Interferon Beta Immunogenicity

Daniel Kramer / Open EIP Symposium 2012
Interferon Beta

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

- Currently various rhIFNβ products are on the market

<table>
<thead>
<tr>
<th>Licensed Product</th>
<th>Form</th>
<th>MAH</th>
<th>Administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Betaseron / Betaferon</td>
<td>IFNβ-1b</td>
<td>Bayer Schering</td>
<td>Every other day 250 μg s.c.</td>
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<tr>
<td>Extavia</td>
<td>IFNβ-1b</td>
<td>Novartis</td>
<td>Every other day 250 μg s.c.</td>
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<tr>
<td>Avonex</td>
<td>IFNβ-1a</td>
<td>Biogen Idec</td>
<td>Once weekly 30 μg i.m.</td>
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<tr>
<td>Rebif</td>
<td>IFNβ-1a</td>
<td>Merck Serono</td>
<td>Thrice weekly 22 or 44 μg s.c.</td>
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RhIFNβ – Immunogenicity Overview

- All licensed rhIFNβ products are quite immunogenic despite a high sequence homology to the endogenous protein
- 3 to 18 months after the start of rhIFNβ 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 rhIFNβ
  - NAbs abrogate the clinical efficacy of rhIFNβ and create the need to switch to another therapy
  - So far no clinical impact of anti-rhIFNβ antibodies cross-reacting with endogenous hIFNβ have been reported

- Both rhIFNβ-1a product are generally considered showing the lowest incidence of anti-drug antibodies, while rhIFNβ-1b displays the highest immunogenicity
RhIFNβ – Immunogenicity
Potential Explanation

- RhIFNβ is a highly hydrophobic and consequently quite prone to aggregation
- RhIFNβ-1b contains up to 60% large, soluble protein aggregates whereas less than 2% aggregates were found in rhIFNβ-1a products
- Dissociation of rhIFNβ-1b aggregates using high hydrostatic pressure considerably reduced immunogenicity in transgenic mice
- De-glycosylation of rhIFNβ-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

Aggregates of therapeutic protein

B-cell receptor

Activation

Endosome

Bruton’s Kinase

Signal

MHC-II

TCR

T-Helper Cell

Differentiation/Proliferation