Drug-Drug Interactions (DDI) of Therapeutic Proteins (TP)

By Rashim Singh, Shufan Ge and Ming Hu
University of Houston
• Introduction to Therapeutic Proteins
• Status of FDA approval of TP
• Market Share of TP
• Drug-Drug Interaction of TP
• Mechanism of TP-DDI
• Types of DDI studies for TP
• Assessment of TP-DDIs
Introduction

• **Therapeutic proteins (TP)** are **proteins** that are engineered in the laboratory for pharmaceutical use. Insulin was the first **therapeutic protein** to be introduced to treat diabetes in the 1920s.

• **Protein-based therapies are used in the treatment of** Cancer, Infectious Diseases, Hemophilia, Anemia, Multiple Sclerosis, Hepatitis B/C etc.

• Therapeutic proteins permit an **individualized treatment** approach by supporting a specifically targeted therapeutic process by compensating the deficiency of an essential protein.

• The fastest growing class of therapeutic proteins is monoclonal antibodies.
Protein Therapeutics

• Replace a protein that is deficient or abnormal

• Augment an existing pathway

• Provide a novel function or activity

• Interfere with a molecule or organism, or deliver a payload such as a radionuclide, cytotoxic drug, or protein effector
Overview of currently marketed protein therapeutics from a protein engineering perspective

Between 2010 and first quarter of 2015, FDA approved 41 NME for cancer indications, out of which 13 were large molecules (9 monoclonal antibodies (mAbs), 2 antibody drug conjugates (ADCs), 1 enzyme, and 1 fusion protein).

Clinical Indications targeted by Therapeutic Proteins

Ref: Kinch (2015), Drug Discovery Today, 20(4):393-398
# Therapeutic Proteins in Market

<table>
<thead>
<tr>
<th>Cytokines</th>
<th>Growth factors</th>
<th>Enzymes</th>
<th>Monoclonal antibodies</th>
<th>Miscellaneous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aldesleukin</td>
<td>Becaplermin</td>
<td>Agalsidase beta</td>
<td>Abciximab</td>
<td>Abatascept</td>
</tr>
<tr>
<td>Denileukin diftitox</td>
<td>Darbepoetin alfa</td>
<td>Alglucosidase alfa</td>
<td>Adalimumab</td>
<td>Abobotulinumtoxin A</td>
</tr>
<tr>
<td>IFNα-2a</td>
<td>Epoetin alfa</td>
<td>Alefacept</td>
<td>Alemtuzumab</td>
<td>Akeflex</td>
</tr>
<tr>
<td>IFNα-2b</td>
<td>Filgrastim</td>
<td>Asparaginase</td>
<td>Basiliximab</td>
<td>Anakinra</td>
</tr>
<tr>
<td>IFN α/β-1a</td>
<td>Peginterferon alfa</td>
<td>Collagenase</td>
<td>Bevacizumab</td>
<td>Botulinum toxin type A</td>
</tr>
<tr>
<td>IFN γ-1b</td>
<td>Opiniflucin</td>
<td>Collagenase clostridium histolyticum</td>
<td>Canakinumab</td>
<td>Botulinum toxin type B</td>
</tr>
<tr>
<td>Peg-IFNα-2a</td>
<td>Palifermin</td>
<td>Dornase alfa</td>
<td>Capromab pendetide</td>
<td>Botulinum toxin complex</td>
</tr>
<tr>
<td>Peg-IFNα-2b</td>
<td>Pegfilgrastim</td>
<td>Drotrecogin alfa</td>
<td>Certolizumab pegol</td>
<td>Ecallantide</td>
</tr>
<tr>
<td>IFN α-1b</td>
<td>Romiprincipide</td>
<td>Galactosidase</td>
<td>Cetuximab</td>
<td>Etanercept</td>
</tr>
<tr>
<td>Peg-IFNα-2b</td>
<td>Sargramostim</td>
<td>Idursulfase</td>
<td>Daclizumab</td>
<td>Rituximab</td>
</tr>
</tbody>
</table>

Market Share of Therapeutic Proteins

- For 2013, 7 of the top 8 best-selling drugs were Biologic Therapeutics.
- 6 of the top 8 blockbuster Biologic Therapeutics are Mab.

