THE BIOANALYSIS, IMMUNOGENICITY, PHARMACOKINETICS AND PHARMACODYNAMICS OF FUSION PEPTIDES AND PROTEINS

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Pharmacokinetics of fusion proteins and peptides

**Absorption**
- Mostly parenteral administered
- Enhance by adding delivery moiety

**Distribution**
- Limited distribution to tissue
- Enhance by adding targeting moieties

**Metabolism**
- Proteolysis
- Nonspecific endocytosis
- Fcγ receptor-mediated clearance
- Target-mediated clearance
- Decrease by adding carrier moiety which recycles

**Excretion**
1) Increase MW
2) Recycle with Albumin
- Significant renal excretion for proteins with MW < 30 kD
- Decrease renal excretion by increasing molecular weight
- Or by binding to plasma protein
Pharmacodynamics of fusion proteins and peptides

- Joining protein domains may impair the intrinsic bioactivity.
- It is often seen that the potency of fusion protein is decreased compared to the single protein.

**In vitro potency**

**In vivo pharmacodynamics:**

- Prolonged half-life
- Intrinsic potency
- Pharmacological effect
Immunogenicity of fusion proteins and peptides

What is Immunogenicity?
- The ability of therapeutic proteins to provoke an unwanted immune response

Important indicator
- Anti-drug-antibodies (ADA) (bind to the drug)
- Neutralizing antibodies (block the pharmacological effect of drug)

Clinical impacts
- Altered pharmacokinetics
- Diminished clinical efficacy
- Hypersensitivity or allergic reactions
- Cross-reaction with endogenous protein
Fusion protein-specific attributes for immunogenicity

New immunogenic epitopes at the junctional region

Enhanced antigen presentation

Antigen presenting cells

Altered processing

e.g. Fc receptors

e.g. MHC
# Bioanalytical considerations for fusion proteins

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<th>Use to obtain</th>
<th>Considerations for fusion proteins</th>
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<td>Immunoassay</td>
<td>Total, free, intact drug</td>
<td>PK profile</td>
<td>Can differentiate the PK of protein domain vs. fusion protein</td>
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<td>Radioactivity counting</td>
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<td>PK profile</td>
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<td>MS</td>
<td>Total, free, intact and degradants</td>
<td>PK profile</td>
<td>Can differentiate the PK of protein domain vs. fusion protein</td>
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<td>Auto-radiography</td>
<td>Total, intact and degradants</td>
<td>Tissues distribution</td>
<td>Proteins with targeting moieties</td>
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<td>Activity of targets, biomarker, ex vivo /in vivo efficacy</td>
<td>PD endpoints</td>
<td>Protein domains with pharmacological effect</td>
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<tr>
<td>ADA assays</td>
<td>ADA</td>
<td>Immunogenicity</td>
<td></td>
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<tr>
<td>Neutralizing Ab assays</td>
<td>Neutralizing Ab</td>
<td>Immunogenicity</td>
<td></td>
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</table>
## Current FDA-approved fusion proteins and peptides

<table>
<thead>
<tr>
<th>Name (trade name)</th>
<th>Effector protein</th>
<th>Secondary domain</th>
<th>Function of secondary domain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alefacept (Amevive;)</td>
<td>Human leukocyte function antigen-3 (LFA-3)</td>
<td>Fc</td>
<td>Prolong half-life</td>
</tr>
<tr>
<td>Etanercept (Enbrel;)</td>
<td>TNFαR</td>
<td>Fc</td>
<td>Prolong half-life</td>
</tr>
<tr>
<td>Abatacept (Orencia)</td>
<td>Cytotoxic T-lymphocyte antigen-4 (CTLA-4)</td>
<td>Fc</td>
<td>Prolong half-life</td>
</tr>
<tr>
<td>Romiplostim (Nplate)</td>
<td>Peptide</td>
<td>Fc</td>
<td>Prolong half-life</td>
</tr>
<tr>
<td>Rilonacept (Arcalyst)</td>
<td>Ligand-binding domains of IL-1 receptor and IL-1 receptor accessory protein (IL-1RAcP)</td>
<td>Fc</td>
<td>Prolong half-life</td>
</tr>
<tr>
<td>Alprolix</td>
<td>Coagulation Factor IX</td>
<td>Fc</td>
<td>Prolong half-life</td>
</tr>
<tr>
<td>Albiglutide (Tanzeum)</td>
<td>Glucagon-like peptide-1 (GLP-1) dimer</td>
<td>Albumin</td>
<td>Prolong half-life</td>
</tr>
</tbody>
</table>
# Case study 1

**albiglutide - pharmacokinetics**

<table>
<thead>
<tr>
<th>Form</th>
<th>Size (kD)</th>
<th>Half-life</th>
<th>Degradation /elimination</th>
<th>Mechanism for half-life extension</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>GLP-1</strong></td>
<td>Natural peptide</td>
<td>3.4</td>
<td>1.5-2 mins</td>
<td>Enzyme (DPP-IV, NEP)</td>
</tr>
<tr>
<td></td>
<td>Liraglutide</td>
<td>3.8</td>
<td>13 hrs</td>
<td>Enzyme</td>
</tr>
<tr>
<td></td>
<td>Albiglutide</td>
<td>73</td>
<td>5 days</td>
<td>Enzyme</td>
</tr>
</tbody>
</table>

