:

Case Study: Kadcyla™ (Ado-Trastuzumab Emtansine)

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

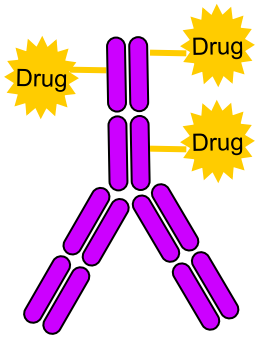
20th June, 2017, Lisbon

Surinder Kaur, Ph. D.
Director, ADC Programs & MS
BioAnalytical Sciences, Genentech
S. San Francisco, California

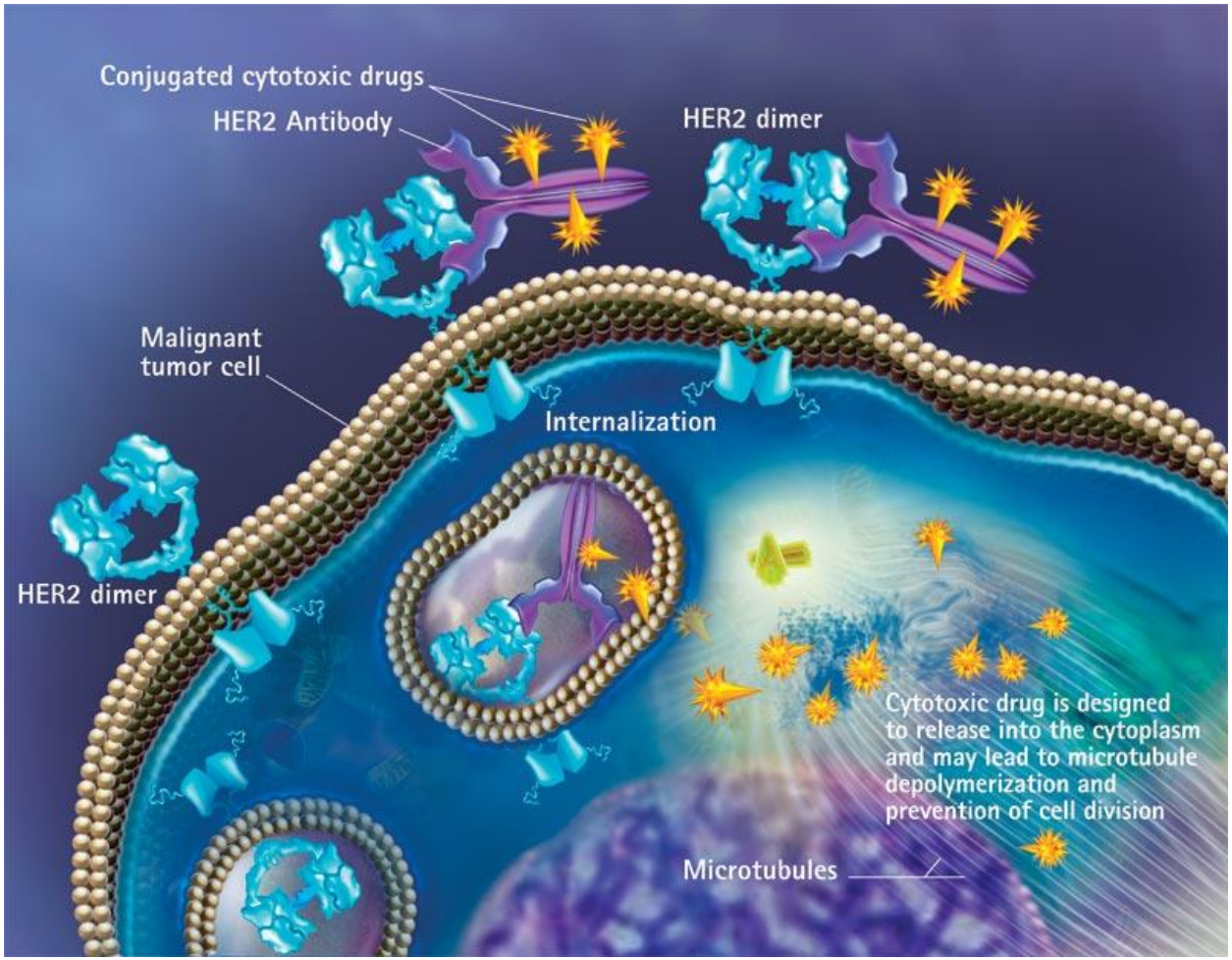
Overview

- 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)?



Antibody-Linker-Drug



- **Targeted and selective cancer treatment with reduced side effects**

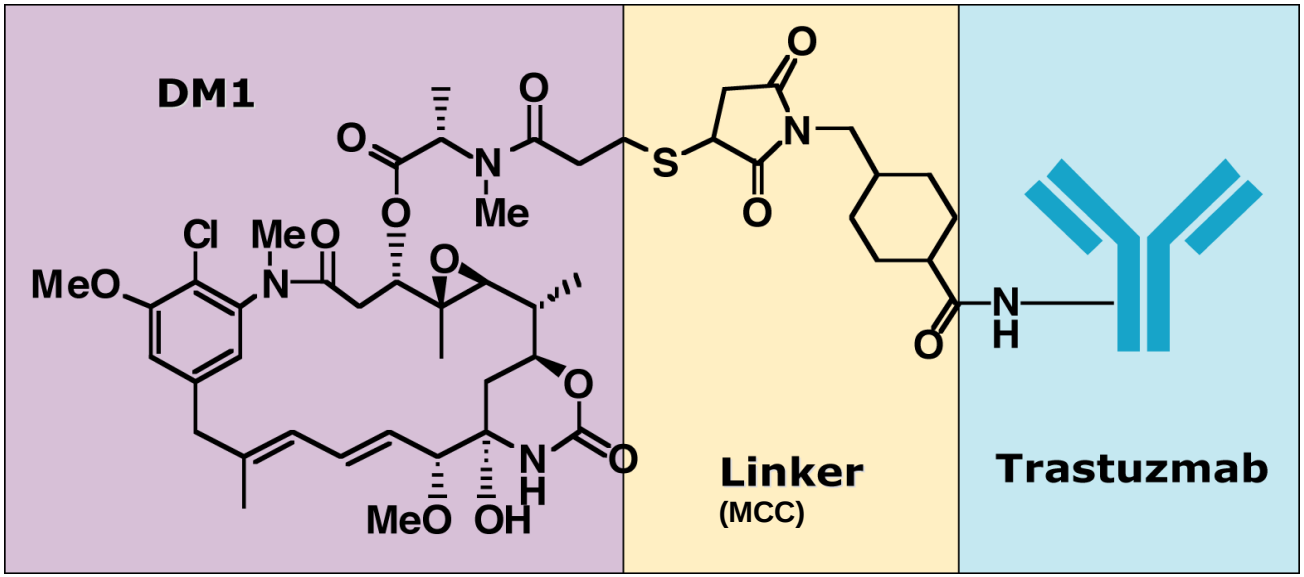
ADCs Approved by the Food and Drug Administration

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Footnote: Mylotarg® (gemtuzumab ozogamicin), accelerated approval 2000 for acute myeloid leukemia (AML), withdrawn 2010 after results from subsequent trial raised safety concerns and failure to demonstrate clinical benefit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

