Isla Bartolomé

ADCs Bioanalysis—
LBA and LC-MS methods, a changing paradigm?

Rand Jenkins
PPD® Laboratories

EBF 9th Open Symposium
Barcelona Spain, 16 Nov 2016

HELPING DELIVER LIFE
CHANGING THERAPIES

PPD®

Overview

1. ADC—what do we mean?
2. ADCs landscape
3. ADCs bioanalysis—what to measure?
4. A model ADC BA case study
5. LBA or LC-MS?
6. Final thoughts

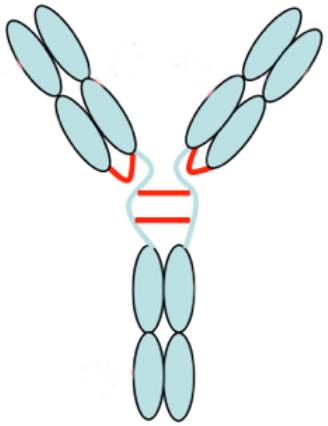
What do we mean?

ADC

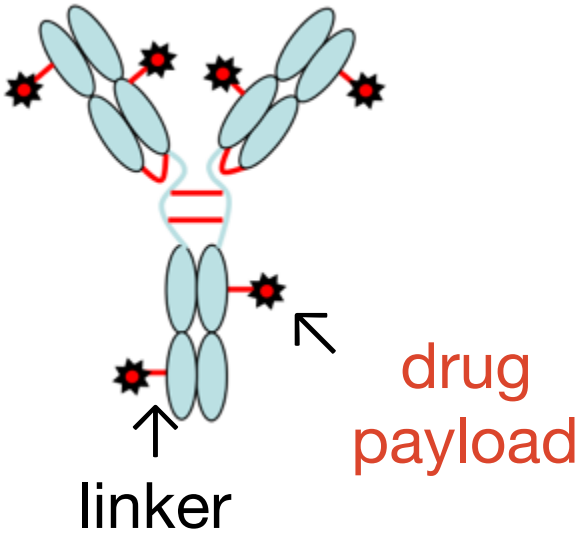
"Antibody"-drug conjugate

A *simple* concept?

moderately
(or in)effective
mAb



potent
'weaponized'
ADC



The 5Rs of ADCs:

The
right target,
right linker,
right warhead,
right antibody and
right conjugation site
to enhance the intratumor delivery of ADCs while minimizing serious adverse effects.

- Rakesh Dixit (Medimmune)

ADC therapeutic *challenges*

- Dosing with a narrow therapeutic index (near MTD)
- Staying in circulation long enough (but not too long?) without degrading
- Minimizing 'on-target' and off-target toxicity (i.e., against normal healthy cells)
- Reaching the intended tumor cells
- Efficient binding to the target receptor/antigen
- Efficient internalization and lysosomal trafficking
- Minimizing FcRn mediated recycling
- Efficient payload release via cleavage/digestion
- Adequate payload potency to induce cell death

ADCs in clinical development

"As of 2016, >55 ADCs, sponsored by 24 different companies, are in clinical testing. The overall success rate of the ADC approach for cancer treatment is still quite low, and at least 27 ADCs have been discontinued from clinical development."

