A PHYSICOCHEMICAL APPROACH TO CHARACTERIZING ANTIBODY-DRUG CONJUGATES THROUGH STABILITY INTO TARGET VALIDATION

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Background

- Trastuzumab (Herceptin®) is approved for use in human epidermal growth factor receptor HER2-positive cancers (i.e., breast, stomach). Maytansine, a cytotoxic drug, increases application by binding to tubulin to prevent microtubule formation.

- Antibody Drug Conjugates: A discriminatory therapeutic with high potency.
Experimental

- On-bead or off-bead preparation.
- On-bead advantage: smaller batches, mL of 0.5 mg/mL
- On-bead question: Did the bead occlude favorable modification sites?
- Modification question: After adding a drug to the mAb does its conformation and binding profile change?
A Panel of Techniques to Answer the Questions

A panel of methods were used to establish comprehensive characterization of antibody-drug conjugates (ADCs).

- Liquid Chromatography
- Mass Spectrometry
- Size Exclusion Chromatography
- Microfluidic Modulation Spectroscopy
- Differential Scanning Calorimetry
- Grating Coupled Interferometry
- Isothermal Titration Calorimetry
Biochemical Characterization

**Intact LC MS: Drug-Antibody Ratio**
- Waters Acquity UPLC with Xevo G2-QToF
- Sample Prep: deglycosylation & Desalting with a MassPREP column

**Peptide Mapping: Modification ID**
- Waters Acquity UPLC with Xevo G2-QToF
- Peptide Mapping After Trypsin Digest. Acquity UPLC with Xevo G2-S, QToF
Biophysical Conformational Stability and Characterization

Domain Stability & Heterogeneity: DSC

- TA Instruments NanoDSC with a Capillary Cell

<table>
<thead>
<tr>
<th></th>
<th>Tmax1 (°C)</th>
<th>FWHM1 (°C)</th>
<th>Tmax2 (°C)</th>
<th>ΔH1 (kJ/mol)</th>
<th>ΔH2 (kJ/mol)</th>
<th>ΔH_total (kJ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tras(avg)</td>
<td>68.4</td>
<td>5.7</td>
<td>81.4</td>
<td>574</td>
<td>3185</td>
<td>3853</td>
</tr>
<tr>
<td>T DM (avg)</td>
<td>64.4</td>
<td>8.5</td>
<td>80.6</td>
<td>251</td>
<td>2389</td>
<td>2698</td>
</tr>
</tbody>
</table>

Secondary Structural Changes & Aggregation: MMS

- RedShiftBio MMS
- Loss of parallel beta sheet to anti-parallel beta sheet, which typically indicates an increase in aggregation
**Binding Affinity and Stability**

**Affinity, Enthalpy, Entropy, Stoichiometry: ITC**

- TA Instruments Affinity ITC LV
- Affinity, Enthalpy, Entropy, Stoichiometry

**Affinity, k\textsubscript{on}, k\textsubscript{off}: GCI**

- Creoptix WAVE GCI
- Amine-coupled
- k\textsubscript{on} agreement, similar K\textsubscript{d} for Her2Fc

### Avg Values (n=2)

<table>
<thead>
<tr>
<th></th>
<th>K\textsubscript{d} (nM)</th>
<th>n</th>
<th>ΔH (kJ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tras</td>
<td>3 ± 1</td>
<td>0.8 ± 0.2</td>
<td>-101 ± 5</td>
</tr>
<tr>
<td>T-DM on</td>
<td>3.3 ± 0.3</td>
<td>0.64 ± 0.1</td>
<td>-99 ± 2</td>
</tr>
<tr>
<td>T-DM off</td>
<td>4 ± 2</td>
<td>0.66 ± 0.02</td>
<td>-98 ± 2</td>
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Conclusion

- The complex was modified, but did it change?
  - Stability Changes
  - Binding remained Intact

- This type of combined biophysical and biochemical analysis amplifies and solidifies the confidence of the reported results while decreasing bias.

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