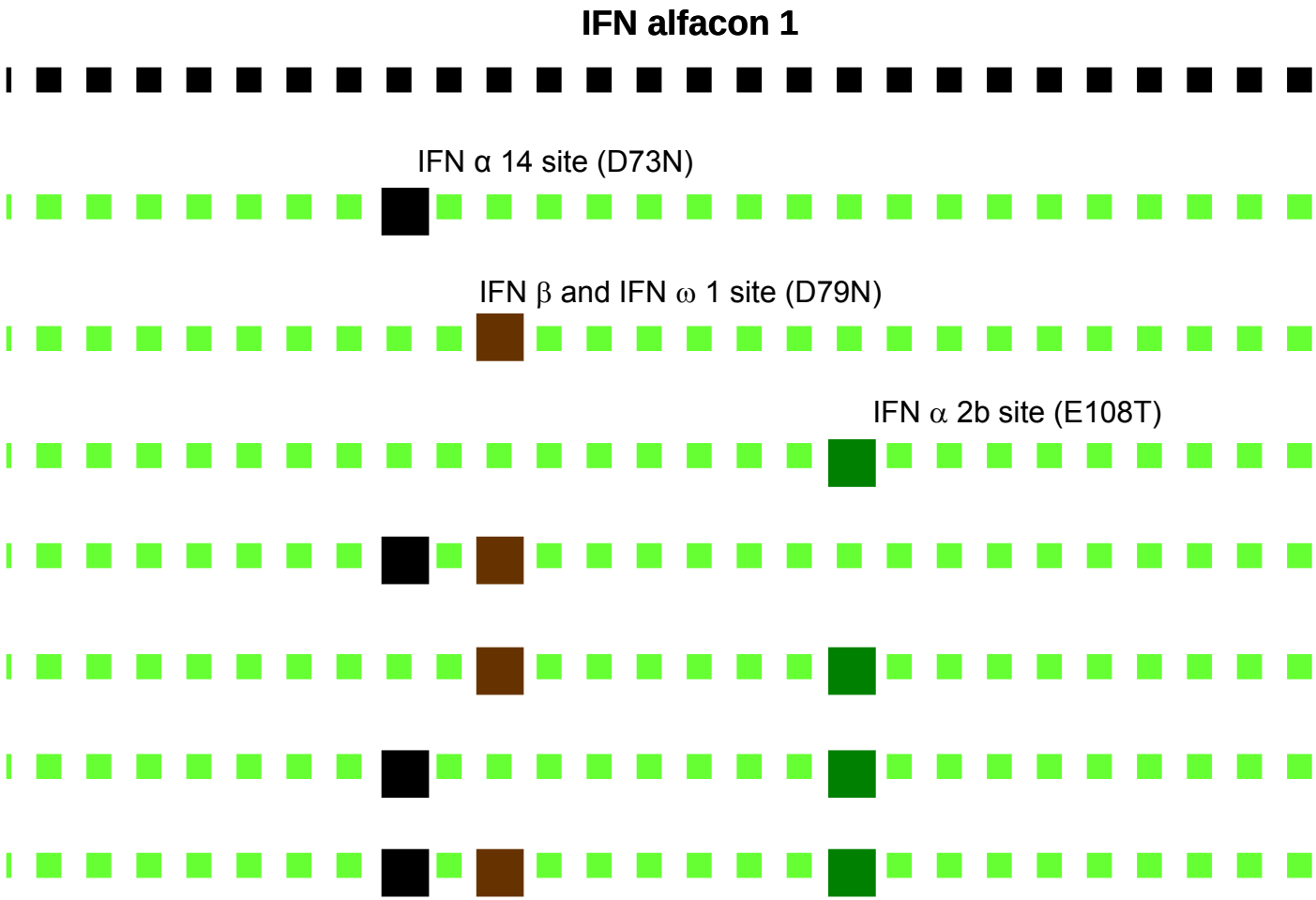
*Approximate serum max.

Erickson A, et al. Manuscript in preparation.

