Agilent’s Solutions for:

Biosimilars & Antibody Drug Conjugates

Gurmil Gendeh, Ph.D.
Biopharma & Biosimilars Markets
Life Sciences Group
Agilent Technologies
Outline

• Biopharma Market, Workflows & Analytical Challenges
• Biosimilars
  – Definitions & Regulations
  – How similar is similar enough
  – Case study: Comparability data between a biosimilar and its innovator reference
• ADCs
  – Rational
  – Heterogeneity in ADC
  – Key quality attributes and analytical methods for ADCs
• Summary
Outline

• Biosimilars
  – Definitions & Regulations
  – How similar is similar enough
  – Case study: Comparability data between a biosimilar and its innovator reference

• ADCs
  – Rational
  – Heterogeneity in ADC
  – Key quality attributes and analytical methods for ADCs

• Summary
Traditional Pharma business model is changing

It’s a mAb!

It’s a large Pharma!
Big Pharma – becoming Big Biopharma
Biologics in development by product category and development phase

mAb Dominates – 3 out of 4 Protein Therapeutics are mAbs

Pharma 2013 Report, Medicines in Development – Biologics
Biologics medicines – by Therapeutic categories

mAbs for Cancer therapeutics dominates

Pharma 2013 Report, Medicines in Development – Biologics
Attributes & Combinatorics

- Pyro-Glu (2)
- Deamidation (3 x 2)
- Methionine oxidation (2 x 2)
- Glycation (2 x 2)
- High mannose, G0, G1, G1, G2 (5)
- Sialylation (5)
- C-term Lys (2)

\[(9600)^2 \approx 10^8\]  \[2 \times 6 \times 4 \times 4 \times 5 \times 5 \times 2 = 9600\]
Biologics manufacturing is highly complex & requires analysis at every step.

Establishment of genetically engineered cells (e.g. CHO) that produce desired product.

"the process is the product"
Analytical groups are tasked with methods development, sample analysis & methods transfer.

Establishment of genetically engineered cells (e.g. CHO) that produce desired product.

Analytical Groups
Develop analytical methods; analyze samples from other functions, methods transfer.
Typical workflows in biopharma analytical labs — from cell culture harvest to clone selection

Samples from cell culture & purification process development, formulation, stability studies, QA/QC

**Sample Purification**
- Cell culture fluid
  - Titer check
    - Passed
      - Prep Protein A capture of mAb
        - Fraction collection
          - Neutralization
            - Dilution
  - Failed
    - Reject

**Sample Preparation**
- Prep Protein A capture of mAb
  - Papain, FabRICATOR Pepsin
    - Peptide Digestion
      - C-Terminal Lys removal by CpB
        - Stability Studies
          - Glycan Release & Labeling
            - HOS Sample Prep e.g. HDX

**LC Characterization**
- Size Analysis
  - Fragment Analysis
    - Peptide Mapping
      - Charge Variant Analysis
        - PTM (Oxidation, Deamidation)
          - Glycan Analysis
            - HOS Analysis

**LC/MS characterization**
- Intact Mass & Glycoforms
  - Middle-Up, -Down Analysis
    - Amino Acid Sequencing
      - Impurity Analysis
        - PTM Analysis
          - Glycan ID & Quant
            - Higher Order Structure (HOS) Analysis

**Clone selection**

Samples from cell culture & purification process development, formulation, stability studies, QA/QC

Agilent Technologies
New trends in Biopharma presents new analytical challenges

• Biosimilars
  – Need to demonstrate similarity/comparability between biosimilar to its innovator molecule

• Antibody Drug Conjugates (ADCs)
  – Increased analytical complexity due conjugate, linker & conjugation chemistries
BIOSIMILARS
Definitions

• Innovator biologic
  – Novel clinically-validated biologic on which biosimilars or biobetters are designed

• Biosimilar
  – Biologic molecule with identical primary amino acid sequence as innovator biologic and developed with intention to be as close to the innovator product as possible

• Biobetter
  – Biologic molecule based on the innovator molecule but with improvements intended to increase efficacy, potency, marketability, safety, or patient compliance

• Next-generation
  – Biologic molecule based on same validated target as innovator biologic, but with novel VH/VL chains and (typically) different epitope, with intent of making an improved biologic against the validated target
Biosimilars—*The Race is “ON”*

**Insight & Intelligence™ : Dec 19, 2011**

**Firms Are Upping the Stakes on mAb Biosimilar Development**

As originators try to defend their patents, companies make larger investments in biosimilars.