These GLP-1 receptor agonists are developed to treat type 2 diabetes, by helping maintaining glycemic control.
Case study 1
albiglutide – pharmacodynamics

In vitro potency

- Design of dimer to improve receptor interaction in the presence of albumin
- A reduced EC$_{50}$ (0.2 vs. 20 nM) relative to the GLP-1R agonist exendin-4

In vivo pharmacodynamics

- Slightly lower efficacy with weekly dosing compared to liraglutide with daily dosing
Case study 1
albiglutide - immunogenicity

- Albiglutide has mostly natural sequences, except an amino acid substitution (Ala to Gly) on GLP-1.
- Immunogenicity rate is low, 2 - 4%.
- The anti-drug antibodies appeared to be largely transient, non-neutralizing and low titer.
- No obvious association between ADA and either efficacy or safety.
Albiglutide could be a suitable alternative to liraglutide

- **Efficacy**: Both albiglutide and liraglutide treatment led to clinically meaningful efficacy end points, although albiglutide did not meet the prespecified non-inferiority margin.

- **Safety**: Both treatments were associated with low rates of hypoglycaemia.

- **Tolerance**: Less nausea and vomiting occurred with albiglutide than with liraglutide.

- **Dosing frequency**: once weekly for Albiglutide, while once daily for lariglutide.
Romiplostim is indicated as a treatment for chronic idiopathic (immune) thrombocytopenic purpura (ITP), to increase the number of platelets in order to decrease the risk of bleeding.
Pharmacodynamics-Mediated Drug Disposition (PDMDD)

- Mean pharmacokinetic profiles of Romiplostim after single IV bolus or SC dose in healthy subjects
As a result of the high potency of this molecule (Kd~0.5 nM, in vitro EC$_{50}$ ~ 3-10 ng/mL), and the long half-life, significant PD response was observed at low doses (0.1-10.0 µg/kg).
Case study 2
Romiplostim- Immunogenicity

- **Design:** No sequence homology with endogenous TPO
  - potential anti-drug-antibody risk
  - reducing the potential for cross-reacting antibodies

- **Clinical observation:**
  - Immunogenicity rate: ADA rate ~11%, neutralizing Ab ~ 0.4%
  - The antibodies against romiplostim did not cross-react with TPO and vice versa.
  - No neutralizing antibodies to endogenous TPO
Case study 3
Alprolix (rFIXFc fusion) - PK

<table>
<thead>
<tr>
<th>Form</th>
<th>Size (kD)</th>
<th>Half-life</th>
<th>Mechanism for half-life extension</th>
<th>Dosing frequency</th>
</tr>
</thead>
</table>
| Enzyme (FIX)          | 120       | 3.5 days (vs. 1.4 days for FIX) | • Decreased renal clearance
                       |            |           | • FcRn-mediated recycling         | • Every 1-2 weeks (vs. 2-3 times weekly for FIX) |
| Fc                    |           |           |                                   |                                       |

- A recombinant rFIXFc fusion with a prolonged half-life was developed to reduce the dosing frequency in hemophilia B patients.
Case study 3
Alprolix (rFIXFc fusion) - PK

Pharmacokinetic profile in human following the injection of 50 IU/kg of rFIX or rFIXFc.

<table>
<thead>
<tr>
<th>Pharmacokinetic Parameter</th>
<th>rFIXFc Geometric Mean (95% CI)</th>
<th>rFIX Geometric Mean (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terminal half-life (h)</td>
<td>82.1 (71.4, 94.5)</td>
<td>33.8 (29.1, 39.2)</td>
</tr>
<tr>
<td>CL (ml/h/kg)</td>
<td>3.2 (2.8, 3.6)</td>
<td>6.3 (5.6, 7.1)</td>
</tr>
<tr>
<td>AUC/dose (IU*h/dl per IU/kg)</td>
<td>31.3 (27.9, 35.2)</td>
<td>15.8 (14.0, 17.7)</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>98.6 (88.2, 110.3)</td>
<td>41.2 (36.0, 47.2)</td>
</tr>
<tr>
<td>Vss (ml/kg)</td>
<td>314.8 (277.8, 356.8)</td>
<td>261.1 (222.9, 305.9)</td>
</tr>
</tbody>
</table>
### Summary of Efficacy in Control of Bleeding Episodes

<table>
<thead>
<tr>
<th>Total no. of new bleeding episodes</th>
<th>636</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of injections to treat bleeding episodes, n (%)</td>
<td></td>
</tr>
<tr>
<td>1 injection</td>
<td>575 (90.4)</td>
</tr>
<tr>
<td>2 injections</td>
<td>44 (6.9)</td>
</tr>
<tr>
<td>3 injections</td>
<td>17 (2.7)</td>
</tr>
<tr>
<td>Median dose per injection to treat a bleeding episode, IU/kg (IQR)</td>
<td>46.07 (32.86, 57.03)</td>
</tr>
</tbody>
</table>
Case study 3
Alprolix (rFIXFc fusion)-Immunogenicity

- Design: sequence derived from endogenous protein

- Clinical observation:
  - No ADA was detected in phase III trial, including patients who received chronic dosing (50 or more exposure days).
Summary

What the body does to a drug?

Pharmacokinetics

Immunogenicity

Pharmacodynamics

What a drug does to the body?

What adverse immune response against the drug?