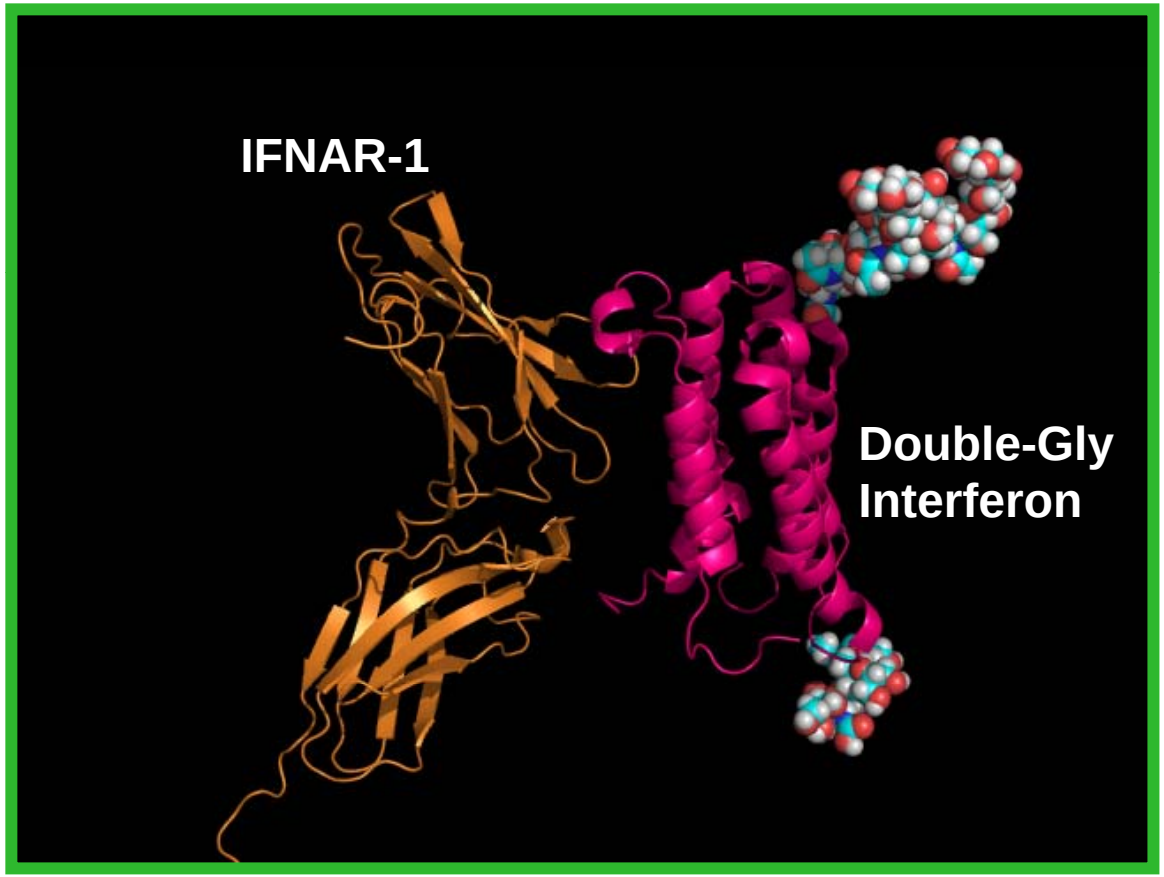
Addition of Glycosylation Sites Cannot Interfere With Binding of the IFN to its Receptor (IFNAR-1 and IFNAR 2)



IFN Glycosylation Mutants Derived From Natural Glycosylation Sites In IFN Non-Allelic Subtypes