Patricia F. Dimond, Ph.D.

Despite delays by the FDA and some opposition from originator companies, biosimilars now represent one of the most rapidly evolving areas of product development in the biopharmaceutical industry. The EU already has legislation in place for the approval of biosimilars, and the FDA has publicly committed to publishing biosimilar guidelines by the end of this year.

Judging from the feverish activity among potential biosimilar marketers, mAb follow-on proteins will be the hottest competitive area. At $6.6 billion in 2010 sales, Ritu-xan is the largest revenue-producing biologic yet to be targeted by biosimilar developers. This anti-CD20 chimeric mAb is approved for chronic lymphocytic leukemia, non-Hodgkin’s lymphoma, and RA and is due to come off patent in 2015.

South Korea’s Celltrion has initiated clinical trials of CT-P13, its Ritu-xan biosimilar. Sandoz, Novartis’ genetics arm, has a Phase II RA trial with its own version of Ritu-xan. Teva Pharmaceuticals and Spectrum Pharmaceuticals are also working on Ritu-xan biosimilars; Teva obtained therapeutic protein production capacity and expertise through its 2009 joint venture agreement with Lonza focused on biosimilars.
Biosimilars—“Foot-in-the-Door” for Emerging Markets

Mumbai, Hyderabad: Cipla said on Tuesday it would invest $65 million (around Rs 300 crore) to acquire stakes in two biotech companies in India and China, as it joins Indian peers like Wockhardt and Biocon to tap the $90-billion biogenerics (generic versions of biotech drugs, also called ‘biosimilars’) opportunity across the globe.

The company's board has approved acquisition of a 40% stake in Indian biotech company, MabPharm, for $40 million. The biotech firm is setting up a state-of-the-art facility for biosimilar products in Goa. Cipla will have rights to market all biosimilar products of the company in India and in the international markets.

The second is the acquisition of a 25% stake in BioMab, a biotech company in Hong Kong, for around $25 million. Here the investment will be made through a wholly-owned overseas subsidiary. The biotech company is setting up a state-of-the-art facility for biosimilar products in Shanghai through its wholly owned...
...and large corporations like Samsung
Tuesday, February 19, 2013

Amgen's Biosimilars Gambit

About a week ago, Amgen rocked the biotech industry’s proverbial boat with their announcement that they’d be entering the biosimilars market. Multiple news outlets like Yahoo!, Forbes, and CNBC report that Amgen, starting in 2017, will be making six generic versions of blockbuster biologics:

- Abbvie's Humira
- Janssen's Remicade
- Roche's Avastin, Herceptin and Rituxan
- Eli Lilly's Erbitux

This comes as a surprise to many, because for years, Amgen has been saying that biologics really can't be copied.
Biologics are falling off the patent cliff too!

* 12-year data exclusivity period ends in 2014
BPCI* Act defines Biosimilar or Biosimilarity

• Biosimilar or Biosimilarity means:
  – that the biological product is highly similar to the reference product notwithstanding minor differences in clinically inactive components; and
  – there are no clinically meaningful differences between the biological product and the reference product in terms of the safety, purity, and potency of the product

• FDA Biosimilars Guidance Outlines ‘Stepwise’ Development Approach
  – The FDA has issued three long-awaited biosimilars guidance documents, recommending a stepwise approach to showing biosimilarity that could allow eased trial requirements if a sponsor can demonstrate biosimilarity in earlier steps

*Biologics Price Competition and Innovation Act of 2009
The challenge
How similar is similar enough?

• Which attributes matter and which don’t?

• If differences are seen, they need to be shown, somehow, not to be clinically meaningful

• To predict clinical similarity from the analytical data, need to understand relationship between protein quality attributes and the clinical safety & efficacy profile of the specific product

• What does the demonstration of “highly similar” get you?
  – An Abbreviated Licensure Pathway
    • Licensure based on less than the full complement of product-specific non-clinical and clinical data that would normally be required for a new biological entity
The stepwise approach should start with extensive structural and functional characterization of both the proposed product and the reference product, which serves as the foundation of a biosimilar development program.

