Structure and Dynamics of a Site-Specific Labeled Human Fc Fragment with Tuned Effector Functions

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Evolution of Antibody-Based Medicines

**Top 10 Drugs by Sales for 2017, $85 Billion:**
Seven are Biologics, Six are mAbs

<table>
<thead>
<tr>
<th>Product</th>
<th>2017 Sales</th>
<th>2017 vs 2016</th>
<th>Use</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Humira (Adalimumab)</td>
<td>$18,427 M</td>
<td>14.6%</td>
<td>Anti-inflammatory</td>
<td>mAb</td>
</tr>
<tr>
<td>Rituxan (Rituximab)</td>
<td>$9,238 M</td>
<td>2.0%</td>
<td>Anti-Cancer</td>
<td>mAb</td>
</tr>
<tr>
<td>Revlimid (Lenalidomide)</td>
<td>$8,187 M</td>
<td>17.4%</td>
<td>Anti-Cancer</td>
<td>Small molecule</td>
</tr>
<tr>
<td>Enbrel (Etanercept)</td>
<td>$7,885 M</td>
<td>-11.1%</td>
<td>Autoimmune diseases</td>
<td>Protein/IgG</td>
</tr>
<tr>
<td>Herceptin (Trastuzumab)</td>
<td>$7,441 M</td>
<td>3.4%</td>
<td>Anti-Cancer</td>
<td>mAb</td>
</tr>
<tr>
<td>Eliquis (Apixaban)</td>
<td>$7,395 M</td>
<td>46.3%</td>
<td>Anticoagulant</td>
<td>Small molecule</td>
</tr>
<tr>
<td>Remicade (Infliximab)</td>
<td>$7,152 M</td>
<td>-13.1%</td>
<td>Autoimmune diseases</td>
<td>mAb</td>
</tr>
<tr>
<td>Avastin (Bevacizumab)</td>
<td>$7,096 M</td>
<td>-1.4%</td>
<td>Anti-Cancer</td>
<td>mAb</td>
</tr>
<tr>
<td>Xarelto (Rivaroxaban)</td>
<td>$6,589 M</td>
<td>11.3%</td>
<td>Anticoagulant</td>
<td>Small molecule</td>
</tr>
<tr>
<td>Eylea (Aflibercept)</td>
<td>$6,034 M</td>
<td>9.4%</td>
<td>Macular degeneration</td>
<td>Fusion protein</td>
</tr>
</tbody>
</table>

Quality control and reproducibility ➔ need for data and standards ➔ NIST mAb
Antibody-Drug Conjugates

Anatomy of an ADC

Where to attach?
Trend is toward...
* carefully chosen specific site(s)
* protected locations
* carefully controlled conjugation
Structure of this Talk

1. Intro to ADC insertion mutant Fc_C239i
2. Fc_C239i crystal structures
3. Dynamics
4. Receptor interactions
5. Conclusions

IgG1 heavy chain sequence:

-- hinge -- Fc...
Fab.......CPPCPAPELGGPSVF...

Cys inserted after Ser239
Cys inserted here, after Ser 239
How does the inserted Cys affect the local structure?

Crystals of the Fc fragment with the C239i mutation.

Diffraction statistics for Fc wt and two Fc_239i adduct structures. All are in space group 19 with unit cell approximately 50, 80, 135 Å.

<table>
<thead>
<tr>
<th>Structure</th>
<th>#non-H atoms</th>
<th>resolution</th>
<th>Rsym</th>
<th>refined R/Rf</th>
<th>rmsd-ideal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fc wt (5vgp)</td>
<td>3765</td>
<td>2.1 Å</td>
<td>0.09</td>
<td>0.20/0.25</td>
<td>0.013</td>
</tr>
<tr>
<td>Fc239i+cys</td>
<td>3652</td>
<td>2.3 Å</td>
<td>0.07</td>
<td>0.22/0.27</td>
<td>0.014</td>
</tr>
<tr>
<td>Fc239i+maleim</td>
<td>3628</td>
<td>2.6 Å</td>
<td>0.10</td>
<td>0.26/0.31</td>
<td>0.010</td>
</tr>
</tbody>
</table>

0. Wild type:

(Gly 236) → Gly 237 → Pro238 → Ser239 → Val240

1. Our naïve expectation:

(Gly 236) → Gly 237 → Pro238 → Ser239 → Val240

2. What we found:

Gly 237 → Pro238 → Ser239 → Val240

Cys 239i causes a shift upward, making the hinge longer.
The extra Cys structurally replaces Ser239, forcing 239 and 240 upward and extending the hinge by 1 residue.
Adducts attached to Cys239i:

1. extra Cysteine

2. Maleimide
Local electrostatics may affect linker stability
Structure of this Talk

1. Introduce Fc_C239i -> Done
2. Crystal structures of proto-linker adducts -> Done

3. Dynamics
4. Receptor interactions
5. Conclusions
Hydrogen-Deuterium Exchange

HDX coverage and heat maps

[Diagram showing HDX coverage and heat maps with protein sequences and respective HDX values.]
Two ways to measure dynamics in Fc_239i
(both are normalized to wild-type)

Dynamics by HDX

Dynamics by diffraction
Receptor Interactions  

FcRn, FcR-gamma, C1q

Extensive measurements reported in 2017 paper:

receptor:  FcRn  FcR-gamma  C1q  
binding:  +  -  -
Biological role of FcRn
helps protect, preserve, recycle, distribute Abs.

Various immune and tissue cells

Helps import Abs into cells for recycling, etc.
Wild-type Fc binding to FcRn (PDB: 4N0U)
Model of Fc_239i binding to FcRn

The binding interface is far from the mutation.
Biological role of FcR-gamma-IIIa forms activating complexes, leading to ADCC, etc.
Wild type Fc in complex with FcRIIla complex (PDB: 3AY4)

FcR bends the hinge sharply
Fc_239i is sterically blocked from binding to FcRIII.
Shifting Pro 238 in place of Gly 237 has a strong disruptive effect.

Pro can’t do what Gly can.
Conclusions

1. The ADC-engineered Fc fragment Fc_C239i crystal structure shows the molecular basis for several observed favorable properties, including:
   * general protein stability and conjugate stability (sheltered site)
   * non binding of Fc-gamma receptors, so that ADCC is not induced
   * normal binding to FcRn

2. Key features appear to be:
   * the sheltered-but-accessible conjugation site near to the FcR interface
   * the specific secondary structure at the start of the Fc: ...GGPS(C)...

3. Structure at 2.3 Angstrom resolution is deposited as 6xxx.pdb
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Collaborators:

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- Joomi Ahn | MedImmune
- Robert Brinson | NIST
- Nazzareno Dimasi | MedImmune