Docking of Double-Gly IFN to IFNAR-1: No steric hindrance

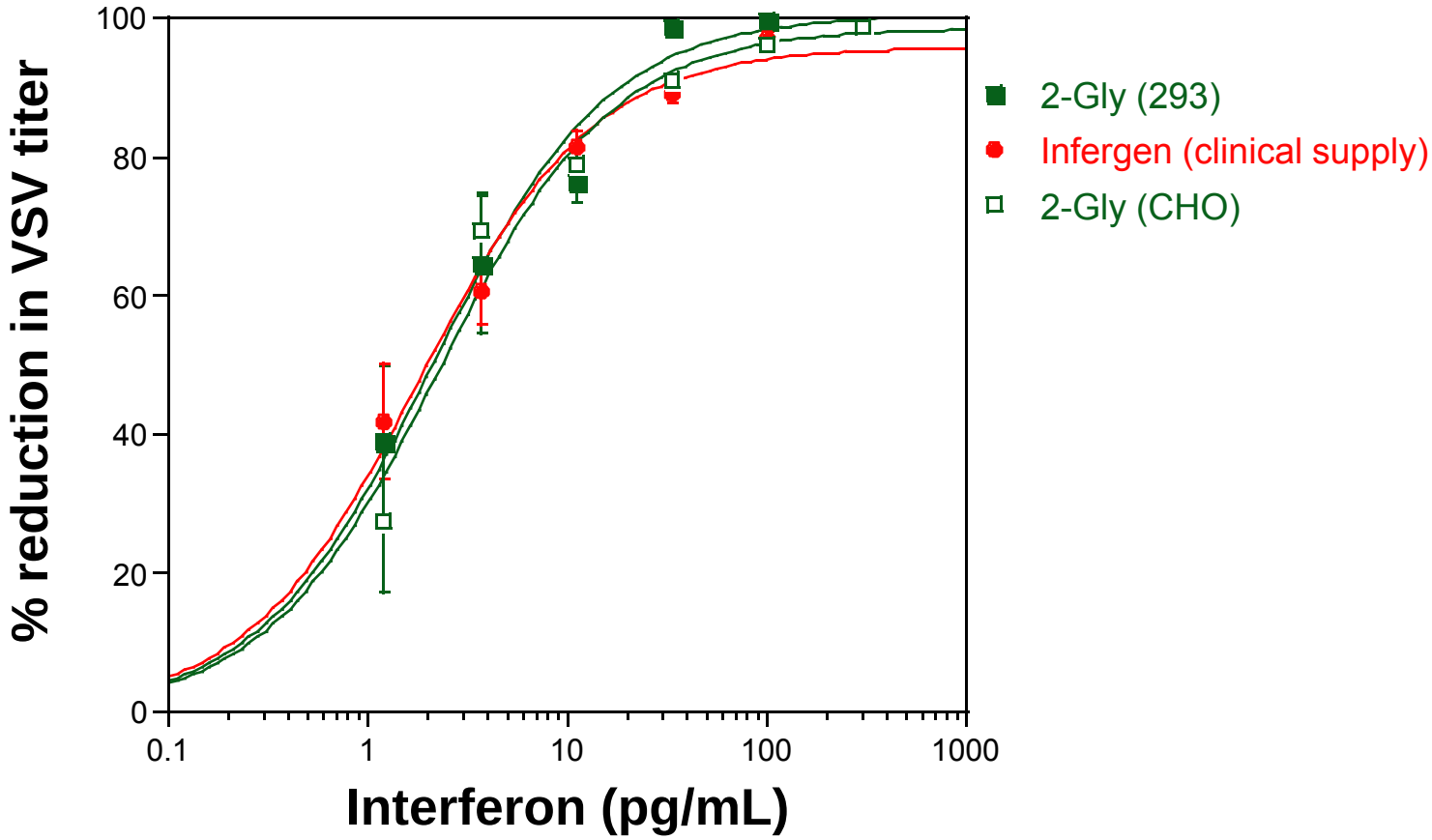


Comparison of Single and Double Glycosylation Variants For Biological Potency with IFN alfacon 1 (VSV)