Highly similar analytical & PK/PD data = ↓ Risk of clinical differences
- Reduce requirements for clinical studies
What does extensive structural and functional characterization means?

- All need to be evaluated as part of analytical similarity studies
Different process, different product
Biosimilar that is different from the originator

Same gene as innovator

Establishment of genetically engineered cells (e.g. CHO) that produce desired product

DNA Vector

Cell Culture Scale-up & Production
Process- and product-related substances and impurities?
Raw material analysis?
Centrifuge

Did host cell protein impurity profile change?
Glycosylation profile?

Did host cell protein impurity profile change?
Glycosylation profile?

Even if a biosimilar uses the same human gene as its innovator, it will differ in other parts of manufacturing process

Same amino acid seq as innovator?
N- or C-terminal truncation?
Any modification because of genetic stability?

Formulation & excipients
Effect on stability, safety & efficacy?

Stability studies
Product degradation profile?

Formulation

Downstream Processing & Purification

Chromatography Column #1
Chromatography Column #2
Chromatography Column #3

Market
Manufacturing
Clinical
Pre-clinical
## Analytical tools to evaluate biosimilarity are the same but focus on comparability features

<table>
<thead>
<tr>
<th>Attributes</th>
<th>Analytical tools</th>
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<tbody>
<tr>
<td>Amino acid sequence and modifications</td>
<td>Mass spectrometry (MS), peptide mapping, chromatographic separations</td>
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<tr>
<td>Folding</td>
<td>S-S bonding, calorimetry, HDX and IM-MS, NMR, circular dichroism, Fourier transform &amp; Raman spectroscopy, fluorescence, interaction chromatographies</td>
</tr>
<tr>
<td>Subunit interactions</td>
<td>Chromatography, IM-MS</td>
</tr>
<tr>
<td>Heterogeneity (size, charge, hydrophobicity)</td>
<td>Chromatography resins; gel &amp; capillary electrophoresis, light Scattering, IM-MS</td>
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<tr>
<td>Glycosylation</td>
<td>Anion exchange, enzymatic digestion, peptide mapping, CE, MS</td>
</tr>
<tr>
<td>PEGylation &amp; isomers</td>
<td>Chromatography, peptide mapping</td>
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<tr>
<td>Bioactivity</td>
<td>Cellular and animal bioassays; ligand &amp; receptor binding (ELISA, surface plasmon resonance), signal transduction</td>
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<tr>
<td>Aggregation</td>
<td>Analytical ultracentrifugation, size-exclusion chromatography, field flow fractionation, light scatter, microscopy</td>
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<tr>
<td>Proteolysis</td>
<td>Electrophoresis, chromatography, MS</td>
</tr>
<tr>
<td>Impurities (HCP, DNA)</td>
<td>LC, LC/MS, LBAs, PCR, metal (ICP-MS) &amp; solvent analysis</td>
</tr>
</tbody>
</table>
We have the most comprehensive portfolio of analytical instrumentation & solutions for biosimilars.
Comparison of follow on biologics to an innovator mAb by HPLC, SEC and Peptide Mapping

Samples:
- Innovator – **Ristova** (Rituximab/Roche)
- Biosimilar – **Reditux** (Rituximab/Dr. Reddy’s)
  - Samples purchased from local Pharmacy in Bangalore, India

Analytical tools:
- The Agilent 1260 Bio-inert LC
- Biocolumns
- Match Compare Software
RP HPLC of Biosimilar and Innovator mAb
Agilent 1260 Infinity Bio-inert LC using Poroshell 120 SB C18 4.6x150 mm, 2.7 μm column

DAD1 B, Sig=280, Ristova (Innovator)

DAD1 B, Sig=280, Reditux (Biosimilar)
Agilent Match Compare tool for comparison

Compare an unknown sample, by selecting the sample chromatogram, in data analysis within OpenLAB CDS.