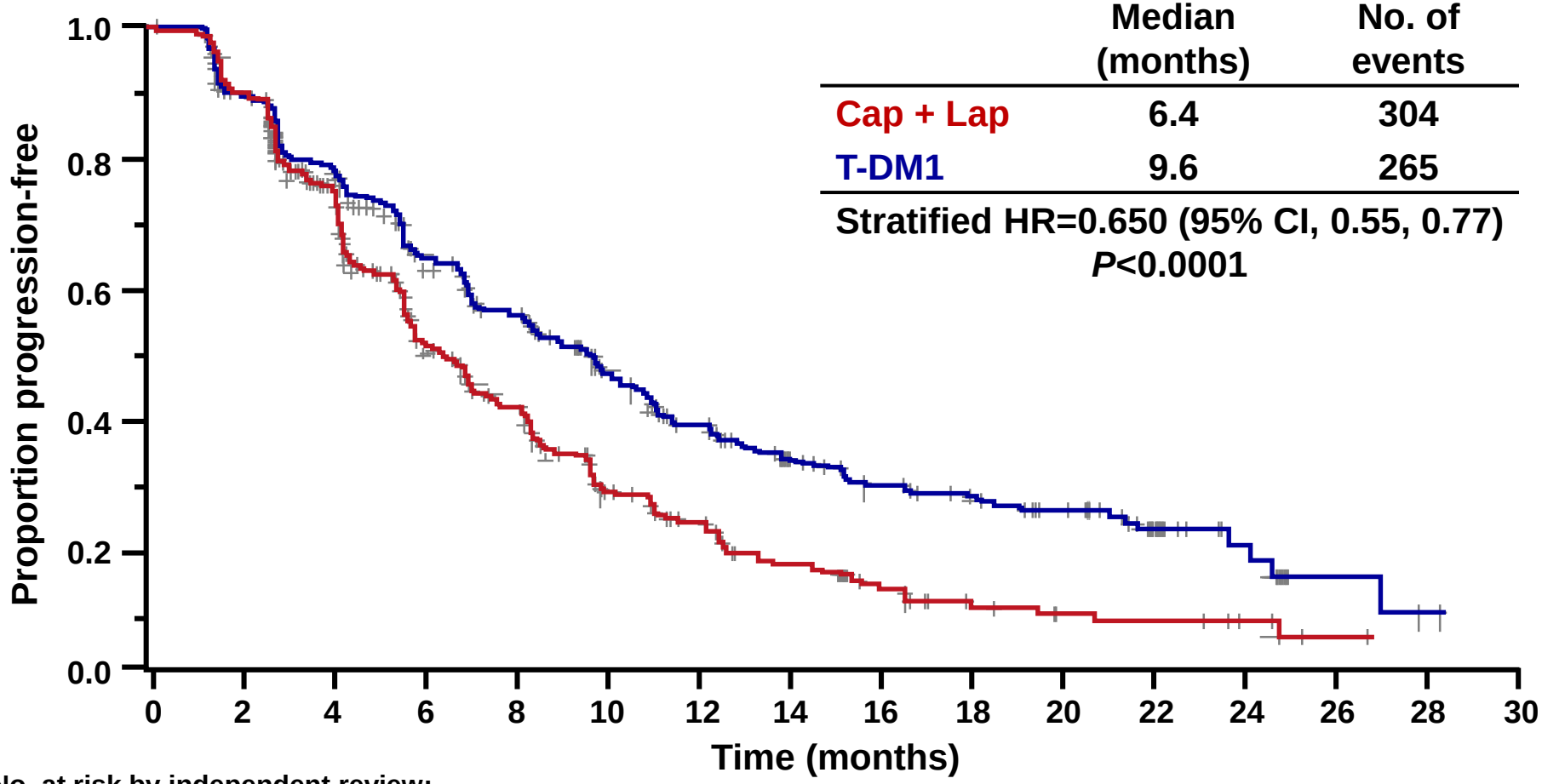
HER2-positive
(centrally confirmed)
Locally advanced or
metastatic BC
previously received
trastuzumab-based therapy
(n = 980)

T-DM1
3.6 mg/kg q3w

Lapatinib
(1250 mg/day) Days 1–21 +
Capecitabine
(1000 mg/m^2) Days 1–14 q3w

Primary end points: PFS by IRF, OS, Safety
Secondary end points: Quality of life

Progression-free survival by independent review



	Median (months)	No. of events
Cap + Lap	6.4	304
T-DM1	9.6	265

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

No. at risk by independent review:

Cap + Lap	496	404	310	176	129	73	53	35	25	14	9	8	5	1	0	0
T-DM1	495	419	341	236	183	130	101	72	54	44	30	18	9	3	1	0

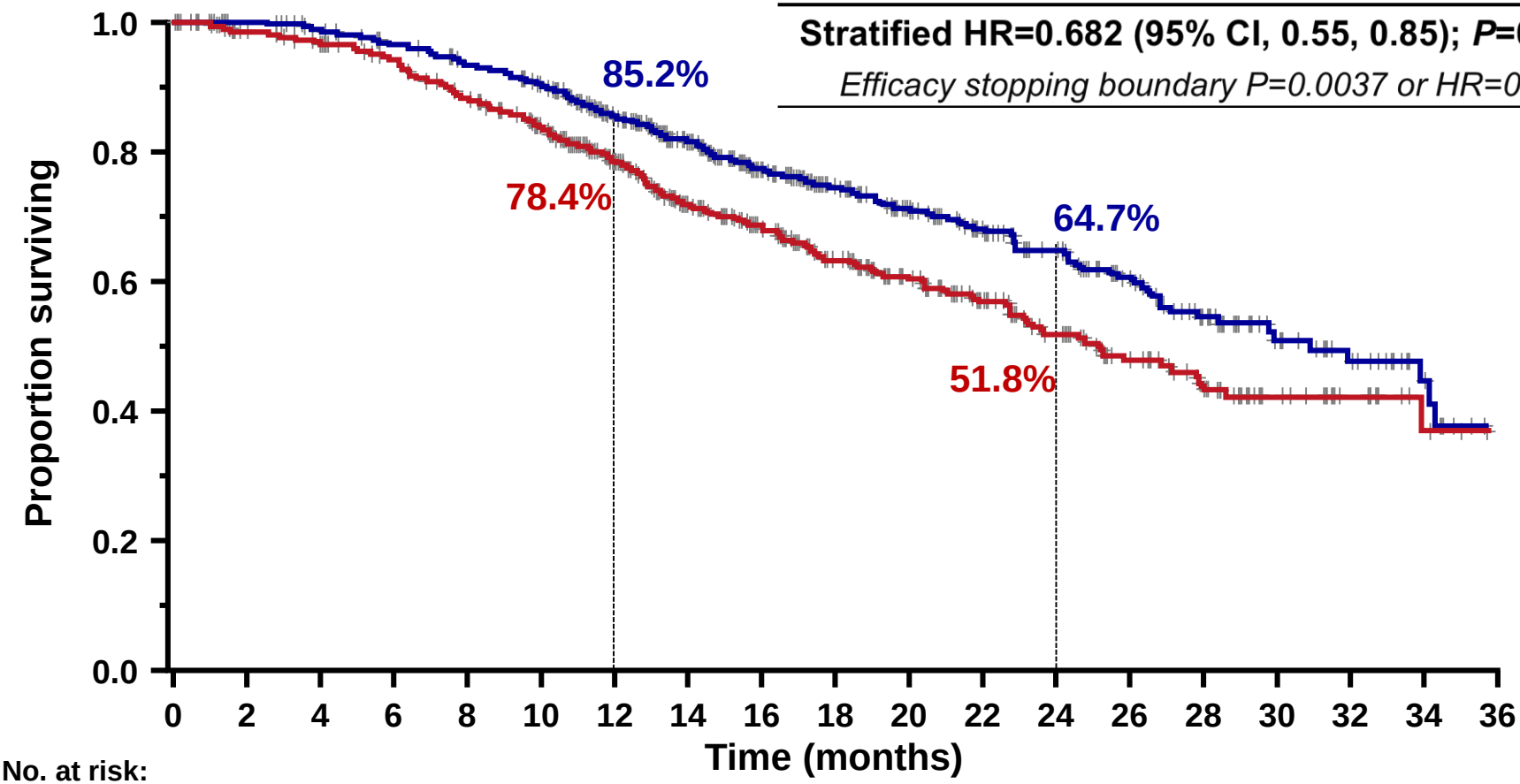
Unstratified HR=0.66 (*P*<0.0001)

Overall Survival: Confirmatory Analysis

	Median (mo)	No. of events
Cap + Lap	25.1	182
T-DM1	30.9	149

Stratified HR=0.682 (95% CI, 0.55, 0.85); *P*=0.0006

Efficacy stopping boundary P=0.0037 or HR=0.727



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

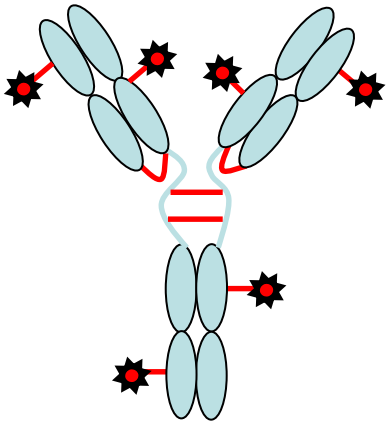
Data cut-off July 31, 2012; Unstratified HR=0.70 (*P*=0.0012).