Annual sales of 8 top selling monoclonal antibody products.

Ref: Ecker et. al (2015), mAbs, 7 (1): 9-14

Ref: http://www.gabionline.net/Biosimilars/General/Top-8-blockbuster-biologicals-2013
Therapeutic Protein Drug Drug Interactions

Table II. Summary of drug interaction studies included in US FDA-approved package inserts for new molecular entity therapeutic proteins approved by the end of February 2010

<table>
<thead>
<tr>
<th>Category</th>
<th>Cytokines</th>
<th>Growth factors</th>
<th>Enzymes</th>
<th>Monoclonal antibodies</th>
<th>Miscellaneous</th>
<th>Total [n (%)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dedicated studies [n]</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>6</td>
<td>0</td>
<td>9 (12)</td>
</tr>
<tr>
<td>Some description [n]</td>
<td>7</td>
<td>2</td>
<td>7</td>
<td>13</td>
<td>6</td>
<td>35 (46)</td>
</tr>
<tr>
<td>No information [n]</td>
<td>2</td>
<td>8</td>
<td>9</td>
<td>10</td>
<td>3</td>
<td>32 (42)</td>
</tr>
<tr>
<td>Total [n (%)]</td>
<td>11 (14)</td>
<td>10 (13)</td>
<td>17 (22)</td>
<td>29 (38)</td>
<td>9 (12)</td>
<td>76 (100)</td>
</tr>
</tbody>
</table>

• Out of 76 BLAs approved by the US FDA by the end of February 2010,

• only 9 BLAs (12%) included information on TP- drug interactions in the labeling from dedicated clinical studies

• 35 BLAs (46%) described some potential for TP- drug interactions without citing any data.

• 32 BLAs (42%) contained no information on drug interactions.

Ref: Lee et.al (2010), Clin Pharmacokinet, 49(5):295-310
Therapeutic Protein Drug Drug Interactions

- Low potential of direct TP-SM DDI as follow different elimination pathways

- Only Indirect pharmacokinetic DDI possible
Possible Mechanisms of TP-SM DDI

1) Alteration of inflammatory/immune/disease processes, thereby affecting CYPs expression. **Example:** Tocilizumab have shown to increase CYP3A4-mediated clearance of simvastatin by over 2-fold in RA patients.

2) Alteration of immunogenicity (influence of immunosuppressive agents on the PK of Mabs) **Example:** Methotrexate can significantly decrease the accelerated clearance of infliximab by suppressing immunogenicity of infliximab in patients with immunogenic reaction, although a role of the Fcγ receptor in this interaction cannot be excluded.

3) Alteration of target physiology or target-mediated disposition by influencing the distribution of TP by TP/SM. **Example:** Tumor uptake of trastuzumab decreases with concomitant administration of an anti-VEGF antibody due to the reduction in both tumor blood flow and vascular permeability to macromolecules.

## DDI with TP as Victim

<table>
<thead>
<tr>
<th>Therapeutic Protein</th>
<th>Small Molecule</th>
<th>Effect on PK of Therapeutic Protein</th>
<th>Reference</th>
</tr>
</thead>
</table>
| Adalimumab          | Methotrexate/ Infliximab | 29-44% reduction of adalimumab clearance  
| Trastuzumab         | Paclitaxel      | 1.5-fold increase in serum level of TP  
| Heparin             | Palifermin      | Palifermin ↑AUC 4- to 5-fold and half-life ↓by 40%-45%, suggesting 70%-80% decrease in palifermin Cl and Vd.  
| Basiliximab         | Azothiopurine / Mycophenolate mofetil | 22% and 51% reduction in clearance by Azothiopurine and Mycophenolate mofetil, respectively  
| Efalizumab           | Triple Immunospressive agents (Cyclosporin, sirolimus, prednisone) | 50% reduction in clearance  
*Mechanism: Reduction in Target-mediated drug disposition by suppression of CD11a + T-cells* | Zhou and Meibohm, Book: Drug-Drug Interactions for Therapeutic Biologics |
# DDI with TP as Perpetrator