To start the comparison, select “Compare current chromatogram” under the Match Compare menu item.
Agilent Match compare analysis of intact mAbs – RP HPLC

100% identical

Similarity 1.000
RP HPLC of reduced biosimilar and innovator mAb
Agilent 1260 Infinity Bio-inert LC using Poroshell 120 SB C18 4.6x150 mm, 2.7 µm column
Agilent Match compare analysis of reduced mAb-HPLC

Similarity 1.000

Area

Innovator

Biosimilar
Intact SEC of Biosimilar and Innovator mAb
Agilent 1260 Infinity Bio-LC using a Bio SEC-3, 300Å, 7.8x300 mm, 3 µm
Agilent Match compare analysis of intact mAbs-SEC

Results table

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<tr>
<th>Code</th>
<th>Name</th>
<th>Rt Samp [min]</th>
<th>Rt Ref [min]</th>
<th>DT</th>
<th>% Samp</th>
<th>% Ref</th>
<th>% Error</th>
<th>Tol [%]</th>
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<td>6.00</td>
<td>Id.</td>
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</table>

0.00 % of peaks out of tolerance represents 0.00 % of total area

0.00 % of unknown peaks represents 0.00 % of total area

Area
SEC of Reduced Biosimilar and Innovator mAb
Agilent 1260 Infinity Bio-LC using a Bio SEC-3, 300Å, 7.8x300 mm, 3 µm
Match compare analysis of reduced mAbs – SEC

100% identical

Similarity 1.000

100.00% Identical
0.00% Out of tolerance
0.00% Ref. only
0.00% Samp. only

Innovator

Biosimilar
Peptide mapping of biosimilar and innovator mAb
Agilent 1260 Infinity Bio-LC using a Poroshell 120 SB C18 4.6x150 mm. 2.7 µm column
Peptide mapping of Biosimilar and innovator mAb
Zoom in of chromatogram; 5 – 20 min
Peptide mapping of Biosimilar and innovator mAb
Zoom in of chromatogram; 20 – 40 min
Zoom in of four representative peaks across the chromatogram to show separation reproducibility
Peptide mapping of Biosimilar and innovator mAb

Comparison of peptide maps of innovator and biosimilar mAb using Agilent OpenLab Match Compare Software (Peaks selected for comparison are annotated)
Peptide mapping of Biosimilar and innovator mAb
Match Compare result

100% Identical
Similarity 1.000

Innovator
Biosimilar
Charge Variant Analysis of Biosimilar & Innovator
Agilent BioMAb WCX Column

Ristova (Originator)

Reditux (Biosimilar)
ANTIBODY DRUG CONJUGATE (ADC)
Rationale for Antibody Drug Conjugates (ADCs)

- Some small molecule drugs have high systemic toxicity, e.g. chemotherapy drugs used for cancer treatment
- Antibodies can target particular cells (e.g. antigen positive tumor cells) quite selectively
- Covalently linking antibodies to small molecule drugs can target the drug and reduce systemic toxicity
- With antibodies that have biological activity, conjugation may increase their effectiveness
Antibody drug conjugates (ADCs)
Targeted cancer therapy
ADC
Surge in INDs

Antibody-Drug Conjugate IND Submissions

Top 10 Leading Sponsors of Ongoing Clinical Trials of Antibody Drug Conjugates

- AstraZeneca
- Sanofi
- UCB
- Agensys
- Astellas
- ImmunoGen
- Takeda
- Pfizer
- Hoffmann-La Roche
- Seattle Genetics

Number of Ongoing Trials as of 10/22/1012
Antibody drug conjugate currently in clinical trials