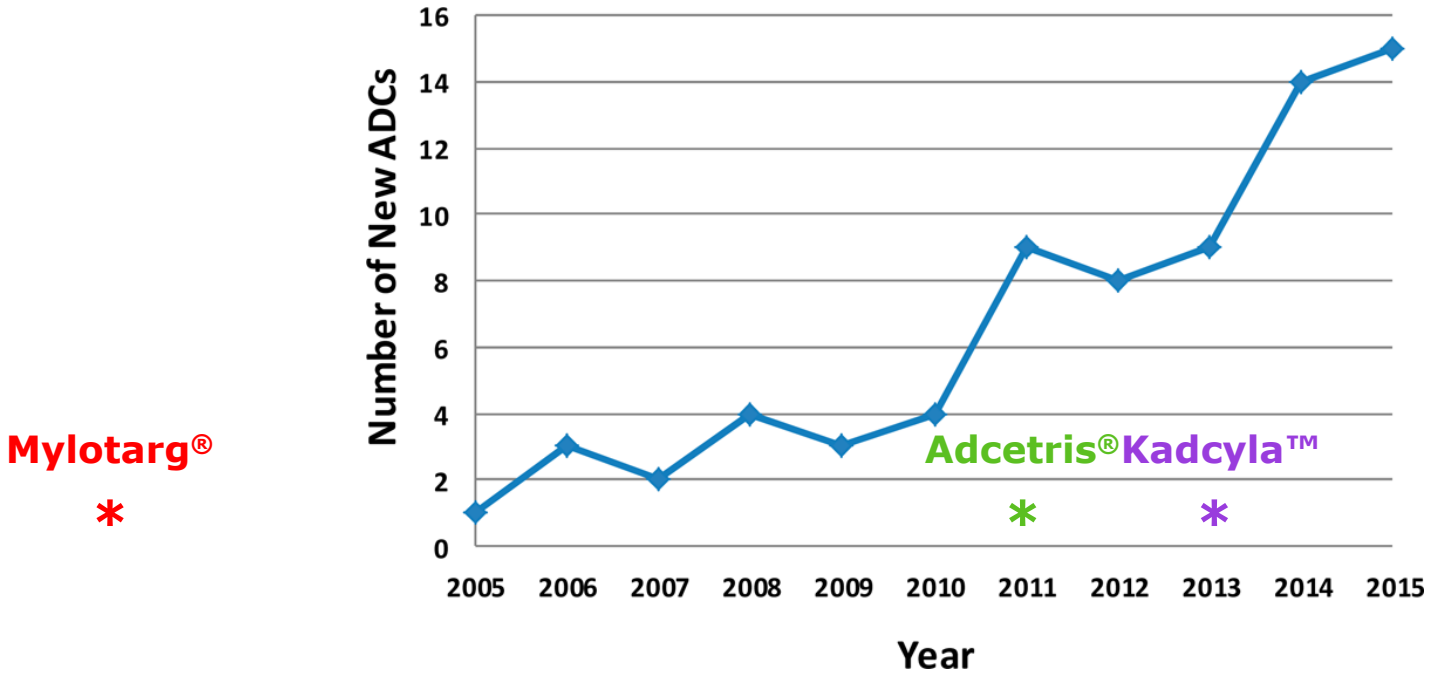
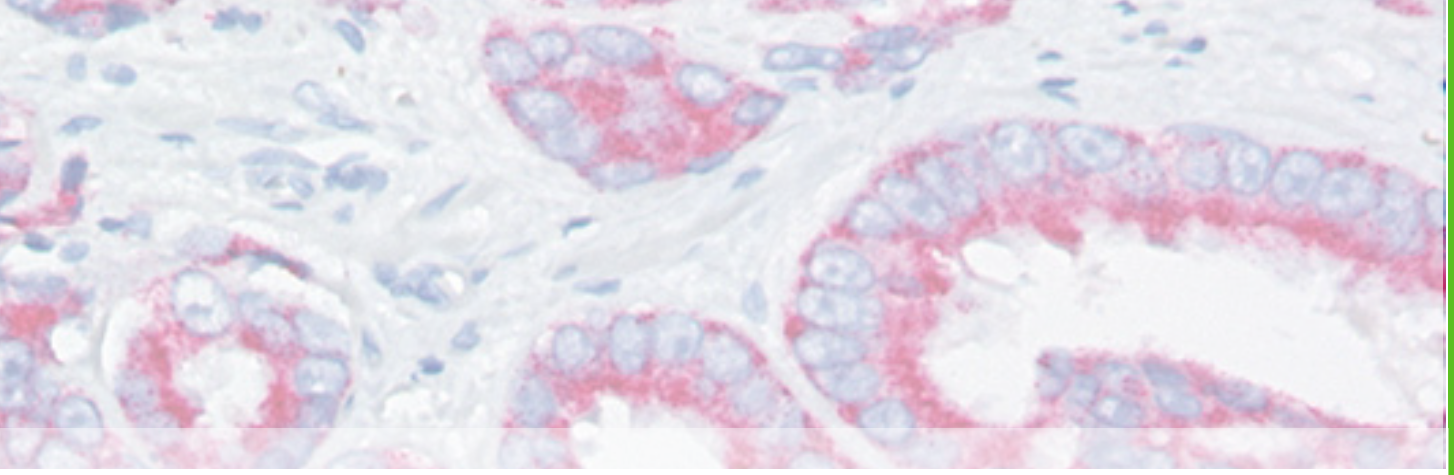
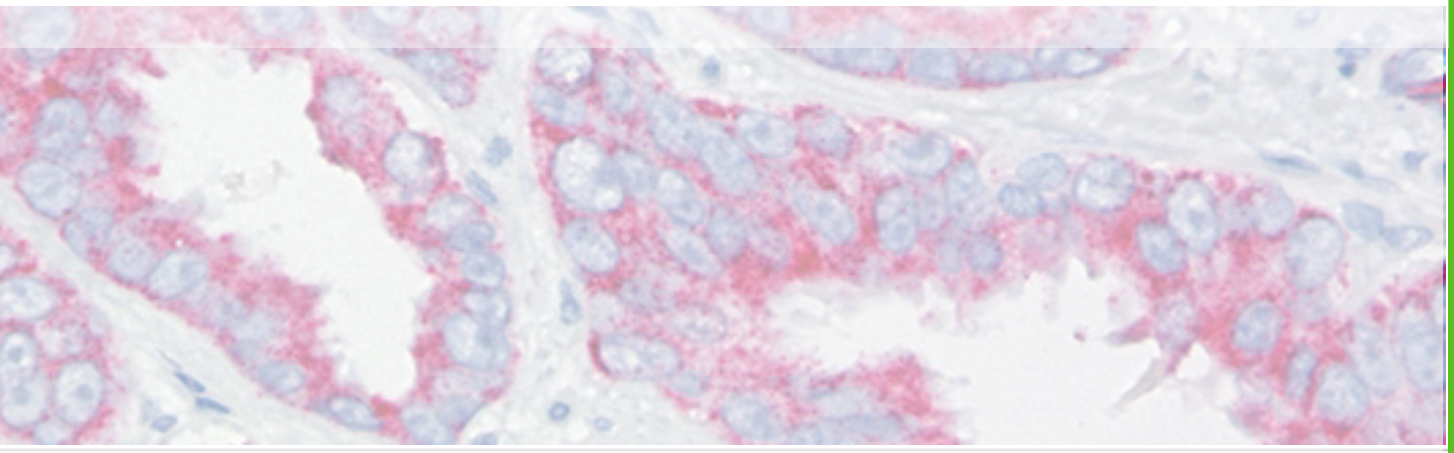


Figure 1. Number of new ADCs entering clinical testing each year.