<table>
<thead>
<tr>
<th>Therapeutic Protein</th>
<th>Small Molecule</th>
<th>Effect on PK of Small Molecule</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trastuzumab</td>
<td>Paclitaxel</td>
<td>25 and 9% reduction of Cmax and AUC of paclitaxel</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>Mechanism: Restore CYPs level down-regulated by disease</strong></td>
</tr>
<tr>
<td>Trastuzumab</td>
<td>CYP2C19 (omeprazole), CYP2D6 (dextromethorphan), CYP3A4 (simvastatin)</td>
<td>1 week after a single IV dose of tocilizumab (10 mg/kg), there was a 57% decrease in exposure (AUC) of simvastatin (a CYP3A4 substrate) [n = 12] and a 28% reduction in omeprazole (a CYP2C19 substrate) [n = 8] but no change in dextromethorphan (a CYP2D6 substrate) [n = 13].</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>Mechanism: Restore CYPs level down-regulated by disease</strong></td>
</tr>
<tr>
<td>Murine mAb CD3</td>
<td>Cyclosporin</td>
<td>Higher cyclosporin trough concentrations among muromonab-CD3-treated patients receiving oral cyclosporin 4 mg/kg twice daily vs ALG-treated patients (265 vs 136 ng/mL) on day 5 of therapy.</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>Mechanism: Down-regulation of CYP3A4</strong></td>
</tr>
<tr>
<td>Interferon-alpha-2b</td>
<td>Theophylline</td>
<td>100% increase in theophylline concentration</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>Mechanism: Inhibition of CYP1A2</strong></td>
</tr>
</tbody>
</table>

## DDI with TP as Perpetrator

<table>
<thead>
<tr>
<th>Therapeutic Protein</th>
<th>Small Molecule</th>
<th>Effect on PK of Small Molecule</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interferon-alpha</td>
<td>Cyclophosphamide</td>
<td>37% reduction of clearance of Cyclophosphamide and 37% increase in half-life and peak plasma concentration. <strong>Mechanism: Inhibition of CYP activity</strong></td>
</tr>
<tr>
<td>Basiliximab</td>
<td>Cyclosporin</td>
<td>Higher whole-blood cyclosporin concentrations within the first 10 days (258 vs 228 ng/mL) with early cyclosporin toxicity. <strong>Mechanism: Down-regulation of CYP3A4,</strong></td>
</tr>
<tr>
<td>Basiliximab (anti-IL-2R mAb)</td>
<td>Tacrolimus</td>
<td>Significant drug-drug interactions Acute tubular necrosis on renal biopsy in half of the basiliximab-treated patients with elevated tacrolimus concentrations. Tacrolimus trough concentrations increased by 63% in basiliximab-treated patients 2 days after basiliximab administration. <strong>Mechanism: Down-regulation of CYP3A4</strong></td>
</tr>
</tbody>
</table>

Types of TP-SM Interactions Studies

Cytokine or Cytokine Modulator

- **In Vitro Study, TP→SM**
  - Effect on CYP or transporters

- **In Vivo Study, TP→SM**
  - Effect on CYP or transporters (Cocktail Individual Study)

TP intended to be used in combination therapy with SM or TP

- **In Vivo Interaction Studies**
  - a) Cross-over Study
  - b) Population PK
  - c) Parallel Study

- **In Vivo Study, TP→SM**
  - 1) TP→SM
  - 2) SM→TP

Studies considered important because of known mechanism or general concern

- **SM→TP**

In Vitro and In Vivo Interaction Studies

- Population PK as initial assessment: may follow up with a formal study

Label describes study results and any important clinical action

Ref: Huang et.al (2010), Clinical Pharmacology & Therapeutics, 87(4):497-503
Ref: 2012 FDA draft DDI guidance, Section IV.B.2.
Cytokine Based Interactions Studies

**DDI studies:**
- Studies should be conducted to determine TP’s effects on CYP enzymes or transporters.
- *In vitro* or animal studies have limited value in this assessment.
- The *in vivo* evaluations with TPs can be conducted with individual substrates for specific CYP enzymes and transporters or using a “cocktail approach”.