<table>
<thead>
<tr>
<th>INN (Isotype)</th>
<th>Drug</th>
<th>Linker</th>
<th>Target</th>
<th>Indication</th>
<th>Sponsor</th>
<th>Clinical Stage (Phase)</th>
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<tbody>
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<td>Gemtuzumab ozogamicin (IgG4)</td>
<td>Calicheamycin</td>
<td>Hydrazine</td>
<td>CD33</td>
<td>AML</td>
<td>Pfizer (Wyeth)</td>
<td>MA2000 (withdrawn 2010)</td>
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<td>Inotuzumab ozogamicin (IgG4)</td>
<td>Calicheamycin</td>
<td>Hydrazine</td>
<td>CD22</td>
<td>NHL</td>
<td>Pfizer (Wyeth)</td>
<td>III</td>
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<tr>
<td>Trastuzumab emtansine (IgG1)</td>
<td>DM1</td>
<td>Thioether</td>
<td>HER2</td>
<td>Breast ca</td>
<td>Genentech</td>
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<td>Lorvotuzumab mertansine (IgG1)</td>
<td>DM1</td>
<td>Thioether</td>
<td>CD56</td>
<td>Myeloma</td>
<td>ImmunoGen</td>
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<td>IMGN-388</td>
<td>DM4</td>
<td>Thioether</td>
<td>αV integrin</td>
<td>Solid tumors</td>
<td>Centocor</td>
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<td>SAR3419</td>
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<td>Disulfide</td>
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<td>Cripto</td>
<td>Breast cancer</td>
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<td>DM4</td>
<td>Disulfide</td>
<td>CD138</td>
<td>Myeloma</td>
<td>Biostat</td>
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<tr>
<td>Brentuximab vedotin (IgG1)</td>
<td>vcMMAE</td>
<td>Valine-Citrulline</td>
<td>CD30</td>
<td>HL</td>
<td>Seattle Genetics</td>
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<td>Glenatumumab vedotin (IgG2)</td>
<td>vcMMAE</td>
<td>Valine-Citrulline</td>
<td>GFNMB</td>
<td>Breast cancer, melanoma</td>
<td>CellDex</td>
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<td>SGN-75</td>
<td>mcMMAF</td>
<td>Maleimidocapryl</td>
<td>CD70</td>
<td>NHL, RCC</td>
<td>Seattle Genetics</td>
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<tr>
<td>PSMA ADC</td>
<td>vcMMAE</td>
<td>Valine-Citrulline</td>
<td>PSMA</td>
<td>Prostate cancer</td>
<td>Progenics</td>
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<td>EphA2</td>
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<td>Medimmune</td>
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<td>ASG-5ME</td>
<td>vcMMAE</td>
<td>Valine-Citrulline</td>
<td>SLC44A4</td>
<td>Pancreatic cancer</td>
<td>Agensys</td>
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<td>MN</td>
<td>Auristatin</td>
<td>NA</td>
<td>MN</td>
<td>Cancer</td>
<td>Bayer Schering</td>
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<td>MDX-1203</td>
<td>Duocarmycin</td>
<td>Dipeptide</td>
<td>CD70</td>
<td>NHL, RCC</td>
<td>BMS (Medarex)</td>
<td>I</td>
</tr>
</tbody>
</table>

Notes:
- approved:
- on market now:
Antibody drug conjugates (ADCs)

Design

T-DM1 (Genentech)

Components:

- Antibody
  - Targeted recognition
  - Abundant target expression & internalization

- Drug
  - Highly potent
  - Validated mechanism of action (microtubule inhibition, DNA damage)

- Linker
  - Stable in plasma
  - Labile upon internalization to release drug

DM1 = ☀ (3 to 4 per IgG) Linker-thioether- Trastuzumab (HzlgG1) -LysNH₂ (random)
Heterogeneity in ADCs
Complexity

T-DM1 (Genentech)

- More complex than mAbs alone
- Dependent on linker & payload stability (hydrolysis, degradation, etc)
- Dependent on the conjugation chemistry
Heterogeneities in ADCs from conjugation
Dependent on conjugation chemistry

Typ DAR=2
Av DAR=3-8

Lysine conjugation
Interchain disulfide conjugation
Site-directed conjugation

# drug / Ab molecule
# drug / Ab molecule
# drug / Ab molecule
Analytical complexity and heterogeneity

• Conjugation introduces heterogeneity on top of that already present in the antibody

• Additional assays required beyond those for an antibody due to presence of cytotoxic agent, e.g.
  – Drug to antibody ratio (DAR)
  – Amount of free and bound cytotoxic agent
Regulatory considerations in developing antibody drug conjugates

• Regulatory Jurisdiction
  – A conjugate is composed of small molecule components (linker and cytotoxic agent) and an antibody
  – What are the roles of divisions with expertise in the components (e.g. ONDQA and DMA)?
  – With divided responsibility, how is review and interaction with the sponsor coordinated?