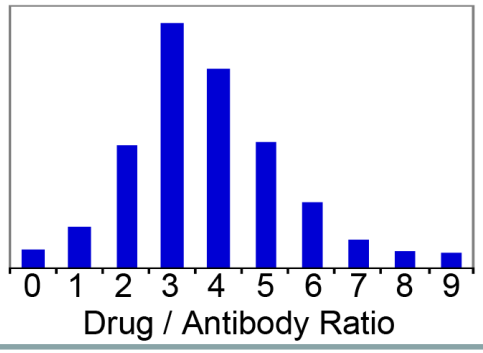
Overview

- 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

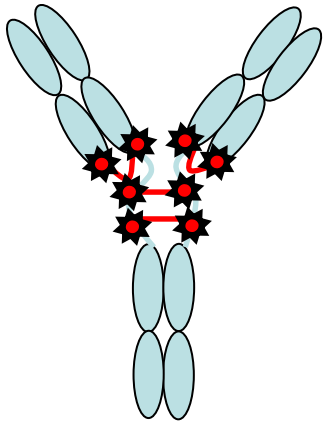
ADC Conjugation Sites & Heterogeneity

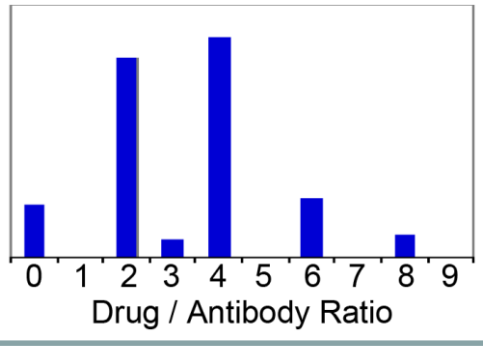
Conjugation through lysines



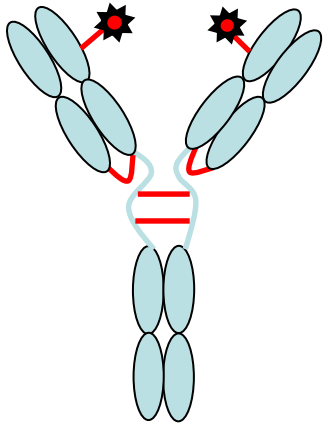


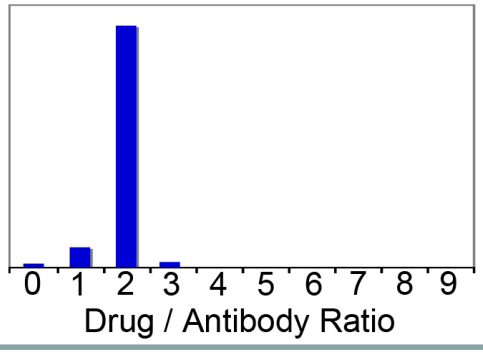
Conjugation through reduced internal disulfide bonds





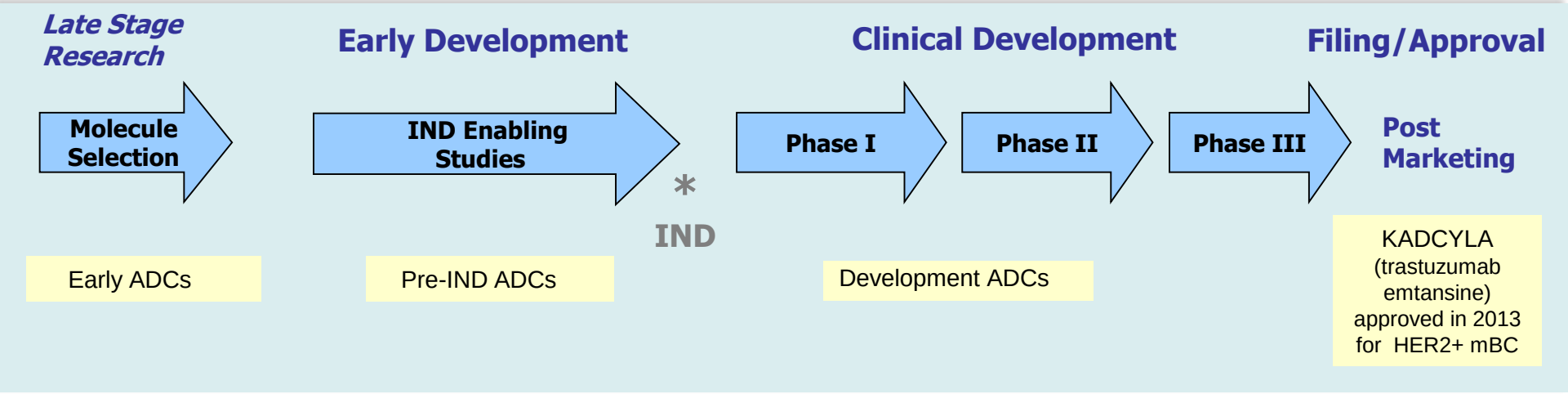
Conjugation through engineered cysteines

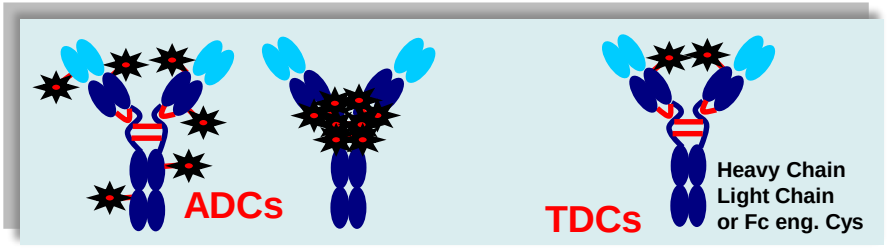




Bioanalytical Strategy Evolved with ADC Pipeline

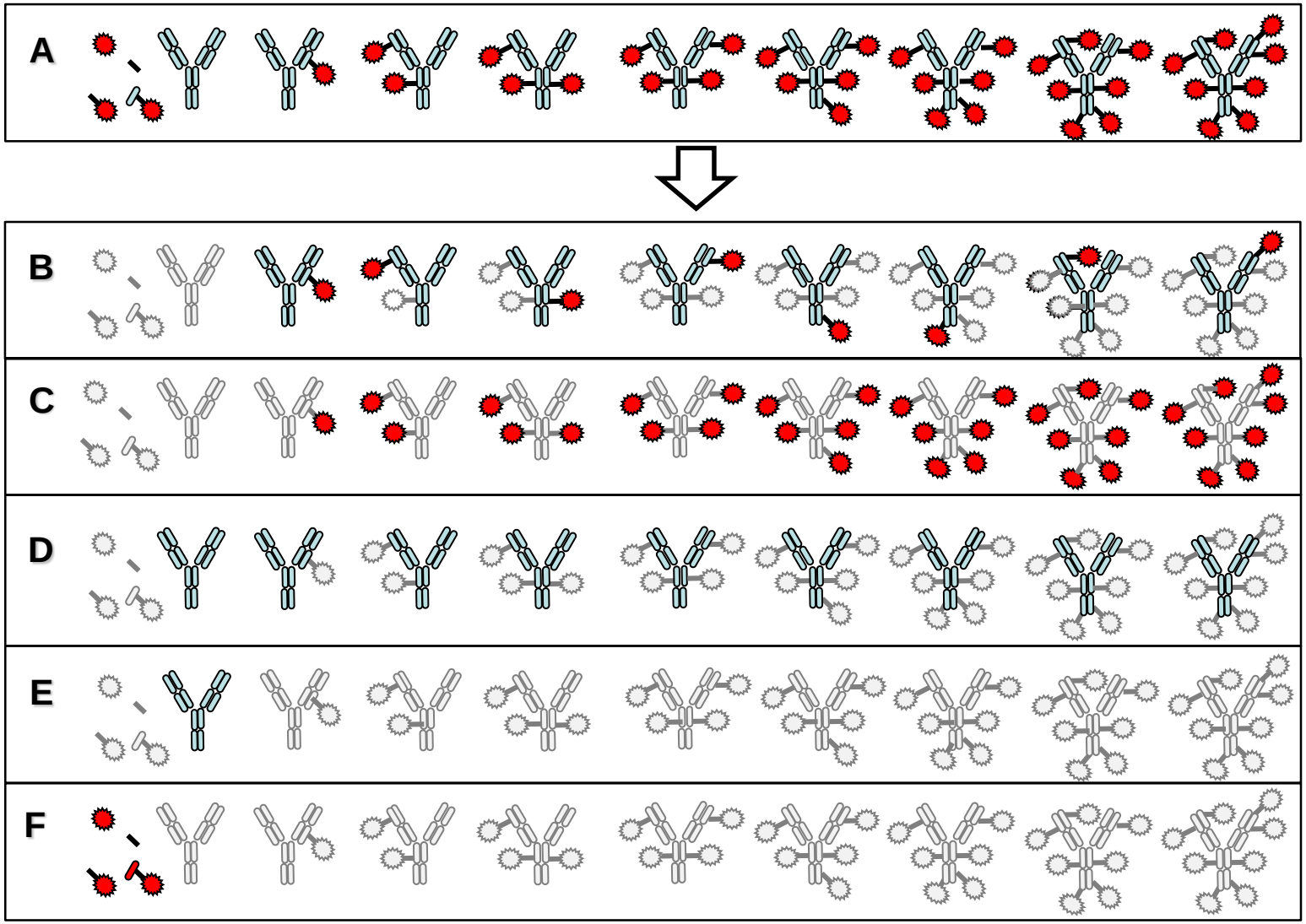
Bioanalytical strategies based on experience from a diverse ADC platform and a rich pipeline





Genentech
A Member of the Roche Group

ADCs are Complex: Many analyte choices….



