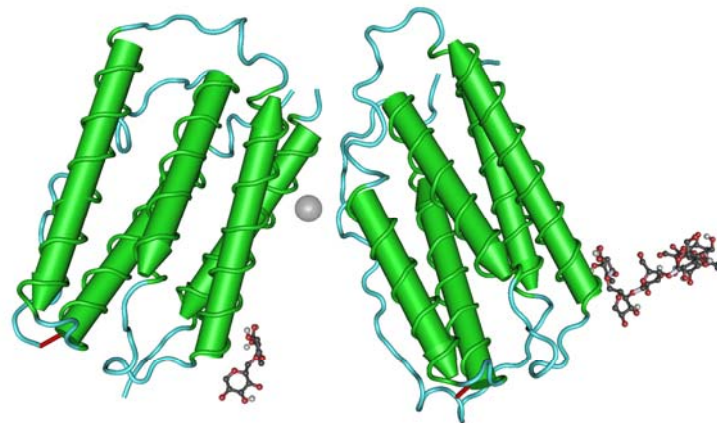


Interferon Beta Immunogenicity

Daniel Kramer / Open EIP Symposium 2012

Interferon Beta

- hIFN β is a 29 kDa glycoprotein with anti-inflammatory properties
- Since 1993 rhIFN β that is administered chronically to reduce the frequency of exacerbations typical for relapsing-remitting multiple sclerosis (MS)
- RhIFN β -1b is produced in *E. coli* and
 - is non-glycosylated
 - lacks the N-terminal methionine compared with the natural human interferon beta (hIFN β) protein
 - its Cys-17 is mutated to Ser-17 to reduce misfolding during downstream processing
- RhIFN β -1a is produced in CHO cells and has a single N-linked carbohydrate at Asn-80 similar to natural rhIFN β



Marketed rhIFN β Products

- Currently various rhIFN β products are on the market

Licensed Product	Form	MAH	Administration
Betaseron / Betaferon	IFN β -1b	Bayer Schering	Every other day 250 μ g s.c.
Extavia	IFN β -1b	Novartis	Every other day 250 μ g s.c.
Avonex	IFN β -1a	Biogen Idec	Once weekly 30 μ g i.m.
Rebif	IFN β -1a	Merck Serono	Thrice weekly 22 or 44 μ g s.c.

RhIFN β – Immunogenicity Overview

- All licensed rhIFN β products are quite immunogenic despite a high sequence homology to the endogenous protein
- 3 to 18 months after the start of rhIFN β therapy patients start forming anti-drug antibodies (BAbs) usually increasing the clearance of the therapeutic protein, leading to reduced efficacy
- Patients persistently showing high levels of binding antibodies are quite susceptible for the formation of neutralizing antibodies (NAbs) against rhIFN β
 - NAbs abrogate the clinical efficacy of rhIFN β and create the need to switch to another therapy
 - So far no clinical impact of anti-rhIFN β antibodies cross-reacting with endogenous hIFN β have been reported
- Both rhIFN β -1a products are generally considered showing the lowest incidence of anti-drug antibodies, while rhIFN β -1b displays the highest immunogenicity

RhIFN β – Immunogenicity Potential Explanation

- RhIFN β is a highly hydrophobic and consequently quite prone to aggregation
- RhIFN β -1b contains up to 60% large, soluble protein aggregates whereas less than 2% aggregates were found in rhIFN β -1a products
- Dissociation of rhIFN β -1b aggregates using high hydrostatic pressure considerably reduced immunogenicity in transgenic mice
- De-glycosylation of rhIFN β -1a resulted in aggregation indicating a key role of the sugar group in stabilizing the protein
- The carbohydrate chain was found to protect a particularly hydrophobic area on the protein surface hindering association with hydrophobic areas of other protein molecules

Immunogenicity - Aggregates