- **Label** describes study results and any important clinical action.
- E.g., Tocilizumab, Actemra® (simvastatin CL 50% ↑, CYP3A4 ↑)
- Pegylated interferon, Pegasys® (theophylline CL 25% ↓, CYP1A2 ↓)

**Cytokine or Cytokine Modulator**

- **In Vitro Study, TP→SM**
  - Effect on CYP or transporters

- **In Vivo Study, TP→SM**
  - Effect on CYP or transporters (cocktail or Individual Study)

- Label may indicate the potential for CYP or transporter interaction with focus on its potential effect on narrow therapeutic range drugs

**Examples:**
- 1) Rilonacept
- 2) Golimumab
- 3) Ustekinumab (Post-marketed DDI studies)
- 4) TP-X (Post-marketed DDI studies)

Ref: 2012 FDA draft DDI guidance, Section IV.B.2.
CYP mediated TP-SM Interactions

List of CYP enzymes with altered activities (decreased, unless noted \textsuperscript{a,b}) in the presence of specific cytokines, cytokine modulators, and human growth hormone, based on \textit{in vitro} and/or \textit{in vivo} studies in humans.

<table>
<thead>
<tr>
<th>CYP enzyme</th>
<th>Cytokines/cytokine modulators</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A2</td>
<td>IFN-(\alpha), IFN(\alpha)-2b, IFN-(\beta), IL-2, IL-6, hGH(^{a})</td>
</tr>
<tr>
<td>CYP2C8</td>
<td>IL-1</td>
</tr>
<tr>
<td>CYP2C9</td>
<td>IL-2, IL-10</td>
</tr>
<tr>
<td>CYP2C19</td>
<td>Tocilizumab(^{b}), IFN(\alpha)-2b, FN-(\beta), IL-2, TNF-(\alpha), IL-6, hGH</td>
</tr>
<tr>
<td>CYP2D6</td>
<td>IFN(\alpha)-2b</td>
</tr>
<tr>
<td>CYP2E1</td>
<td>IL-2, IFN(\alpha)-2b</td>
</tr>
<tr>
<td>CYP3A</td>
<td>Basiliximab, muromonab-CD3, tocilizumab(^{b}), IL-1, IL-2, IL-6, IL-10</td>
</tr>
</tbody>
</table>

Ref: Huang et.al (2010), Clinical Pharmacology & Therapeutics, 87(4):497-503

Approach to assess TP-DDI risk for cytokine or cytokine modulator and effects on CYP enzymes

Ref: Evers et.al (2013), Drug Metab Dispos 41:1598–1609
Tocilizumab (IL-6R blocker) Interactions with CYP450 Substrates

- In vitro studies showed effect on expression of multiple CYP enzymes including CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6 and CYP3A4. Its effects on CYP2C8 or transporters is unknown.

- In vivo clinical studies with omeprazole metabolized by CYP2C19 and CYP3A4, and simvastatin, metabolized by CYP3A4, showed up to a 28% and 57% decrease in exposure one week following a single dose of ACTEMRA, respectively.

Tocilizumab (IL-6R blocker) and Simvastatin

Tocilizumab:
Reverse IL-6-induced suppression of CYP 3A4 activity

Simvastatin:
Metabolized by CYP3A4

Tocilizumab (IL-6R blocker) Interactions with CYP450 Substrates

Package Insert based on DDI findings:

• Clinically relevant for CYP450 substrates with narrow therapeutic index, where the dose is individually adjusted.

• Upon initiation or discontinuation of ACTEMRA, therapeutic monitoring of effect (e.g., warfarin) or drug concentration (e.g., cyclosporine or theophylline) should be performed and the individual dose of the medicinal product adjusted as needed.