S.Kaur, K.Xu, O.M.Saad, R.C.Dere, M.C.Triguero , Bioanalysis, 5(2), 201-226, (2013)

Integrated Strategies Needed for ADC Bioanalysis

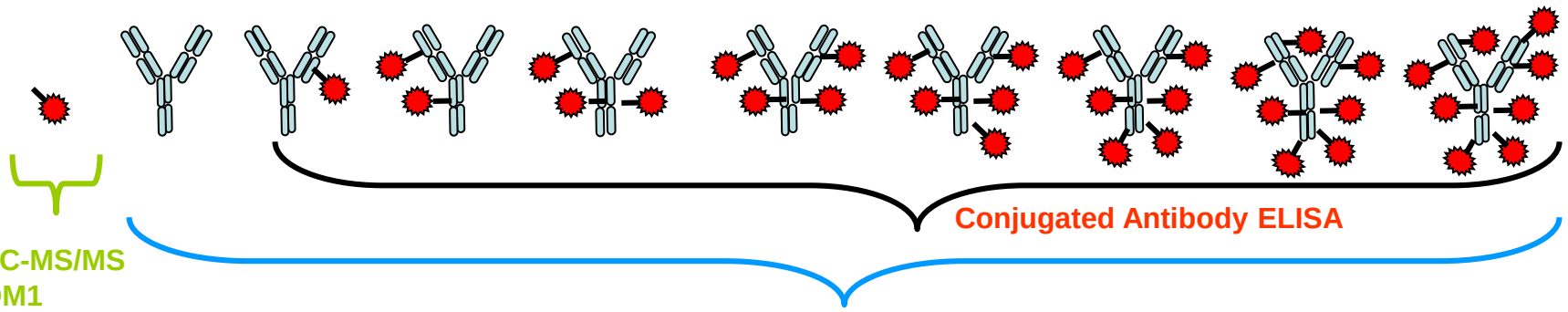
Multi-disciplinary bioanalytical team to enable innovation:

- 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

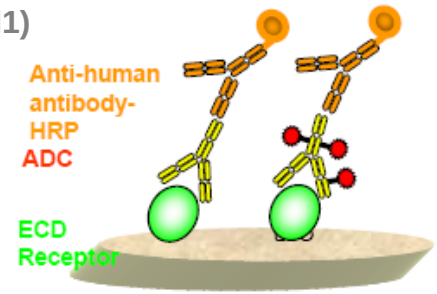
Develop novel methods specifically designed to characterize ADC analytes in vivo to ensure appropriate quantitative assays & biotransformation insights

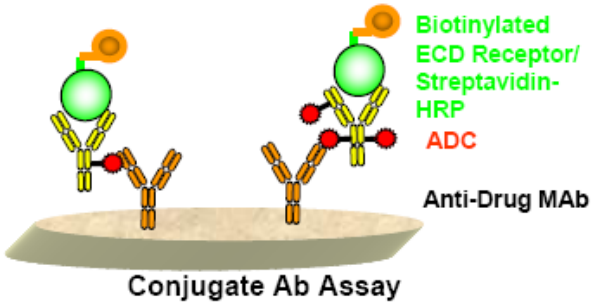
S.Kaur, K.Xu, O.M.Saad, R.C.Dere, M.C.Triguero , Bioanalysis, 5(2), 201-226, (2013)

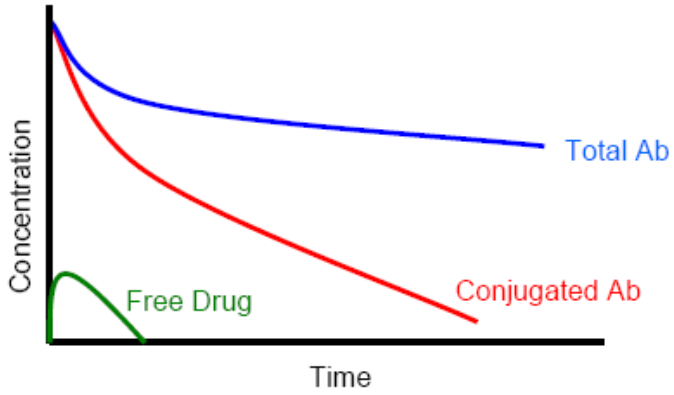
T-DM1 PK Assays in Plasma & Serum, Catabolite Assays in Plasma, Bile & Urine



LC-MS/MS
DM1
(MCC-DM1
Lys-MCC-DM1)