• Classification of components (Antibody, Linker, Cytotoxic Agent) used to manufacture conjugates
  – Is existing terminology appropriate (e.g. starting material, intermediate, API)?
  – What are the consequences for testing, validation, process changes?
### Key quality attributes & methods for ADC

<table>
<thead>
<tr>
<th>Quality attributes</th>
<th>Assays</th>
<th>ADC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Identity</td>
<td>Intact Mass, Peptide Mapping, Sequence</td>
<td>mAb + ADC</td>
</tr>
<tr>
<td>Size heterogeneity (aggregates)</td>
<td>SDS-PAGE, SEC, MALS, MS</td>
<td>mAb + ADC</td>
</tr>
<tr>
<td>Charge heterogeneity</td>
<td>IEF, CEX</td>
<td>mAb + ADC</td>
</tr>
<tr>
<td>PTMs</td>
<td>LC/MS</td>
<td>mAb + ADC</td>
</tr>
<tr>
<td>Drug load (DAR)</td>
<td>UV, HIC, HPLC</td>
<td>ADC</td>
</tr>
<tr>
<td>Drug load distribution</td>
<td>HIC, MS</td>
<td>ADC</td>
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<tr>
<td>Residual drug</td>
<td>ELISA, HPLC</td>
<td>ADC</td>
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<tr>
<td>Potency (for drug)</td>
<td>Cytotoxicity</td>
<td>ADC</td>
</tr>
<tr>
<td>Potency (for mAb)</td>
<td>Antigen binding ELISA</td>
<td>mAb + ADC</td>
</tr>
</tbody>
</table>
Drug to antibody ratio (DAR) by HIC

- HIC is gentle and does not disrupt non-covalent interactions. This retains MAb structures lacking normal disulfide bonds as found in some conjugates.

- Typical HIC eluents
  - A: 2.0 M (NH₄)₂SO₄ in 0.1M NaH₂PO₄, pH 7.0; B: 0.1 M NaH₂PO₄, pH 7.0

- Metal cladded PEEK design
- 600 Bar
DAR-HIC stability studies

- Lyophilized sample - T0
- Solution sample – 2 weeks @ 40C
Analysis of reduced ADC fragments

Figure 4. (A–D) Reversed-phase HPLC analysis of DTT-reduced conjugates produced using different reduction/reoxidation protocols. (E–H) Analysis of the same conjugate samples in (A–D), under non-reducing conditions, using the Agilent Bioanalyzer™, a silicon chip based system for capillary electrophoresis in the presence of SDS (CE-SDS). Adapted with permission from Sun MM, Beam KS, Cerveny CG, Hamblett KJ, Blackmore RS, Torgov MY, et al. Reduction-alkylation strategies for the modification of specific monoclonal antibody disulfides. A Wakankar, Y Chen, Y Gokarn & F Jacobson (Genentech)
Size variant analysis of conjugates

Figure 5. SEC analysis on a TSK 3000SW columns run at 0.5 mL/min and monitored by 280 nm absorbance. (A) Mobile phase is 0.2 M KPi and 0.25 M KCl, pH 6.95. (B) 85% KPi/KCl mobile phase; 15% 2-propanol.
Peptide mapping of ADC

- Enzymatic cleavage of ADCs can be used to identify drug-containing peptides
  - Peptides labeled with a hydrophobic drug would be expected to elute later than their unmodified forms in the RP-HPLC chromatogram due to increased retention by the column
  - Trastuzumab has 88 lysines for possible linkage. DM1 chromophore ($\lambda_{\text{max}} = 252\text{nm}$) can be used to identify peptides containing bound drug. Peptides (~60 new peaks) with drug elute at end of gradient
Simple peptide map; Cys-linked ADC

- 4 major sites of vcMMAE conjugation at interchain disulfide bonds:
  - Cys 220 and Cys 218 (H-L)
  - Cys 226 and Cys 229 (H-H)

Agilent Solutions: 1260 Bio or 1290 Zorbax & AdvanceBio Peptide mapping
Free drug analysis by RP-HPLC

- Free drug species are very low in cysteine-linked ADCs
- Barely detectable increases in free drug during storage, even at elevated temperatures
- Lysine linked conjugates (e.g. T-DM1) show some time dependent release of drug-linker from side-reactions w/other amino acids

Agilent Solutions: 1260 Bio or 1290 Zorbax & AdvanceBio Peptide mapping
Drug distribution & location by LC/MS

- The complexity of the spectrum is due to the presence of DMx molecules and N-linked glycans.

