Identification and Possible Implications of a Human Plasma Purified Anodal Variant of Alpha-1-Antitrypsin

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Alpha-1-Antitrypsin (AAT)

- 394 amino acids
- Glycoprotein
- 3 N-linked carbohydrate attachment sites
- Molecular weight ~52,000 Da
- Single cysteine
- Isoelectric point ~4.2-4.8

Functions:
- Inhibition of serine proteinases
- Inhibition of neutrophil defensins
- Broad anti-inflammatory properties

Also called Alpha-1-Proteinase Inhibitor (A1PI)
Characterization of AAT by Isoelectric Focusing (IEF)

- IEF-Method of separating protein isoforms by charge
- Isoelectric point is the place on the pH scale where a protein has no net charge
- Traditionally used in the laboratory diagnosis of AAT Deficiency
AAT Isoform Microheterogeneity

AAT Isoform

<table>
<thead>
<tr>
<th>Isoform</th>
<th>Sequence</th>
<th>pI</th>
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<tbody>
<tr>
<td>2</td>
<td>NH₂ Glu-Asp-Pro-Gln-Gly</td>
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<td>NH₂</td>
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<tr>
<td>8</td>
<td>NH₂</td>
<td>4.67</td>
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Adapted from Jeppsson, JO. J Chromat 1985; 326: 173-177
Identification of the Anodal Variant in Aralast™

- Individuals with AAT deficiency most commonly are ZZ
- Augmentation therapy IEF pattern - MZ
- “E” region variant was identified by 2 labs
- Genotypically the subjects were ZZ
- All subjects were receiving Aralast™ augmentation therapy

*Subject on Prolastin™
Alpha Therapeutics Corporation (ATC) Pivotal Study

• Aralast™ developed by ATC
• Clinical trial from 1996-1999
• 28 deficient subjects enrolled for 6 months-2 early drop outs
• Comparator: Prolastin™
• Primary Outcome Variable-Not Inferior to Comparator (Total & Functional AAT)
• Central Lab-Brantly-NIH/UF

<table>
<thead>
<tr>
<th></th>
<th>ATC A1PI</th>
<th>Prolastin</th>
<th>ATC A1PI</th>
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<tr>
<td></td>
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<td>24 Weeks</td>
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Anodal Variant Present in Pivotal Study

IEF of Plasma Sample at 6 Wks

Aralast™ Lot LH2009A

pH 4

E4
E6

pH 5

M4
M6
Z4
Z6

SZ Standard
Proportion of E & M Isoforms in Study Samples

- Determine the proportion of “E” (modified variant) compared to M
- Densitometry of IEF isoforms following 6 weeks of study drug
- ~76% of total nadir AAT was modified form
ATC Pivotal Study Results

• Antigenic and functional amount (anti-protease activity) of ATC-A1PI similar to comparator (Prolastin)
• No serious safety signal in small short study
• Following 6 weeks of ATC-A1PI “E” (modified AAT variant) is ~76% of total nadir AAT
• Half-life similar for ATC-A1PI & Prolastin
Explanation for Anodal Variant

- Anodal variant secondary to loss of C-Terminal positive charged Lys
- Loss of C-Terminal residue likely secondary to carboxypeptidase activity
Lys 394 Before and After Protease Cleavage of Reactive Site Loop

1PSI Non-cleaved

9AP1 Cleaved
IEF Comparison of Several Manufactures of Plasma Purified AAT

- No product is identical to native AAT
- Greater than 65% of Trypsone & Aralast is modified AAT variant
- Prolastin and Zemaira have between 2-6% modified AAT Variant
- Other modifications are known to occur in these products
Is There Reason for Concern in Using a Modified Form AAT for Augmentation Therapy?

• Small clinical study established safety profile
• Modified Form of AAT
  – ~35-Fold more in Aralast compared to Prolastin
  – Potential of antigenicity because of the loss of surface amino acid
  – Charge difference may result in a different tissue distribution and/or clearance
  – Active site cleavage of the modified form creates a modified C-terminal fragment
• There is reason to believe that ATC did their animal studies using a different form of A1PI
Summary

• Anodal variant is a modified form of AAT (truncated C-terminus) which occurs during purification
• At least 65% of AAT in Aralast is modified form
• Truncation of AAT may alter
  – antigenicity
  – tissue distribution/clearance
  – stability and/activity of the reactive site loop
• This modified form of AAT may or may not have all functional properties of native AAT
• Careful monitoring of patients taking this group of products is warranted