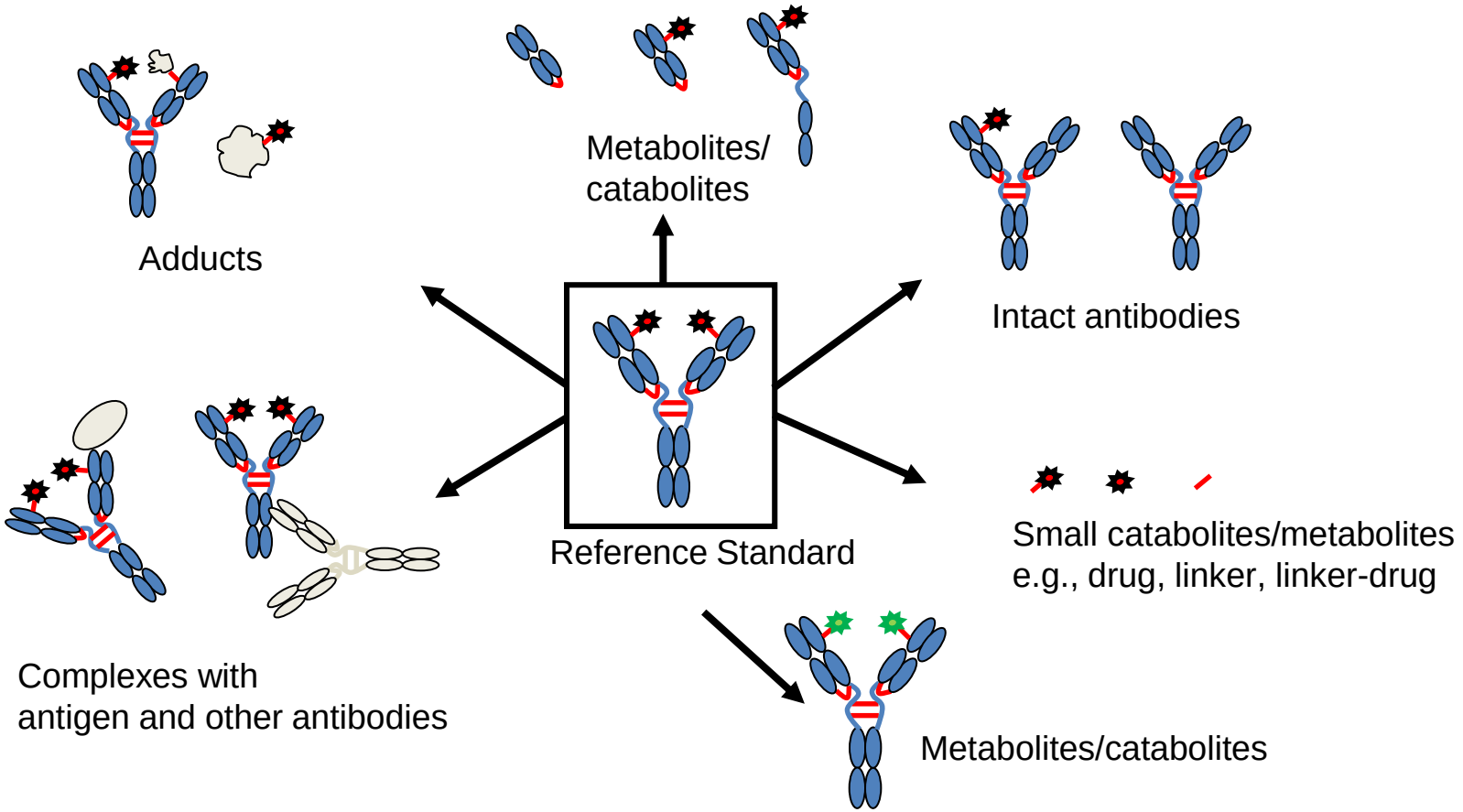






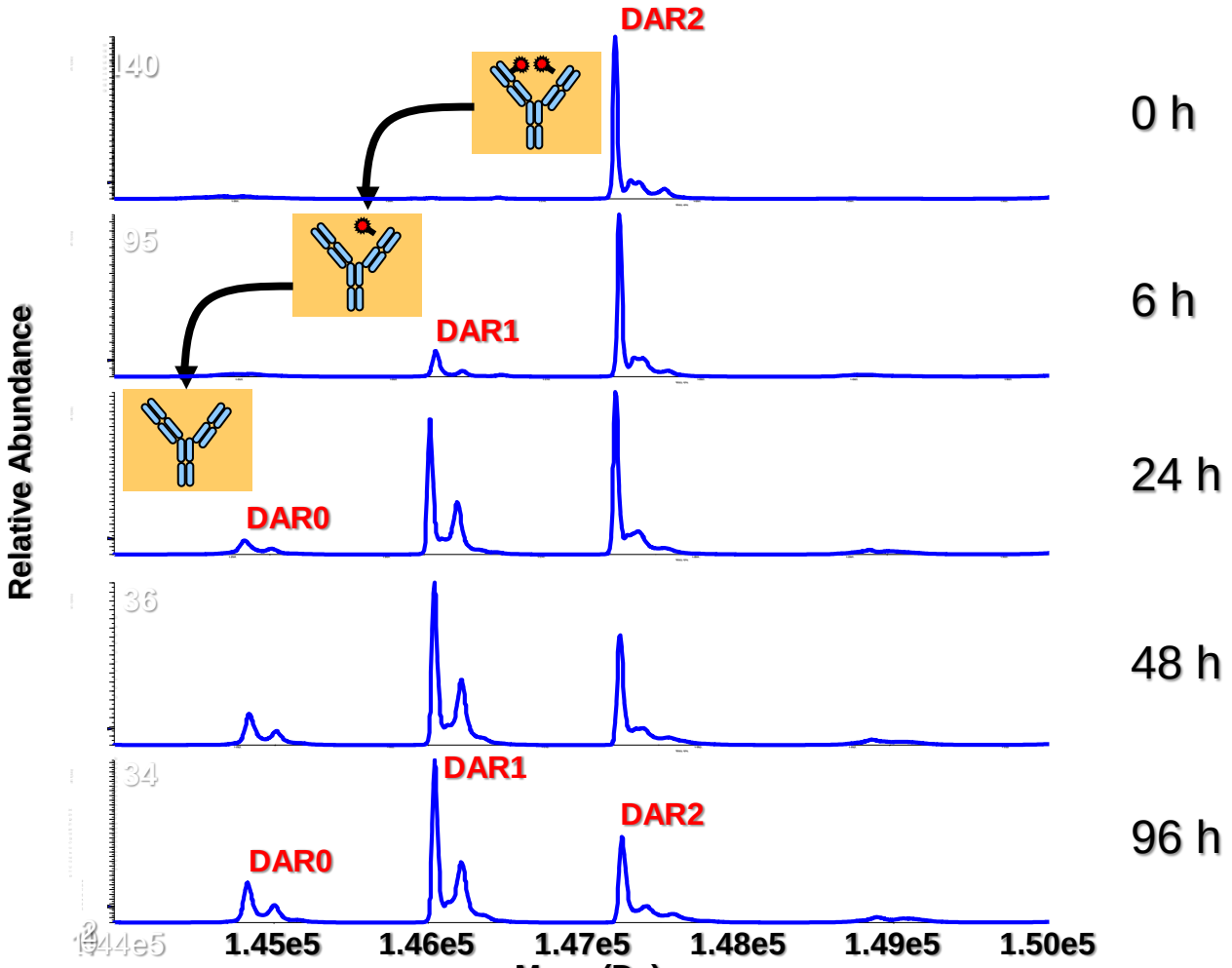
Characterize ELISA reagents to ensure binding to all DARs

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

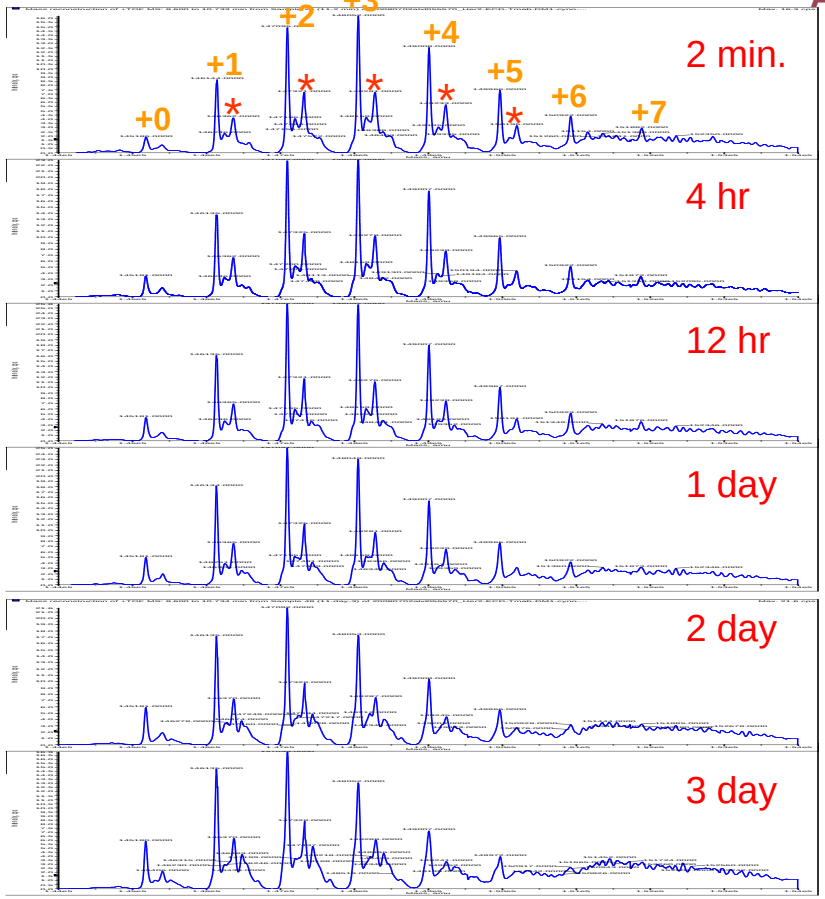


Incubated in rat plasma at 37°C

K. Xu, L. Liu, O.M. Saad, J. Baudys, L. Williams, D. Leipold, B. Shen, H. Raab, J.R. Junutula, A. Kim and S. Kaur, Anal. Biochem., 412, 56-66, (2011)

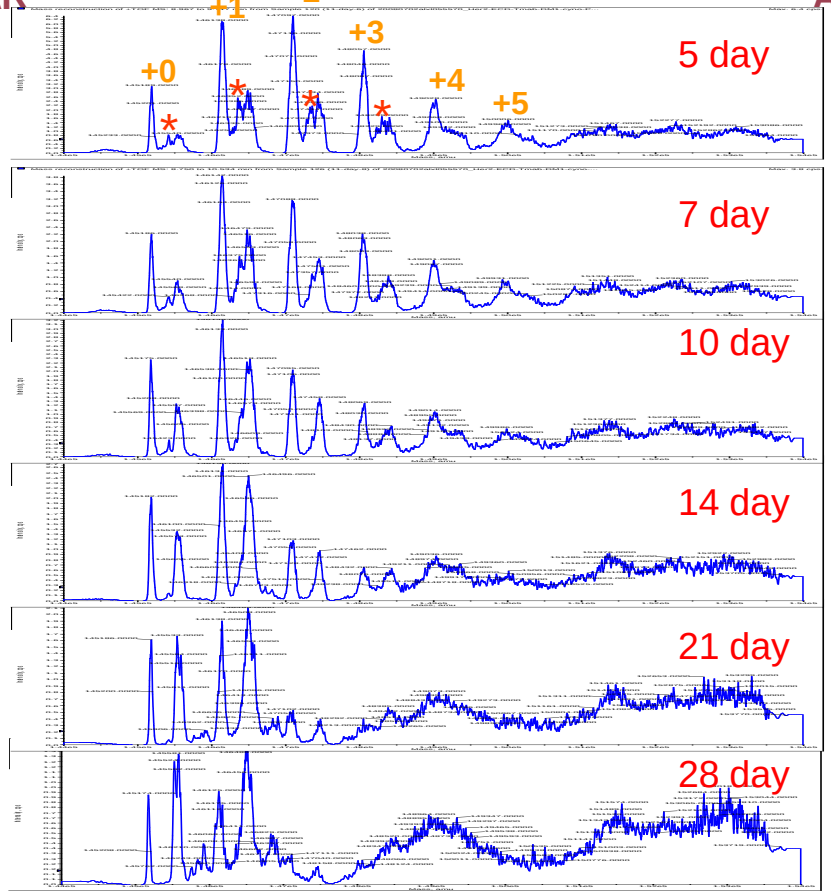
T-DM1 DAR in Nonclinical Study *in vivo*