Chari RVJ. *ACS Med. Chem. Lett.* (2016) in press



ADCs landscape



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'Classic' ADCs

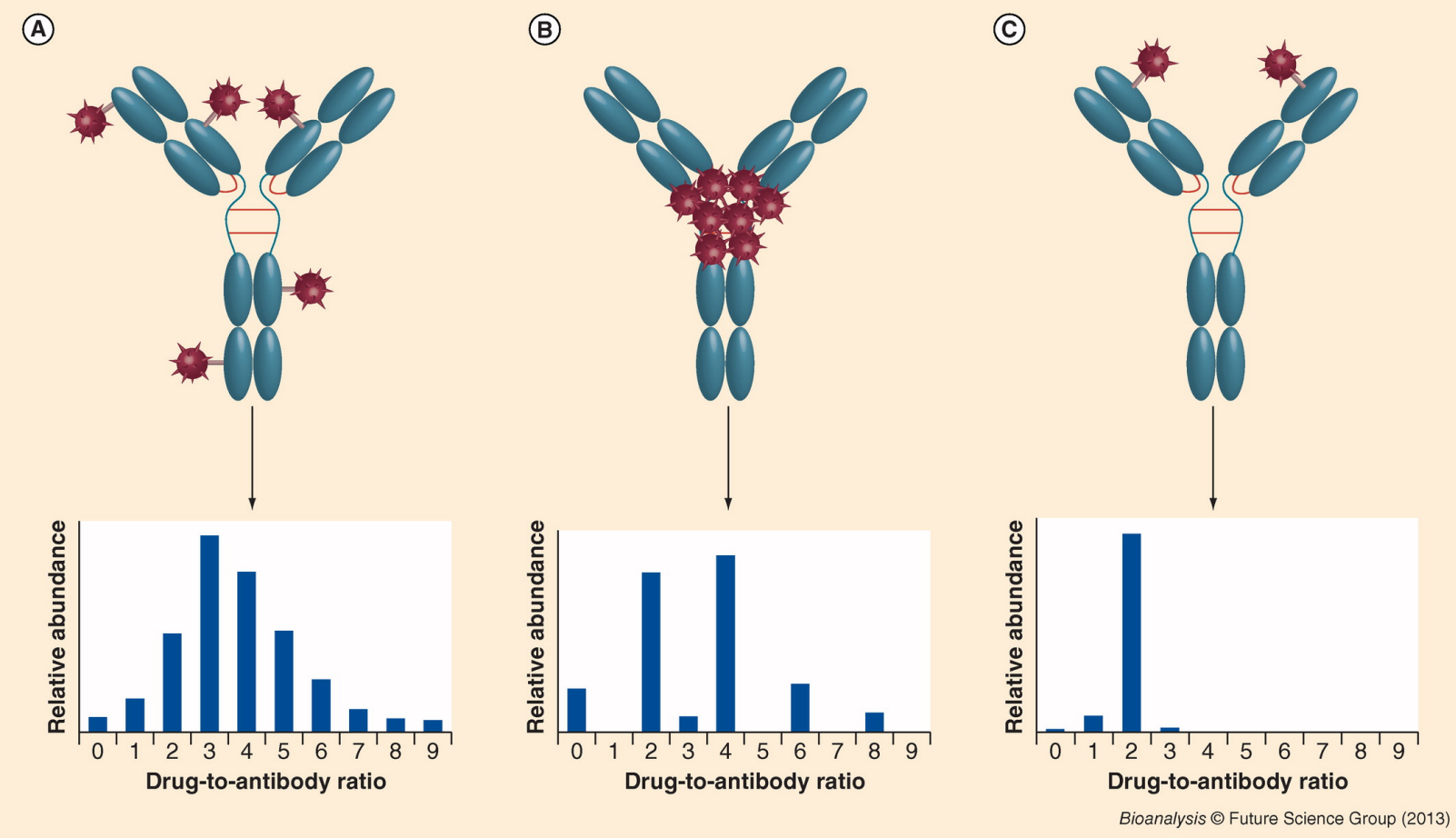


Figure 1. Antibody–drug conjugate conjugation sites and drug-to-antibody ratio heterogeneity. (A) Conjugation through lysines, **(B)** conjugation through reduced interchain disulfide bonds, and **(C)** conjugation through engineered cysteines

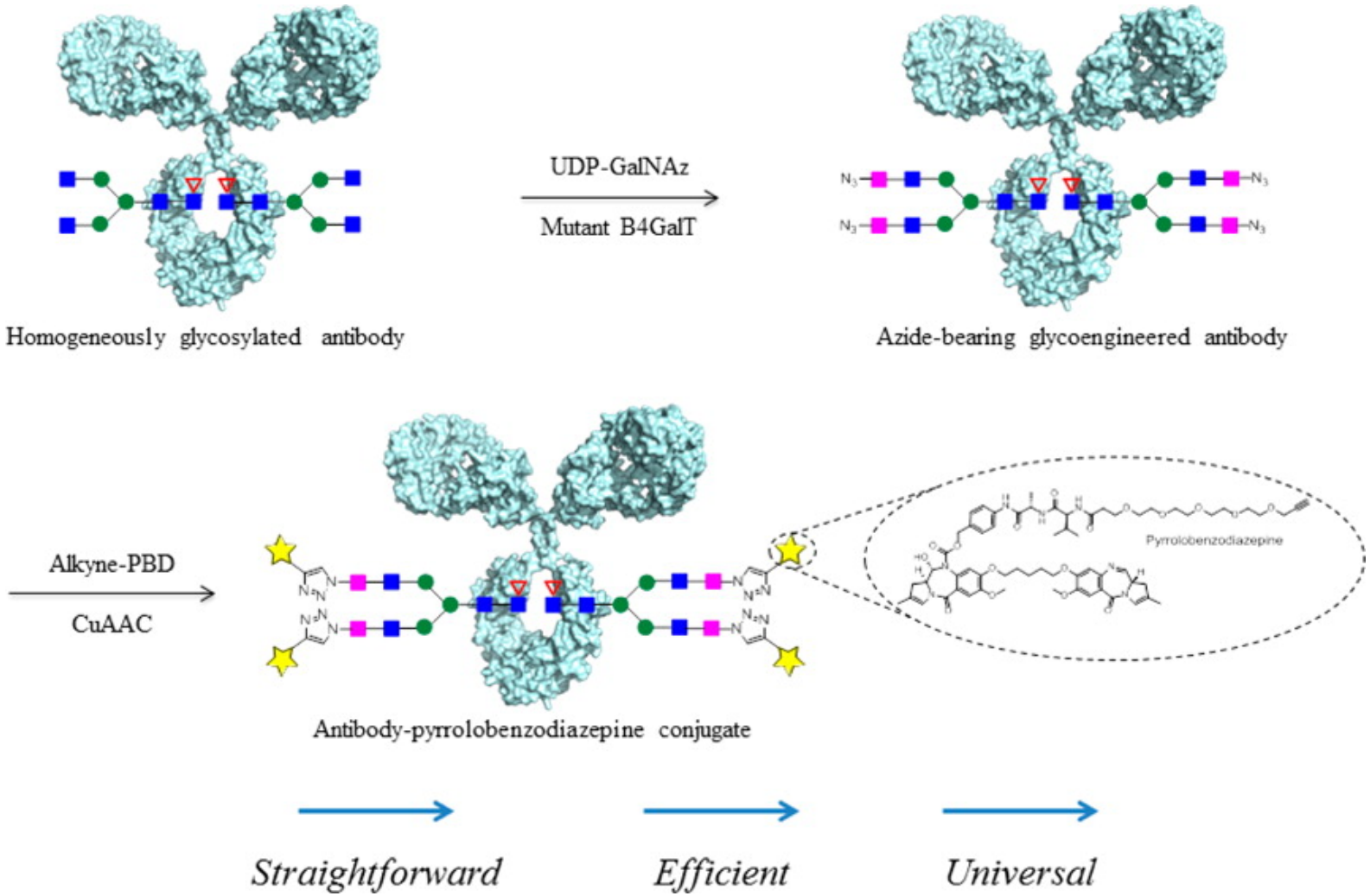
Kaur S, Xu K, Saad OM *et al*. *Bioanalysis,* (2013) 5(2), 201–226

