Case Study: Kadcyla™ (Ado-Trastuzumab Emtansine)

European Bioanalysis Forum – ADC Training Day
Bringing ADC into Practice
Antibody-Drug Conjugate Submissions

20th June, 2017, Lisbon

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Overview

- General Background
- Kadcyla™ approval and highlights of clinical data
- Multiple diverse quantitative assays for pharmacokinetics
- Biotransformation and ensuring appropriate PK assays
- Overview of immunogenicity strategy and data
- Summary and acknowledgments
What Are Antibody Drug Conjugates (ADCs)?

- Targeted and selective cancer treatment with reduced side effects

S. Kaur, EBF ADC Training Day, Case Studies, Lisbon, 06.20.17
FDA NEWS RELEASE: Aug. 19, 2011 (Seattle Genetics)
FDA approves Adcetris® to treat two types of lymphoma
“The U.S. Food and Drug Administration today approved Adcetris® (brentuximab vedotin) to treat Hodgkin lymphoma (HL) and a rare lymphoma known as systemic anaplastic large cell lymphoma (ALCL)”
http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncementsucm268781.htm

FDA NEWS RELEASE: Feb. 22, 2013 (Genentech/Roche)
FDA approves new treatment for late-stage breast cancer
The U.S. Food and Drug Administration today approved Kadcyla® (ado-trastuzumab emtansine), a new therapy for patients with HER2-positive, late-stage (metastatic) breast cancer
http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncementsucm340704.htm

Footnote: Mylotarg® (gemtuzumab ozogamicin), accelerated approval 2000 for acute myeloid leukemia (AML), withdrawn 2010 after results from subsequent trial raised safety concerns and failure to demonstrate clinical benefit
Kadcyla™ Molecular Structure:
Trastuzumab Emtansine (T-DM1)

Ado-trastuzumab emtansine (T-DM1)
**Antibody:** Targets HER-2 positive tumors
**Drug:** Derivative of Maytansine,
**Linker:** Designed to be stable
**Drug/Antibody (DAR):** Approximately 3.5/1
**Complex mixture:** DAR0-DAR8
T-DM1 vs Capecitabine + Lapatinib in HER2+ MBC Phase III Study: TDM4370g/BO21977

**EMILIA**

**Primary end points:** PFS by IRF, OS, Safety

**Secondary end points:** Quality of life

**HER2-positive (centrally confirmed)**
Locally advanced or metastatic BC
previously received trastuzumab-based therapy
(n = 980)

**T-DM1**
3.6 mg/kg q3w

**Lapatinib**
(1250 mg/day) Days 1–21 +
Capecitabine
(1000 mg/m²) Days 1–14 q3w

Kadcyla™ Development Team
Progression-free survival by independent review

<table>
<thead>
<tr>
<th></th>
<th>Median (months)</th>
<th>No. of events</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cap + Lap</td>
<td>6.4</td>
<td>304</td>
</tr>
<tr>
<td>T-DM1</td>
<td>9.6</td>
<td>265</td>
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</table>

Stratified HR=0.650 (95% CI, 0.55, 0.77)  
\( P<0.0001 \)

Unstratified HR=0.66 (\( P<0.0001 \))
Overall Survival: Confirmatory Analysis

<table>
<thead>
<tr>
<th>Time (months)</th>
<th>Proportion surviving</th>
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<tr>
<td>0</td>
<td>1.0</td>
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<tr>
<td>12</td>
<td>0.852</td>
</tr>
<tr>
<td>24</td>
<td>0.647</td>
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<tr>
<td>36</td>
<td>0.518</td>
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<tr>
<td>48</td>
<td>0.45</td>
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<tr>
<td>60</td>
<td>0.38</td>
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<tr>
<td>72</td>
<td>0.28</td>
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<tr>
<td>84</td>
<td>0.13</td>
</tr>
<tr>
<td>96</td>
<td>0.05</td>
</tr>
<tr>
<td>108</td>
<td>0.01</td>
</tr>
</tbody>
</table>

No. at risk:
- **Cap + Lap**: 496, 471, 453, 435, 403, 368, 297, 240, 204, 159, 133, 110, 86, 63, 45, 27, 17, 7, 4
- **T-DM1**: 495, 485, 474, 457, 439, 418, 349, 293, 242, 197, 164, 136, 111, 86, 62, 38, 28, 13, 5

**Data cut-off July 31, 2012; Unstratified HR=0.70 (P=0.0012).**

**Unstratified HR**
- **Cap + Lap**: 0.682 (95% CI, 0.55, 0.85; P=0.0006)
- **T-DM1**: 0.70 (P=0.0012)

**Efficacy stopping boundary**
- P=0.0037 or HR=0.727
Overview

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ADC Conjugation Sites & Heterogeneity

Conjugation through lysines

Conjugation through reduced internal disulfide bonds

Conjugation through engineered cysteines
Bioanalytical strategies based on experience from a diverse ADC platform and a rich pipeline.