T-DM1 Cyno PK Study



Av. DAR

3.16
3.04
2.96
2.76
2.57
2.32



Av. DAR

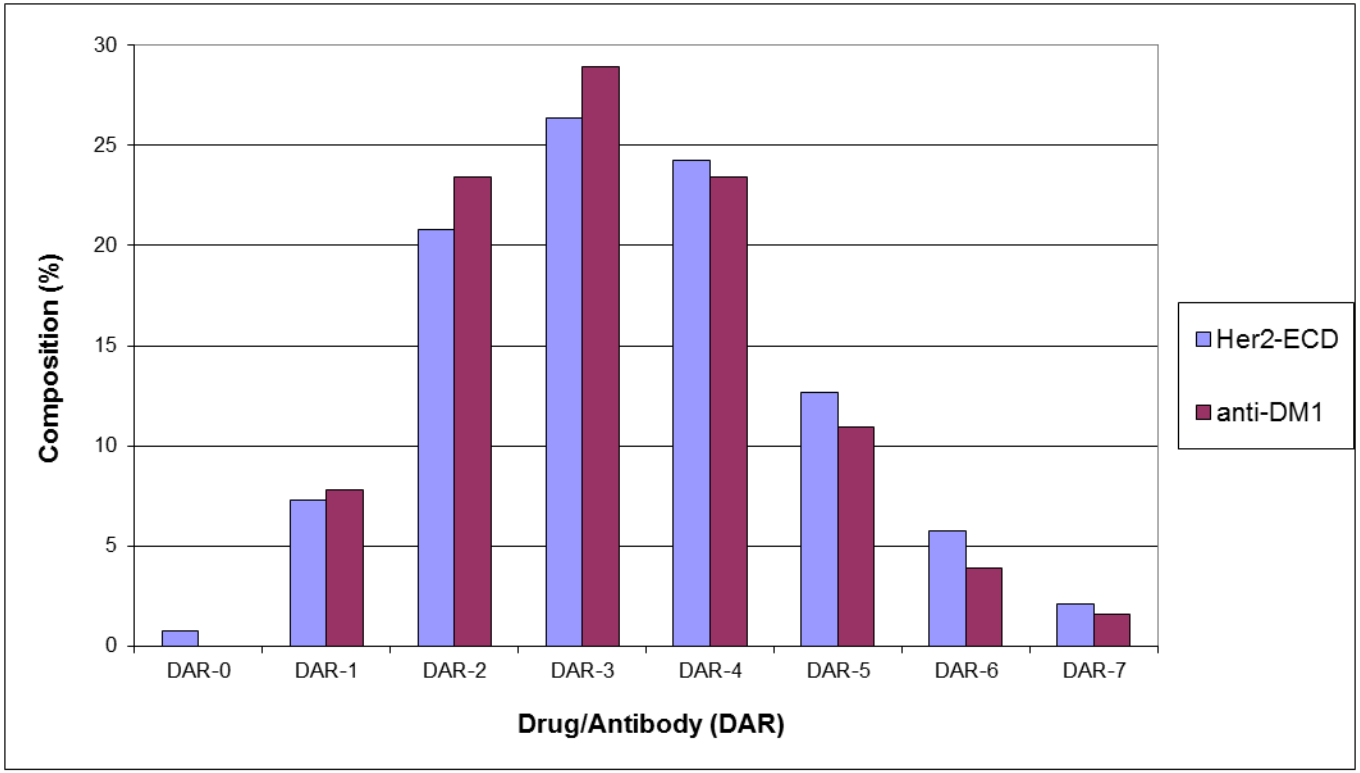
2.32
2.23
1.86
1.21
1.01
0.66

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

L. Liu, K. Xu, D. Leipold, J. Tibbitts, S. Kaur

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

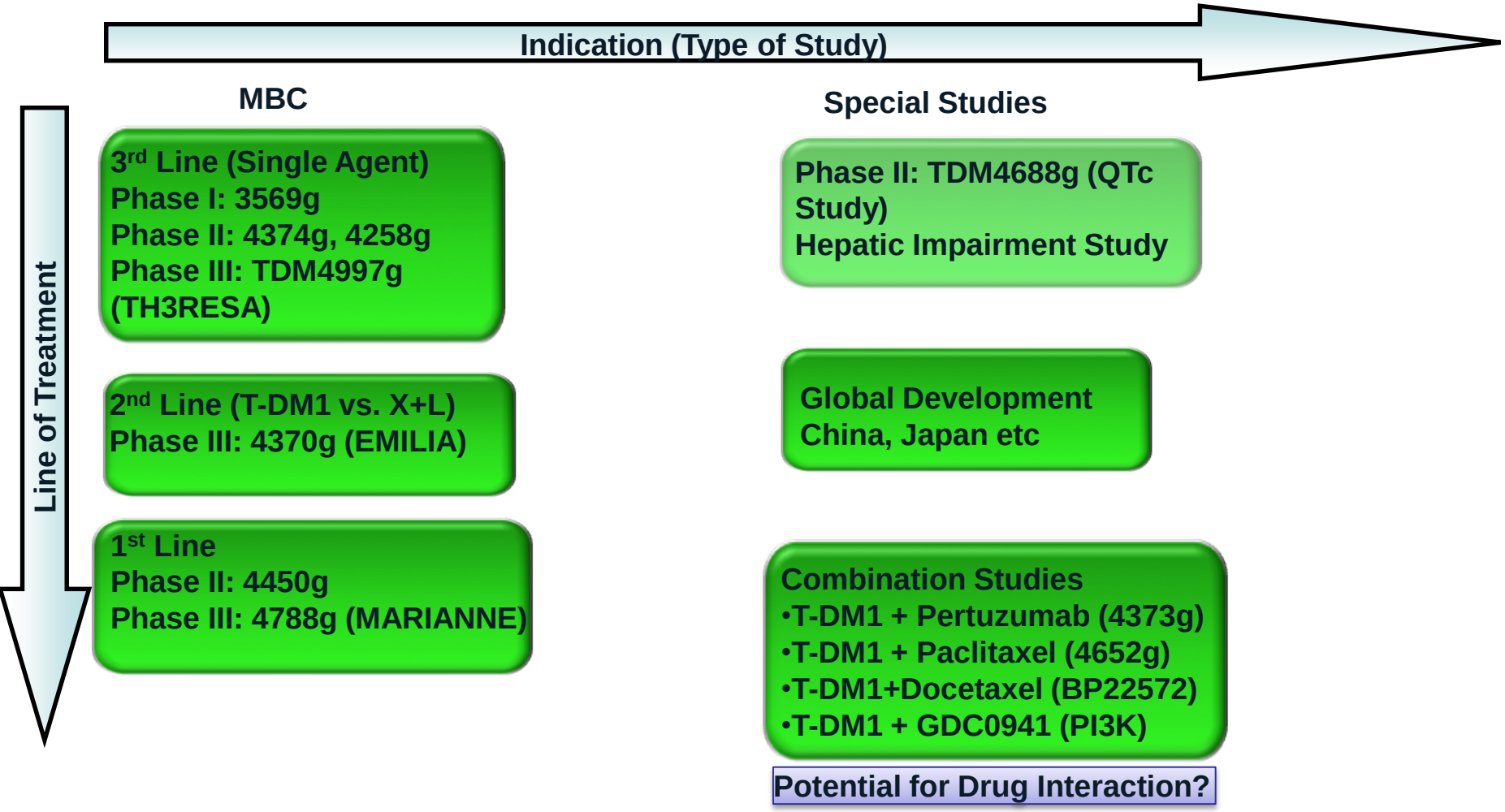
Characterize T-DM1 ELISA Ligand Binding Assay Reagents:
Ensure Anti-DM1 mAb and HER2 ECD Binding to All DARs



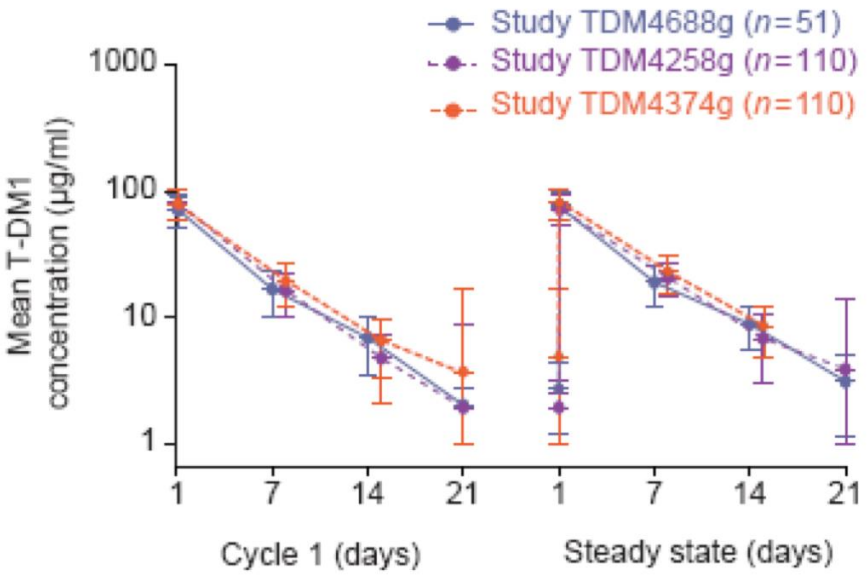
Affinity capture LC-MS of T-DM1 after incubation in human plasma with each capture probe