Evolving ADC constructs

IgG-based carriers

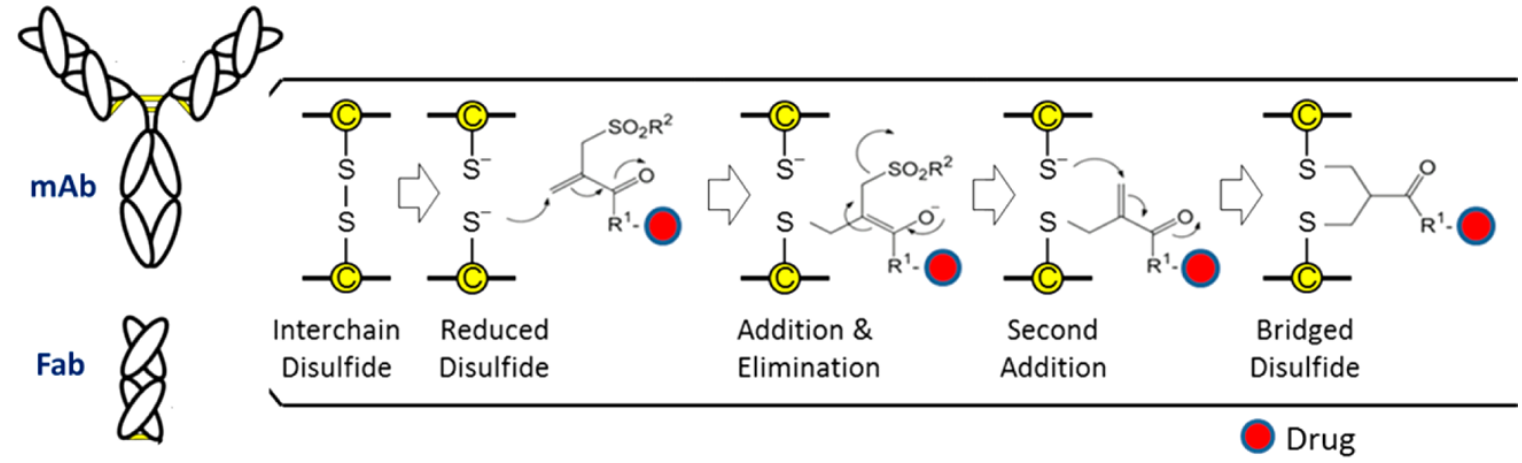
+ *Conventional* bivalent immunoglobulins
+ New conjugation schemes – site-specific emphasis
+ bsADCs
+ 'Prodrug' ADCs
  e.g., CytomX Therapeutics Probody™ 'empowered' drug conjugates (PDCs)
+ Others

Glycoengineered homogeneous site-specific ADCs



P. Thompson *et al*, Straightforward Glycoengineering Approach to Site-Specific Antibody−Pyrrolobenzodiazepine Conjugates, *ACS Med. Chem. Letters* 2016; in press.