Dn represents the number of DMx molecules per MAb.
Deglycosylating the conjugate reveals heterogeneity due to conjugation.

Dn represents the number of DMx molecules per MAb.
dx.doi.org/10.1021/ac203346c | Anal. Chem. 2012, 84, 2843–2849

Native Intact Mass Determination of Antibodies Conjugated with Monomethyl Auristatin E and F at Interchain Cysteine Residues

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Mass Spectrometry. Mass spectral data for mAbs and ADCs was acquired on an Agilent 6510 QTOF (Agilent, Santa Clara, CA) in positive electrospray ionization (ESI) mode in the range 1000–8000 m/z. The drying gas temperature was 350 °C, and flow rates for the drying gas and the nebulizer gas pressure were 12 L/h and 35 psi, respectively. The capillary, fragmentor, and octupole rf voltages were set at 5000, 450, and 750, respectively. The raw data was converted to zero charge mass spectra with a maximum entropy deconvolution algorithm within the MassHunter workstation software version B.03.01.
**UHPLC**: Agilent 1290 Infinity Binary pump, well plate autosampler, thermostatted column compartment, 1200 binary pump

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>PolyLC PolyHYDROXYETHYL A, 1 x 50 mm, 5 μm, 300 Å</td>
</tr>
<tr>
<td>Column temperature</td>
<td>23 °C</td>
</tr>
<tr>
<td>Injection volume</td>
<td>1-5 μL</td>
</tr>
<tr>
<td>Autosampler temp</td>
<td>4 °C</td>
</tr>
<tr>
<td>Needle wash</td>
<td>flushport (50% MeOH in H2O), 10 seconds</td>
</tr>
<tr>
<td>Mobile phase</td>
<td><strong>A = 200 mM Ammonium Acetate (Native)</strong></td>
</tr>
<tr>
<td></td>
<td>B = 0.2% Formic Acid, 30% Acetonitrile in Water (Denaturing)</td>
</tr>
<tr>
<td>Flow rate</td>
<td><strong>0.050 mL/min</strong></td>
</tr>
<tr>
<td>Gradient</td>
<td><strong>ISOCRATIC</strong></td>
</tr>
<tr>
<td>Stop time</td>
<td><strong>5.00 min</strong></td>
</tr>
</tbody>
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Mass measurement of a deglycosylated mcMMAF conjugate ADC
Comparing Two Batches of ADC
Mass distribution profile is a good test of process consistency

- UV based assay gives average drug loading
  - MS provides orthogonal verification
- MS also provides information on relative amounts of antibodies loaded with 1, 2, 3, 4 etc. drugs (without prior separation)
- MS also provides information on unconjugated antibody
- MS can detect other conjugation products, e.g.
  - Cross linked species
  - Species with linker attached but no drug
Mass distribution profile shows comparability after process changes

Other analytical methods also show these batches are comparable
**in vivo** Stability assay for ADCs & their metabolites in serum (PK) by affinity capture LC-MS

**AssayMAP Bravo Platform**
Summary

- Interest in Antibody Drug Conjugates is high and growing
- Conjugates are analytically challenging due to their complexity and heterogeneity
- There is regulatory complexity with conjugates due to split review responsibilities within the FDA
- Agilent has solution to analyze ADCs
  - Bio-Inert 1260 for HIC application for DAR
  - 1290 with RP columns for peptide mapping to located conjugation sites
  - LC-QTOF/TOF can be used for mass distribution profile
    - Heterogeneity due to the conjugate can be distinguished from that of the Antibody
  - AssayMAP/Encore automation solution can be leverage to capture ADC for PK/PD and conjugation stability studies in plasma
  - Bioanalyzer and TapeStation can be used to monitor ADC fragments & oxidation-reduction products during conjugation process
Thank you for your attention!

Q&A