**Late Stage Research**
- Molecule Selection
- Early ADCs

**Early Development**
- IND Enabling Studies
- Pre-IND ADCs

**Clinical Development**
- Phase I
- Phase II
- Phase III

**Filing/Approval**
- Post Marketing
- KADCYLA (trastuzumab emtansine) approved in 2013 for HER2+ mBC

Bioanalytical strategies based on experience from a diverse ADC platform and a rich pipeline.

Bioanalytical strategies based on experience from a diverse ADC platform and a rich pipeline.

Bioanalytical strategies based on experience from a diverse ADC platform and a rich pipeline.
ADCs are Complex: Many analyte choices....

S. Kaur, EBF ADC Training Day, Case Studies, Lisbon, 06.20.17

Multi-disciplinary bioanalytical team to enable innovation:

- Protein structural characterization by affinity capture LC-MS
- Large molecule quantification & immunogenicity by LBA
- Large molecule quantification by hybrid binding LC-MS/MS
- Small molecule LC-MS/MS methods

Develop novel methods specifically designed to characterize ADC analytes in vivo to ensure appropriate quantitative assays & biotransformation insights

Characterize ELISA reagents to ensure binding to all DARs

S. Kaur, EBF ADC Training Day, Case Studies, Lisbon, 06.20.17
Potential for Additional Heterogeneity In Vivo:
Active Large Catabolites & Small Catabolites Possible

Kaur, Xu, Saad, Dere, Triguero, Bioanalysis (2013)
Saad et al., Bioanalysis 7(13), 1583-1604 (2015)
ADC Drug/Antibody (DARs) can Monitor Changes in Plasma


S. Kaur, EBF ADC Training Day, Case Studies, Lisbon, 06.20.17
T-DM1 DAR in Nonclinical Study \textit{in vivo}

T-DM1 Cyno PK Study

Scale is relative to major component at each time point, * Extra MCC linker

- Characterize key ADC analytes \textit{in vivo}: Ensure appropriate PK assays for DARs
- Valuable biotransformation insights: T-DM1 with at least DAR1 up to day 28

L. Liu, K. Xu, D. Leipold, J. Tibbitts, S. Kaur
Characterize T-DM1 ELISA Ligand Binding Assay Reagents: Ensure Anti-DM1 mAb and HER2 ECD Binding to All DARs

Affinity capture LC-MS of T-DM1 after incubation in human plasma with each capture probe

- DAR distributions data confirm ELISA reagents bind all DARs appropriately
- T-DM1 enriched DAR fractions recovered 80-120% in ELISA (data not shown)
Kadcyla™ Clinical Studies and Bioanalysis:
Three PK Assays, Catabolite Assays & ADA Assays

**Indication (Type of Study)**

**MBC**
- **3rd Line (Single Agent)**
  - Phase I: 3569g
  - Phase II: 4374g, 4258g
  - Phase III: TDM4997g (TH3RESA)

- **2nd Line (T-DM1 vs. X+L)**
  - Phase III: 4370g (EMILIA)

- **1st Line**
  - Phase II: 4450g
  - Phase III: 4788g (MARIANNE)

**Special Studies**
- Phase II: TDM4688g (QTc Study)
- Hepatic Impairment Study

- Global Development
  - China, Japan etc

**Combination Studies**
- T-DM1 + Pertuzumab (4373g)
- T-DM1 + Paclitaxel (4652g)
- T-DM1 + Docetaxel (BP22572)
- T-DM1 + GDC0941 (PI3K)

**Potential for Drug Interaction?**
T-DM1 Clinical PK Phase II Studies (3.6 mg/kg q3w)
Showing Data from Two ELISA and One LC-MS/MS Assay