Rebridged stable site-specific disulfide conjugates

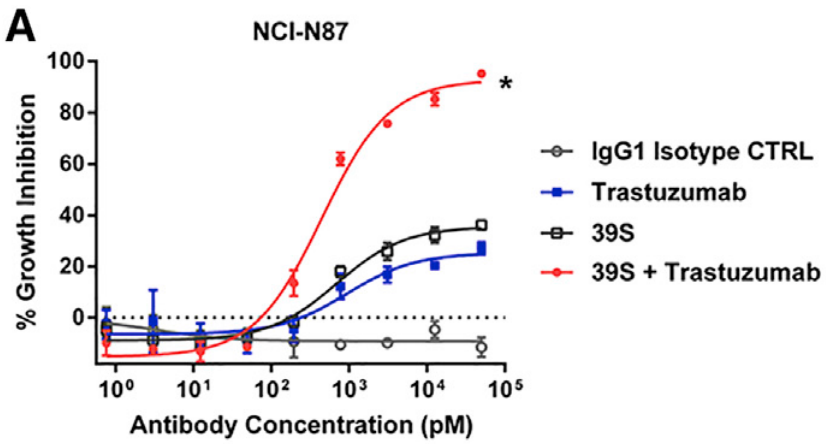


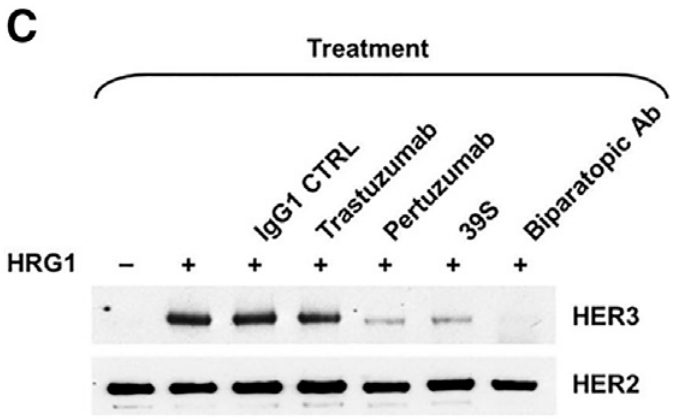
Targeting inter-chain disulfides

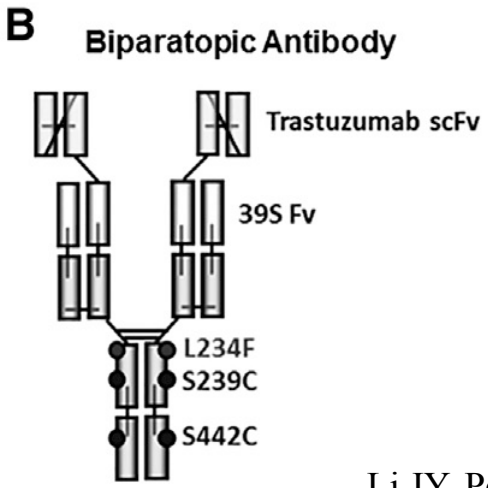
Figure 1. Conjugation of a payload to a disulfide bridge using a bis-alkylating conjugation approach involving a sequence of Michael addition and elimination reactions.

George Badescu *et al*, Bridging Disulfides for Stable and Defined Antibody Drug Conjugates, *Bioconj. Chem.* 2014, 25, 1124-1136

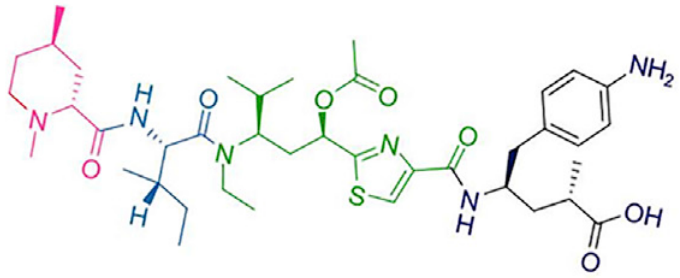
MEDI4276 – a Anti-HER2 Biparatopic ADC







Tubulysin Warhead (AZ13599185)

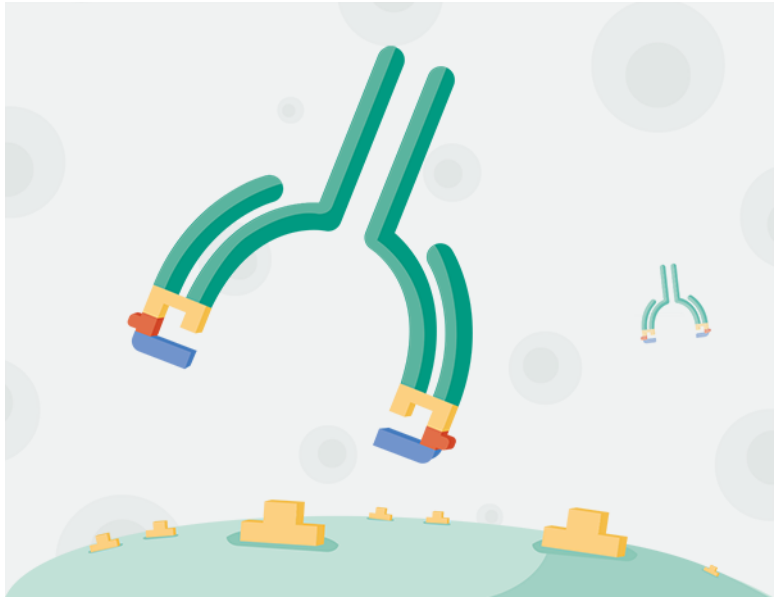


Li JY, Perry SR, Muniz-Medina V *et al.* A Biparatopic HER2-Targeting Antibody-Drug Conjugate Induces Tumor Regression in Primary Models Refractory to or Ineligible for HER2-TargetedTherapy. *Cancer Cell* 2016, 29, 117-129