- DAR distributions data confirm ELISA reagents bind all DARs appropriately
- T-DM1 enriched DAR fractions recovered 80-120% in ELISA (data not shown)

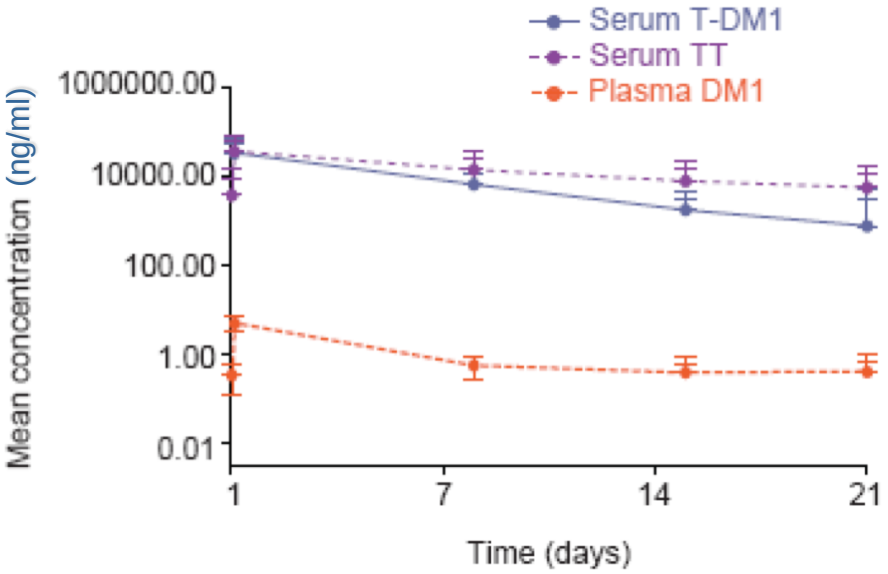
Kadcyla™ Clinical Studies and Bioanalysis:
Three PK Assays, Catabolite Assays & ADA Assays



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



Sandhya Girish, Manish Gupta, Bei Wang, Dan Lu, Ian E. Krop, Charles L. Vogel, Howard A. Burris III, Patricia M. LoRusso, Joo-Hee Yi, Ola Saad, Barbara Tong, Yu-Waye Chu, Scott Holden, Amita Joshi (2011). Cancer Chemother Pharmacol DOI 10.1007/s00280-011-1817-3

Examples of Kadcyla™ Clinical PK from Emilia Study:
Asian Population comparable to Overall Population

	Mean (SD)				
	C_{max} (μg/mL)	AUC_{inf} (day • μg/mL)	$t_{1/2}$ (day)	V_{ss} (mL/kg)	CL (mL/day/kg)
Trastuzumab Emtansine Conjugate					
All Patients (n = 292)	83.4 (16.5)	489 (122)	3.68 (0.886)	29.5 (14.6)	7.81 (2.18)
Patients enrolled in Asia (n= 55)	77.8 (12.0)	439 (87.8)	3.54 (0.651)	31.1 (7.15)	8.55 (2.02)
Total Trastuzumab					
All Patients (n = 291)	86.3 (20.1)	816 (422)	7.80 (4.01)	42.2 (15.6)	5.35 (2.33)
Patients enrolled in Asia(n=53)	80.2 (16.5)	676 (274)	7.00 (2.95)	44.1 (11.3)	6.16 (2.44)

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

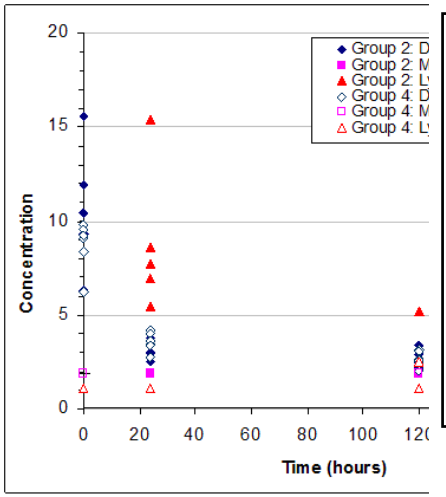
Mean Cycle 1 Cmax for DM1 were 4.61 ng/mL (All) and 4.25 ng/mL (Asian)

Courtesy of Sandhya Girish

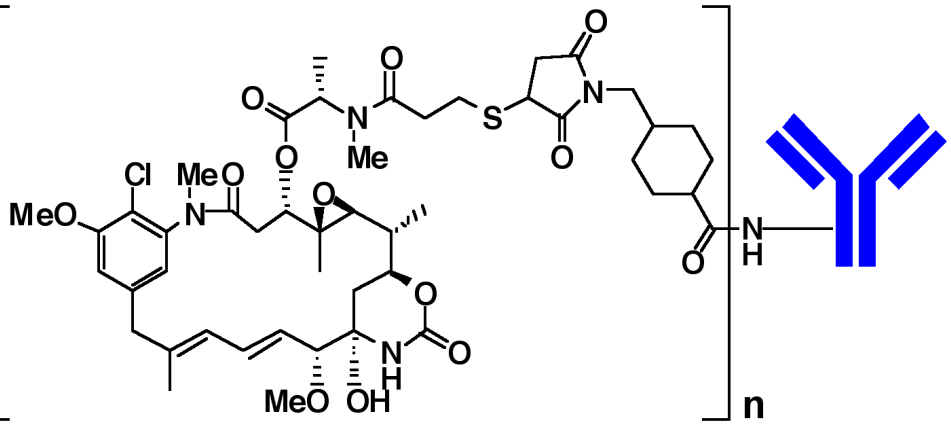
PK of trastuzumab emtansine conjugate, total trastuzumab and DM1 are comparable between patients enrolled in Asia and overall population

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

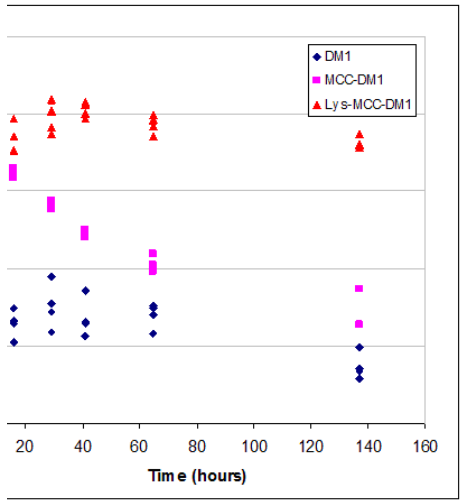
Rat Plasma



Rat Urine



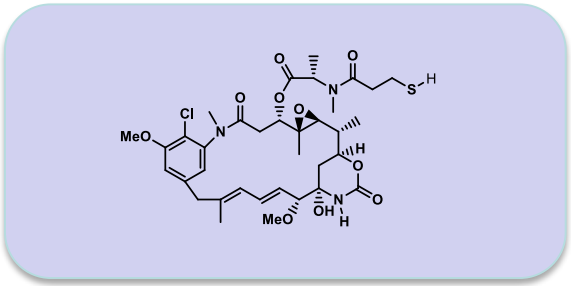
Rat Bile



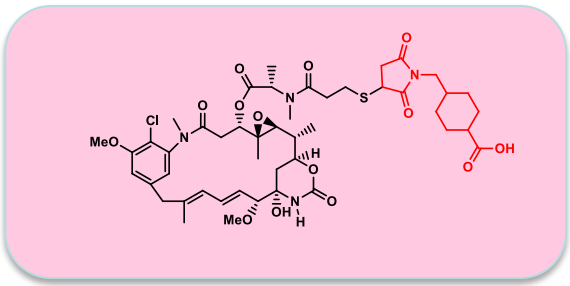
Where n ~ 3.5

Human Plasma:

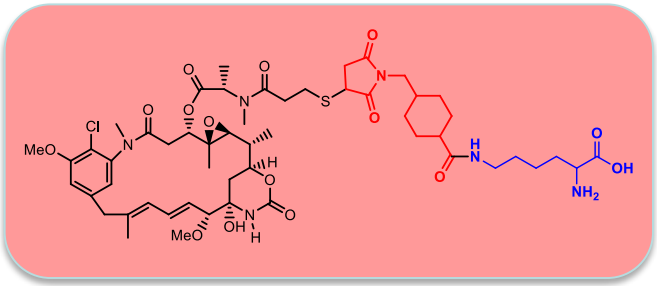
- Same catabolites (DM1, MCC-DM1 and Lys-MCC-DM1) also identified in human plasma



DM1



MCC-DM1



Lys-MCC-DM1

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

Prescribing Information (Kadcyla™ USPI)

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.