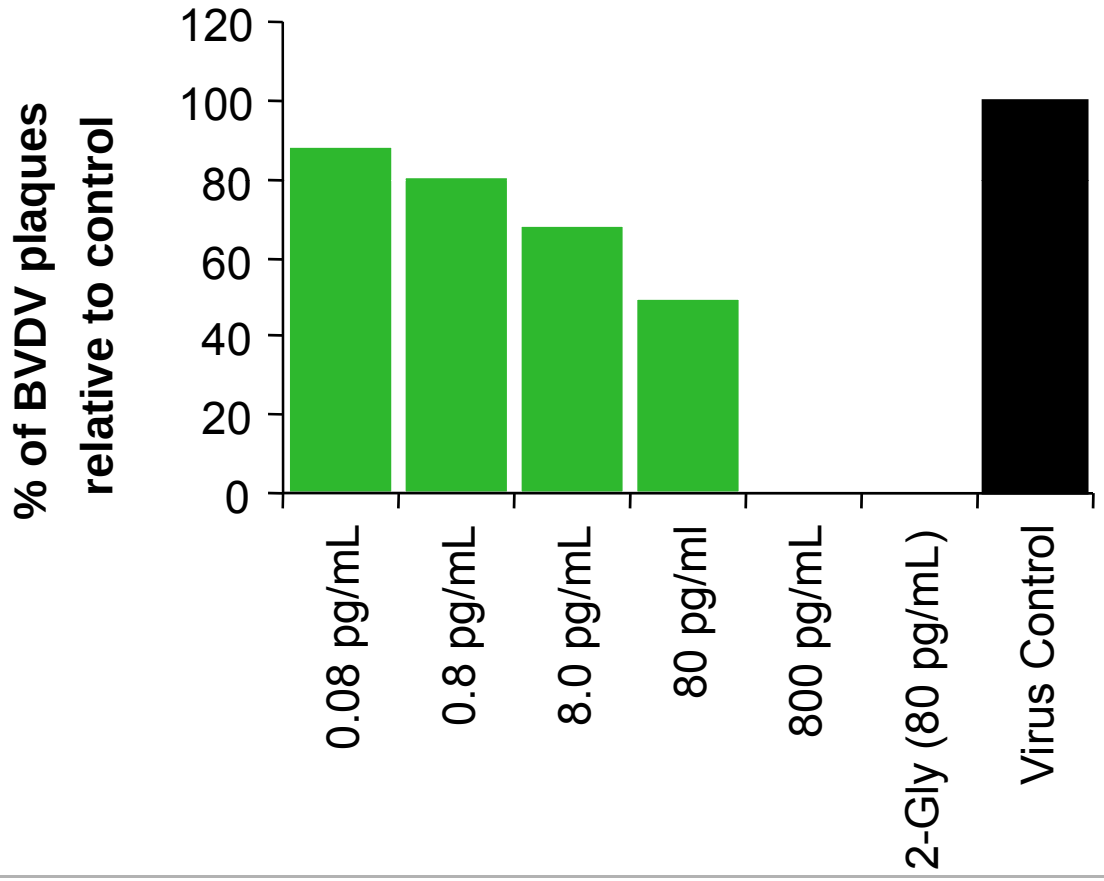
IFN	Glycoprotein source	Construct	EC_{50} (pg/mL)
IFN alfacon 1	293	Infergen	1.83 ± 0.41
1-Gly-A	293	D73N	2.53 ± 0.53
1-Gly-B	293	E108T	3.98 ± 0.85
2- Gly	293	D73N/E108T	2.13 ± 0.50
2-Gly	CHO	D73N/E108T	2.27 ± 0.41
Pegasus (PEG-IFN-α-2a)	NA	NA	1200 ± 600

Glyco- IFN variants retain full antiviral biological potency and are ~3 log_{10} more biologically active compared to Peg-IFN α 2a