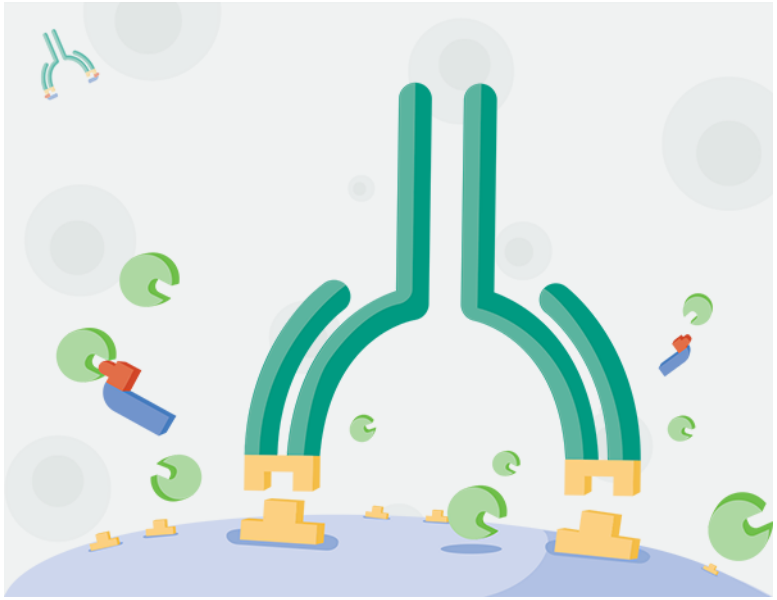
Probody™ therapeutics

CytomX Therapeutics

HEALTHY TISSUE



DISEASED TISSUE

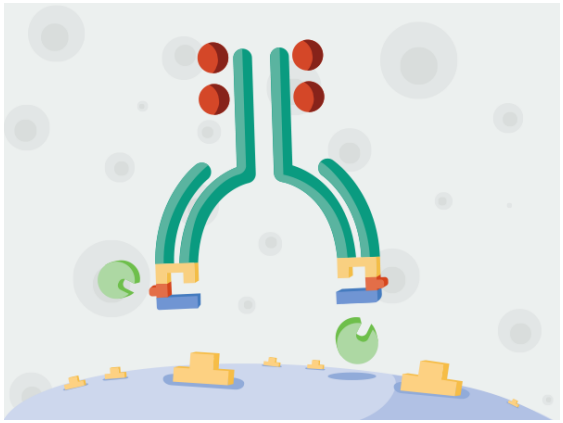


Probody™ drug conjugates (PDC)

CytomX Therapeutics

Based on cleavable peptide blocked CDRs

- Activation of the Probody antibody tightly regulated, binding only when cleaved by tumor-associated proteases
- Promising for previously inaccessible cancer antigens by *greatly reducing collateral damage to normal tissues*
- Example: anti-human CD166 Probody-SPDB-DM4 (CX-2009)
  - CD166 highly expressed in many cancers, but also on normal tissues
  - Well tolerated at 5 mg/kg, with no on-target and off-target toxicity



Evolving ADC constructs

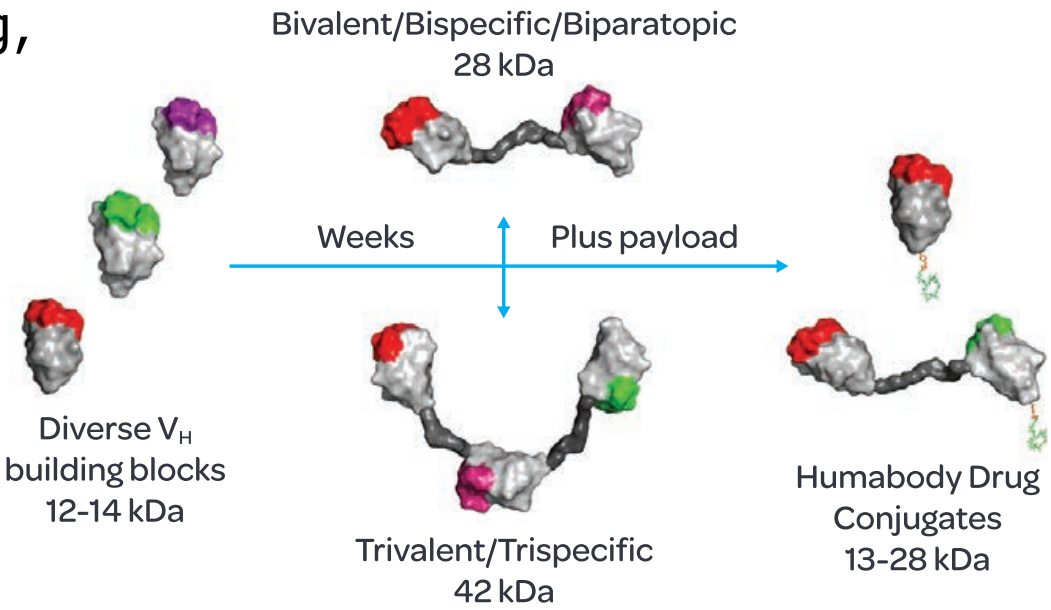
Antibody fragment-based carriers

+ Mono- or di-valent immunoglobulin fragments
+ scFv
+ V_H
  e.g., Crescendo Biologics Humabody™ drug conjugates (HDCs)
+ Others

Humabody™ drug conjugates (HDCs)

Based on fully human V_H building blocks

- Small size for efficient tissue penetration
- Modular 'Plug & Play' engineering options and cost-effective production
- Biparatopic formats for *superior tumor targeting with rapid internalization*
- Rapid onset cell killing, enhanced therapeutic index
- Tuneable serum half-life



Evolving ADC constructs

Targeting ligand-based carriers

+ Receptor/target protein binding ligands
  e.g., Tarveda Therapeutics Pentarins™ miniaturized biologic drug conjugates (mBDCs)
+ Designed ankyrin repeat proteins (DARPins)
+ Others

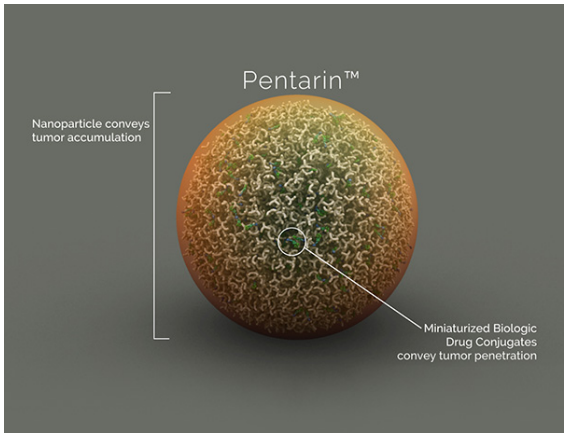
Pentarins™
miniaturized biologic drug conjugates (mBDCs)