• Prescribers should exercise caution when ACTEMRA is co-administered with CYP3A4 substrate drugs where decrease in effectiveness is undesirable, e.g., oral contraceptives, lovastatin, atorvastatin, etc.

• The effect of tocilizumab on CYP450 enzyme activity may persist for several weeks after stopping therapy.
Combination Therapy Based Interactions Studies

DDI studies:

- Studies should evaluate the effect of each product on the other.
- The *in vivo* studies should assess effects on PK and, when appropriate, PD.
- This evaluation is particularly important when the combination drug has a narrow therapeutic range (e.g., chemotherapeutic agents)

In Vivo Interaction Studies to assess effects on PK and PD

- a) Cross-over Study
- b) Population PK
- c) Parallel Study

Label describes study results and any important clinical action

TP intended to be used in combination therapy with SM or TP

1) TP→SM
2) SM→TP

E.g., 1) Bevacizumab and FOLFOX4 combination
2) Trastuzumab + doxorubicin or paclitaxil or docetaxil

Ref: Huang et al (2010), Clinical Pharmacology & Therapeutics, 87(4):497-503
Ref: 2012 FDA draft DDI guidance, Section IV.B.2.
Mechanism Based Interactions Studies

DDI studies:

- Appropriate in vitro and/or in vivo assessment for possible interactions needs to be conducted.

Ref: Huang et al. (2010), Clinical Pharmacology & Therapeutics, 87(4):497-503
Ref: 2012 FDA draft DDI guidance, Section IV.B.2.
Palifermin Interactions with and Heparin (Competitive Binding)

- Two studies were performed in healthy subjects to characterize
  - Effect of palifermin on the PD of heparin (activated partial thromboplastin time)
  - Evaluate the impact of heparin on the PK and PD (Ki67 staining of buccal mucosal tissue) of palifermin.

- Pronounced PK interaction: co-administration of heparin caused large changes in pharmacokinetic of palifermin including:
  - AUC
  - t_{1/2}
  - Cl and V_d

Palifermin Interactions with and Heparin (Competitive Binding)

• Two studies were performed in healthy subjects to characterize
  • Effect of palifermin on the PD of heparin (activated partial thromboplastin time)
  • Evaluate the impact of heparin on the PK and PD (Ki67 staining of buccal mucosal tissue) of palifermin.

• Pronounced PK interaction: co-administration of heparin caused
  • AUC of palifermin ↑4- to 5-fold
  • $t_{1/2}$ ↓ 40%-45%,
  • Cl and $V_d$ ↓ 70%-80%

• No PD interaction:
  • No effect of heparin on PD of palifermin,
  • No effect of palifermin on the anticoagulant activity of heparin,

• Therefore, these results suggest that dose adjustments for heparin and palifermin are not warranted when administered concurrently.

Evaluating DDI for TP: In Vitro and In Vivo Approaches

- **Human-cultured hepatocytes**: effects of TP on CYP enzymes and transporters

- In vitro-in vivo correlation for TP-DIs has not been established and warrant further mechanistic investigation

- **Animal models**: rarely used because of highly species-specific immune responses
Evaluating DDI for TPs: In Vitro and In Vivo Approaches

• Dedicated clinical DDI studies
  ➢ Parallel
  ➢ Crossover
  ➢ Single-sequence crossover

• Cocktail clinical DDI study
  ➢ Screen the effect of a TP on multiple CYP isozymes by using corresponding prototypical CYP probes

• Model-based method: population PK/PD-based DDI study
Summary

• No relevant *in vitro* models are available to predict TP-DDI in vivo. Most commonly used *in vivo* models are Pop-PK studies.

• Clinical Studies must be designed for SM drugs with a narrow therapeutic range that are CYP substrates.

• PK interaction of TP as victim or perpetrator does not necessarily mean PD interaction or vice versa, and as such does not necessarily warrant dose adjustment in patients.

• DDI studies for Therapeutic proteins are increasingly becoming routine in the drug development process.