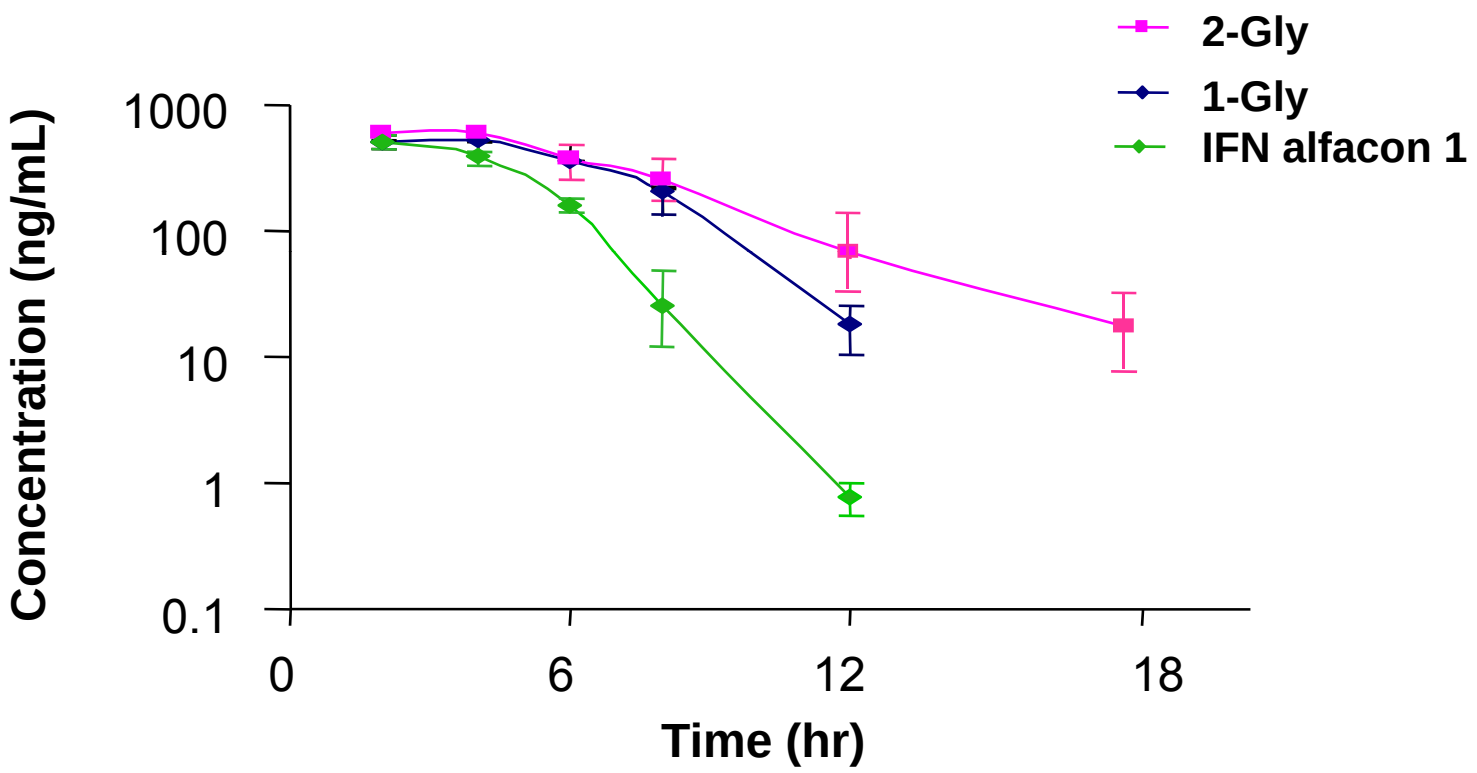
Hyperglycosylated (Double) Interferon Variants Dose Response



Potent effects on Flavivirus (BVDV)



Plasma Pharmacokinetics of prototype Glycosylated IFNs in Hamsters



Overall clearance of 1Gly improved over IFN alfacon 1 and even more significant improvement with 2-Gly

Conclusions

- **A need still exists for IFN-based products with novel biological properties**
- **Addition of Glycosylation Sites**
  - Improved PK
  - Maintained Biological Potency
  - May Address HCV NR
  - Additional applications in HBV and possibly HIV
- **Continuing optimization of glycosylation**
  - Optimization cell culture conditions will improve carbohydrate incorporation
  - More glycosylation sites can be added if needed
- **Further Study is Warranted**