TARVEDA THERAPEUTICS

Pentarins™ miniaturized biologic drug conjugates (mBDCs)

Based on receptor/target protein ligands

- Targeting cell surface receptors or other proteins over-expressed in many cancers
- Small size for *deep penetration in solid tumor tissue*
- Polymeric nanoparticle encapsulation to control systemic circulation half-life, biodistribution and drug release
- Enhanced permeability and retention (EPR) effect



- Example: anti-human SSTR2 somatostatin receptor peptide ligand-DM1 conjugate (PEN-221)
  - For neuroendocrine cancers (e.g., SCLC)
  - Tumor regression with one cycle of 2 mg/kg


Adaptation…

ADCs bioanalysis—*what to measure?*

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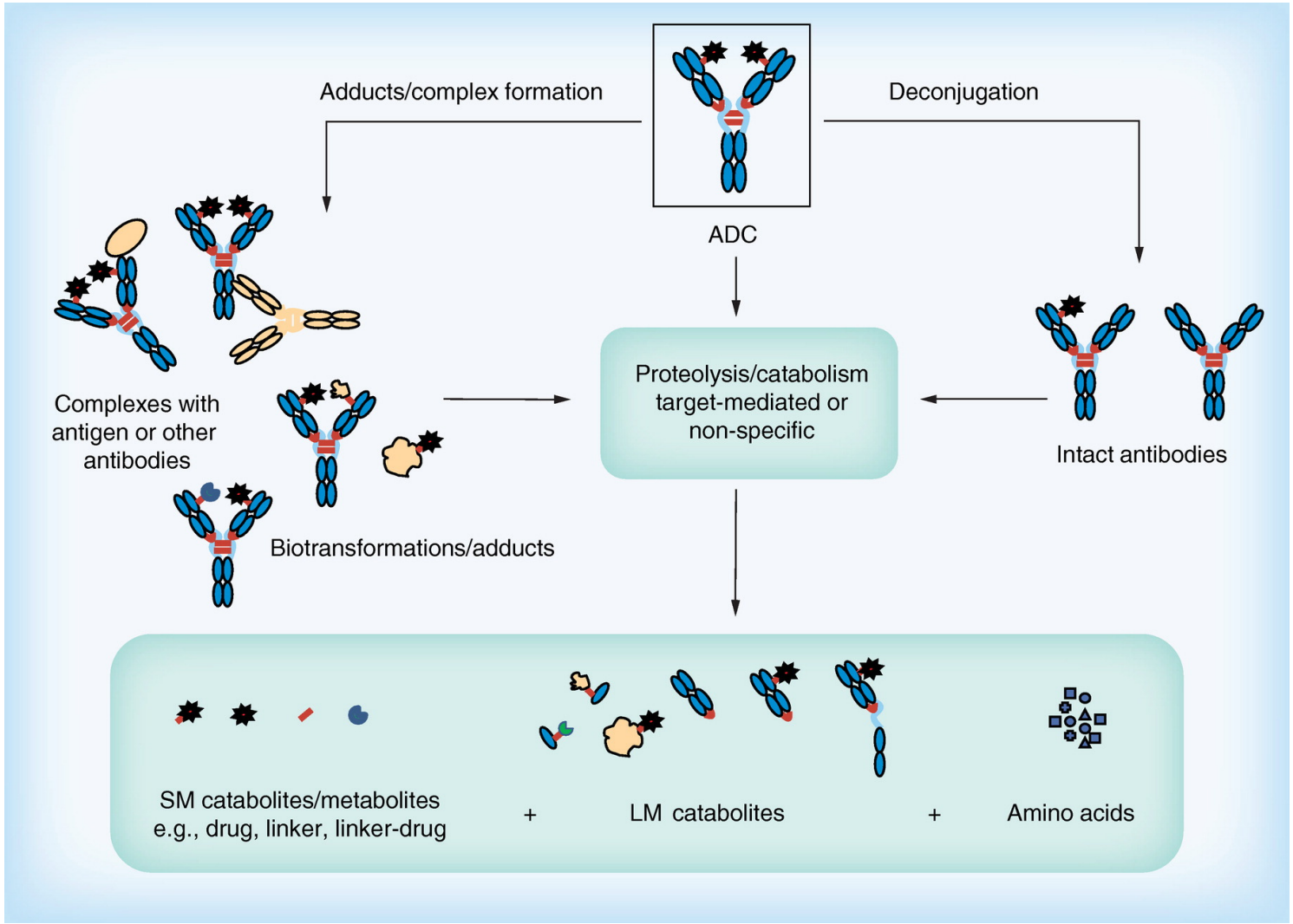
Potential catabolic fate of an ADC