- Consistent PK across 3 Phase II studies
- Consistent low systemic exposure to DM1

| PK of trastuzumab emtansine conjugate, total trastuzumab and DM1 are comparable between patients enrolled in Asia and overall population |

<table>
<thead>
<tr>
<th></th>
<th>$C_{\text{max}}$ ($\mu$g/mL)</th>
<th>$\text{AUC}_{\text{inf}}$ (day $\cdot$ $\mu$g/mL)</th>
<th>Mean (SD)</th>
<th>$t_{1/2}$ (day)</th>
<th>$V_{\text{ss}}$ (mL/kg)</th>
<th>CL (mL/day/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Trastuzumab Emtansine Conjugate</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All Patients (n=292)</td>
<td>83.4 (16.5)</td>
<td>489 (122)</td>
<td>3.68 (0.886)</td>
<td>29.5 (14.6)</td>
<td>7.81 (2.18)</td>
<td></td>
</tr>
<tr>
<td>Patients enrolled in Asia (n=55)</td>
<td>77.8 (12.0)</td>
<td>439 (87.8)</td>
<td>3.54 (0.651)</td>
<td>31.1 (7.15)</td>
<td>8.55 (2.02)</td>
<td></td>
</tr>
<tr>
<td><strong>Total Trastuzumab</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All Patients (n=291)</td>
<td>86.3 (20.1)</td>
<td>816 (422)</td>
<td>7.80 (4.01)</td>
<td>42.2 (15.6)</td>
<td>5.35 (2.33)</td>
<td></td>
</tr>
<tr>
<td>Patients enrolled in Asia (n=53)</td>
<td>80.2 (16.5)</td>
<td>676 (274)</td>
<td>7.00 (2.95)</td>
<td>44.1 (11.3)</td>
<td>6.16 (2.44)</td>
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</tr>
</tbody>
</table>

$CL$ = clearance; $C_{\text{max}}$ = maximum plasma concentration; $t_{1/2}$ = half-life; $V_{\text{ss}}$ = volume of distribution at steady state.

Mean Cycle 1 $C_{\text{max}}$ for DM1 were 4.61 ng/mL (All) and 4.25 ng/mL (Asian)

Courtesy of Sandhya Girish
Exploratory LC-MS/MS assays to measure T-DM1 catabolites in Nonclinical and Clinical Studies to support ADME

- Same catabolites (DM1, MCC-DM1 and Lys-MCC-DM1) also identified in human plasma

All LC-MS/MS assays outsourced and validated by CRO

S. Kaur, EBF ADC Training Day, Case Studies, Lisbon, 06.20.17
12 CLINICAL PHARMACOLOGY

• 12.3 Pharmacokinetics

The pharmacokinetics of KADCYLA was evaluated in a phase 1 study and in a population pharmacokinetic analysis for the ado-trastuzumab emtansine conjugate (ADC) using pooled data from 5 trials in patients with breast cancer. A linear two-compartment model with first-order elimination from the central compartment adequately describes the ADC concentration-time profile. In addition to ADC, the pharmacokinetics of total antibody (conjugated and unconjugated trastuzumab), DM1 were also determined. The pharmacokinetics of KADCYLA are summarized below.

• Distribution

- Maximum concentrations (Cmax) of ADC and DM1 were observed close to the end of infusion. In Study 1, mean (SD) ADC and DM1 Cycle 1 Cmax following KADCYLA administration was 83.4 (16.5) g/mL and 4.61 (1.61) ng/mL, respectively.
- In vitro, the mean binding of DM1 to human plasma proteins was 93%. In vitro, DM1 was a substrate of P-glycoprotein (P-gp).
- Based on population pharmacokinetic analysis, the central volume of distribution of ADC was 3.13 L.

• Metabolism

- In vitro studies indicate that DM1, the small molecule component of KADCYLA, undergoes metabolism by CYP3A4/5. DM1 did not inhibit or induce major CYP450 enzymes in vitro. In human plasma, ado-trastuzumab emtansine catabolites MCC-DM1, Lys-MCC-DM1, and DM1 were detected at low levels.
Overview

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Immunogenicity Assays for ADCs

Measure anti-drug antibodies (ADAs) to all components

- ADCs have potential to elicit immune responses *in vivo* and may affect PK, PD, efficacy and safety
- Assays should be sensitive and robust in presence of ADC and measure ADAs to antibody, linker, cytotoxic drug, neo-epitopes
Tiered Approach for ADC Immunogenicity Testing: Detect Anti-Drug Antibodies (ADA) to All Components

- **Screening Assay**
  - ADA Positive
    - Confirmatory Assay
      - Negative result
        - **ADA Positive**
  - ADA Negative
    - Confirm Specificity
      - POSITIVE Sample
        - Characterize +ve Response
          - Titer Assay To mAb or linker-drug? NAb activity?
      - NEGATIVE Sample
        - Therapeutic concentration detectable?
          - Yes
            - NEGATIVE Sample (Therapeutic interference?)
          - No
            - NEGATIVE Sample (Therapeutic interference?)