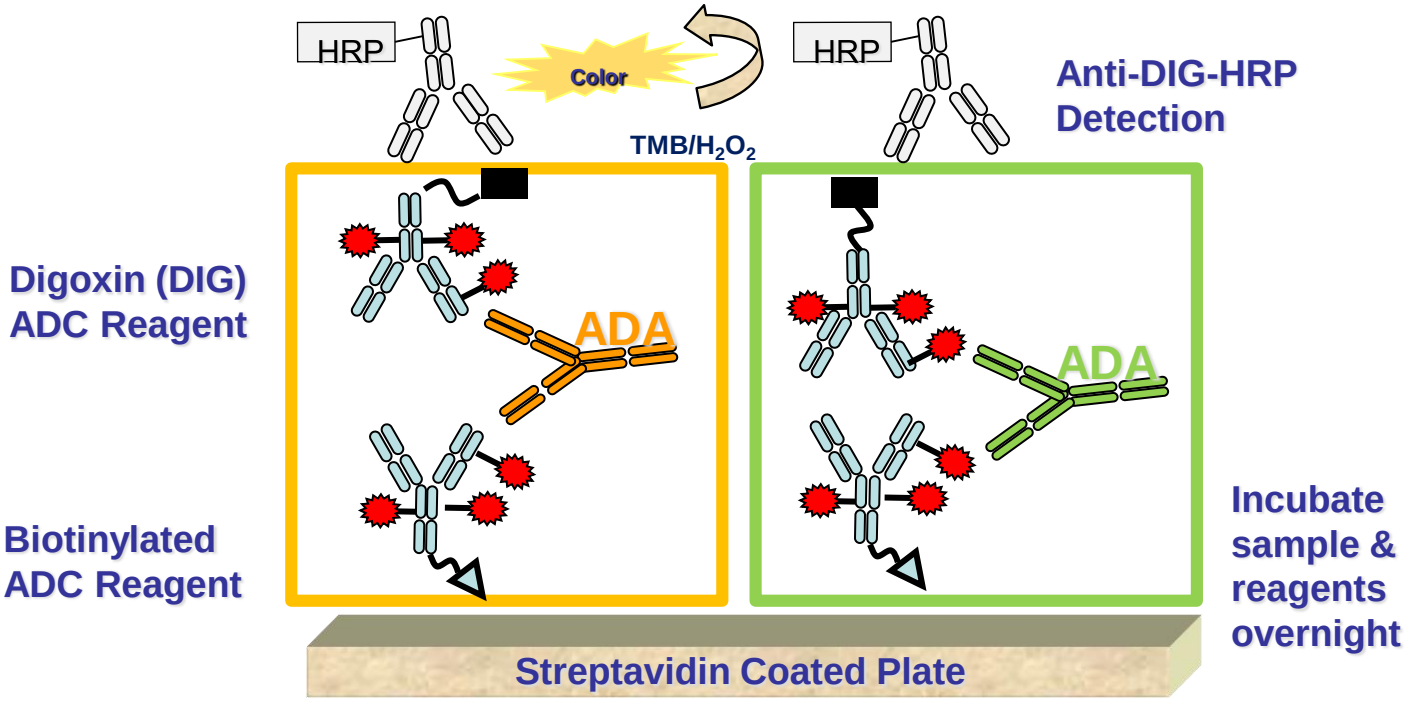
Overview

- 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

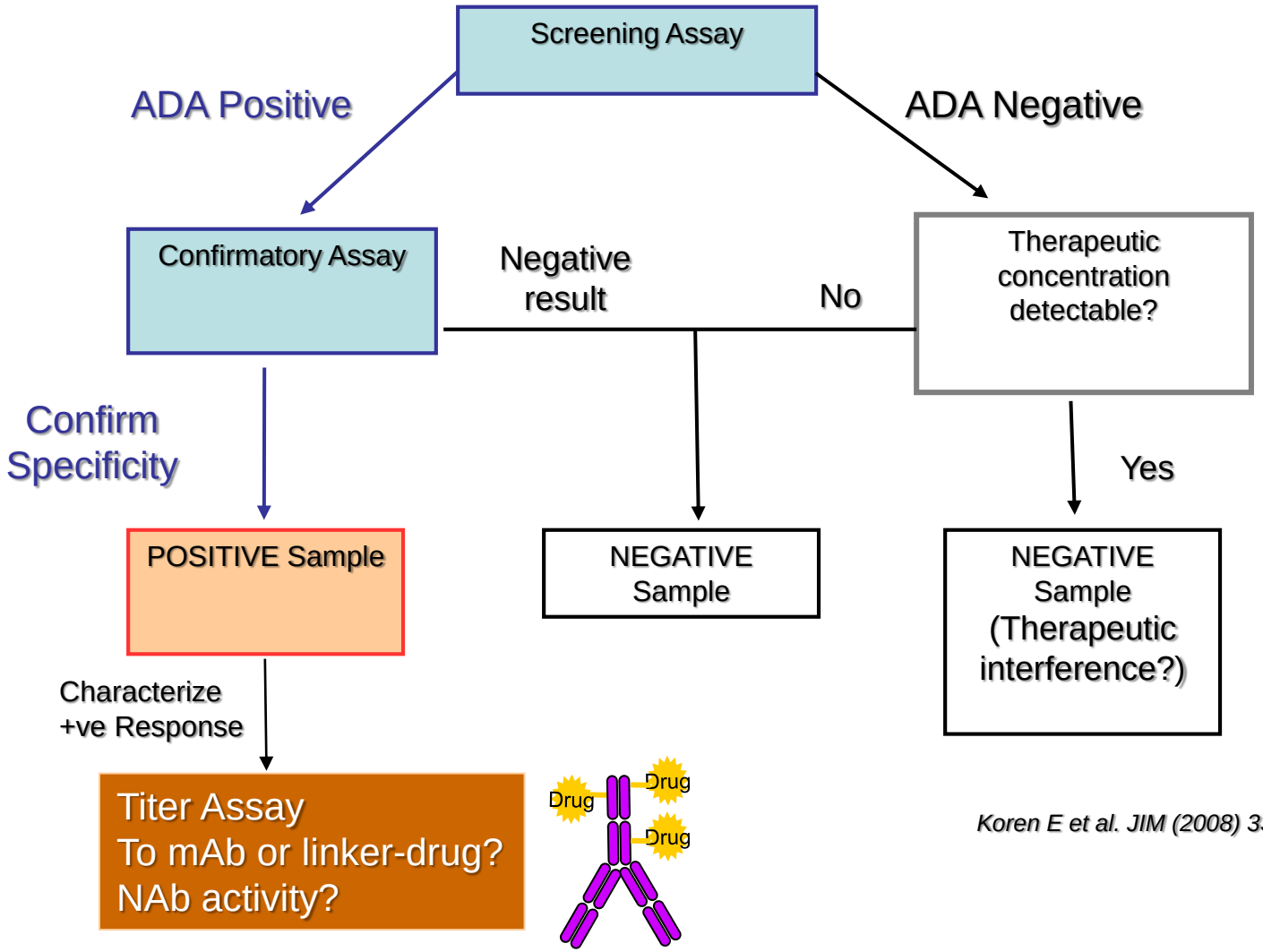
Immunogenicity Assays for ADCs

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



Koren E et al. JIM (2008) 333:1-9

Immunogenicity Overview

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 immunogenecity 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

Prescribing Information (Kadcyla™ USPI)

- **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

- ADCs have complex structures and are dynamically changing mixtures *in vivo*
- Important to understand ADC analyte biotransformations for appropriate assays
- Bioanalysis requires LBA, small molecule LC-MS/MS & hybrid LC-MS/MS methods
- No specific ADC guidances & limited clinical data for choosing analytes for safety/efficacy

- Kadcyla™ bioanalytical three PK assay strategy was accepted by agencies
- Tiered immunogenicty strategy was accepted; Nab assay developed as PMC
- 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

Administrative Assistant
Cassandra Duenas

Dev Sci Management
An Song, Patty Siguenza, Sara Kenkare