Immunogenicity incidence and Impact if any on safety, efficacy and PK

- Sampling for ATA across multiple clinical studies (Phase I – III)
  - Can we streamline PK sampling?
- Assess incidence of ATA in early Phase I studies
  - Assess impact if any on PK, safety
- To assess potential for drug interference we include sampling post termination
  - however, 3 months/6 months post termination can be challenging for oncology patients
  - Assess ethnic insensitivity (differences in incidence of immunogenicity and impact on safety/efficacy/PK if any)
Overview of Immunogenicity of Ado-Trastuzumab Emtansine in Patients

- Overall immunogenicity rate
  - Confirmed ADA responses were detected in 44/836 (5.3%) patients across six clinical studies

- Impact of ADA response on PK, safety and efficacy
  - PK and safety profiles are comparable between ADA positive and ADA negative patients
  - In EMILIA, a lower median PFS and OS were observed in ADA positive patients as compared to overall patients in the T-DM1 arm (PFS: 5.6 vs 9.6 mos, OS: 26.8 vs 30.9 mos) but objective response rates were similar (38.9% vs 43.6%)

Low incidence and robust analysis of the impact of ADA is not possible; therefore, the clinical significance of antibodies to ado-trastuzumab emtansine is unknown at this time
6.2 Immunogenicity

As with all therapeutic proteins, there is the potential for an immune response to KADCYLA.

A total of 836 patients from six clinical studies were tested at multiple time points for anti-therapeutic antibody (ATA) responses to KADCYLA. Following KADCYLA dosing, 5.3% (44/836) of patients tested positive for anti-KADCYLA antibodies at one or more post-dose time points. The presence of KADCYLA in patient serum at the time of ATA sampling may interfere with the ability of this assay to detect anti-KADCYLA antibodies. As a result, data may not accurately reflect the true incidence of anti-KADCYLA antibody development. In addition, neutralizing activity of anti-KADCYLA antibodies has not been assessed.

Immunogenicity data are highly dependent on the sensitivity and specificity of the test methods used. Additionally, the observed incidence of a positive result in a test method may be influenced by several factors, including sample handling, timing of sample collection, drug interference, concomitant medication and the underlying disease. Therefore, comparison of the incidence of antibodies to KADCYLA with the incidence of antibodies to other products may be misleading. Clinical significance of anti-KADCYLA antibodies is not yet known.

PMC: Develop a validated, sensitive, and accurate assay for detection of neutralizing antibodies to TDM1, including procedures for detection in the presence of TDM1 levels in the serum or plasma at the time of patient sampling.
Summary & Conclusions

1. ADCs have complex structures and are dynamically changing mixtures in vivo.
2. Important to understand ADC analyte biotransformations for appropriate assays.
3. Bioanalysis requires LBA, small molecule LC-MS/MS & hybrid LC-MS/MS methods.
4. No specific ADC guidances & limited clinical data for choosing analytes for safety/efficacy.
5. Kadcyla™ bioanalytical three PK assay strategy was accepted by agencies.
6. Tiered immunogenicity strategy was accepted; Nab assay developed as PMC.
7. Catabolites were measured in selected studies.
Acknowledgments

BioAnalytical Sciences/ ADC Group

Mass Spectrometry:  
**Keyang Xu**, Luna Liu, Carl Ng, Dian Su, Jintang He

Mass Spectrometry:  
**Ola Saad**, Neelima Koppada, Violet Lee, Suk-Joon Hyung, Sylvia Wong

Immunoassays:  
Randy Dere

**Montse Carrasco**, Helen Davis, Connie Mahood, Kyu Hong

Collaborator Groups & ADC Teams

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Cassandra Duenas

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An Song, Patty Siguenza, Sara Kenkare