Saad OM *et al*. *Bioanalysis*, (2015) 7(13), 1583–1604

Standard ADC bioanalysis methods

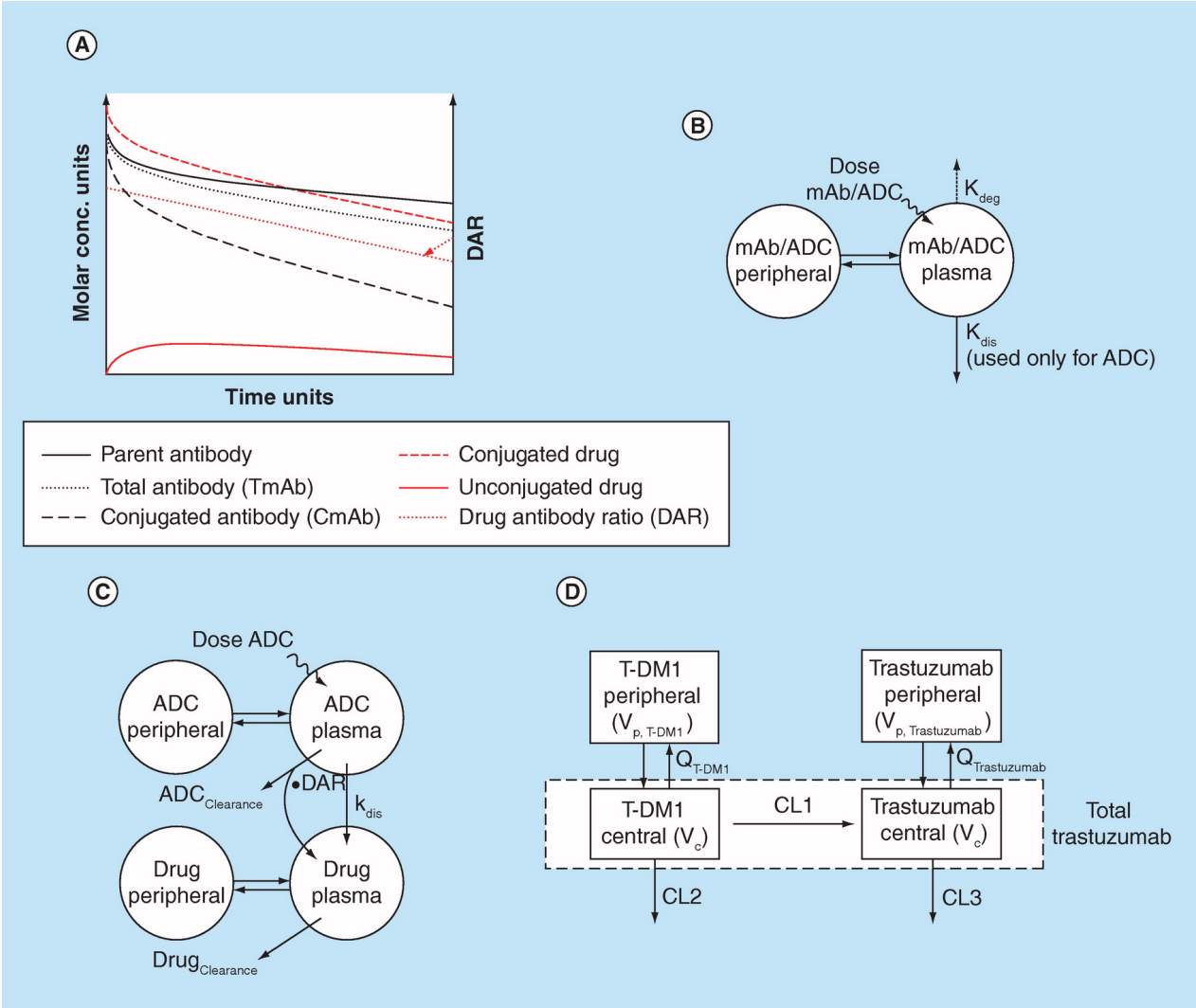
"Some of the most important questions in drug development are: 'What is the exposure–response (E–R) relationship for efficacy?' and 'What are the E–R relationships for safety?'"

Table 1. Analytes commonly assessed for antibody–drug conjugate bioanalysis.

Analyte type	Analyte(s) details	Typical analytical method(s)
Conjugated antibody†	Antibody with minimum of DAR >1	LBA
Total antibody‡	Conjugated, partially unconjugated and fully unconjugated (DAR >0)	LBA
Antibody-conjugated drug§	Total small-molecule drug conjugated to antibody	Affinity LC–MS/MS, LBA
Unconjugated drug¶	Small-molecule drug not conjugated to antibody	LC–MS/MS
Total drug#	Total unconjugated and conjugated drug	LC–MS/MS
Antitherapeutic antibody	Antibodies directed against antibody component of ADC, linker or drug (binding/neutralizing)	LBA

Gorovits B, Alley SC, Bilic S *et al*. *Bioanalysis*, (2013) 5(9), 997–1006

PK-PD modeling/simulation of ADCs



Khot A, Sharma1 S, & Shah DK. *Bioanalysis*, (2015) 7(13), 1633–1648

“Meanwhile, in ADC PK and bioanalysis, questions (remain) …”

Jian Wang *et al*

Wang J, Gu H, Liu A *et al*. Antibody–drug conjugate bioanalysis using LB-LC–MS/MS hybrid assays: strategies, methodology and correlation to ligand-binding assays. *Bioanalysis*, (2016) 8(13), 1383–1401

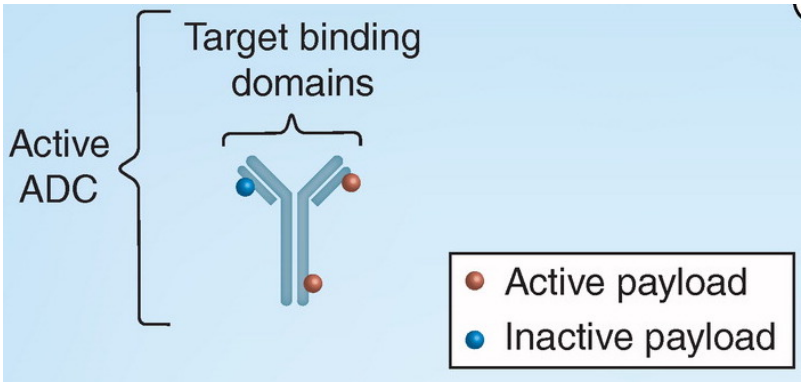
Questions (Wang *et al*)

+ Which assays and analytes are preferred, conjugated-antibody or conjugated-payload?
+ What assay format to use, LBAs or hybrid [LB-LC-MS/MS] assays in preclinical and clinical PK studies?
+ Can hybrid assays play additional roles beyond measuring conjugated-payload?
+ Are hybrid assays viable alternatives to LBA for the analysis of total-antibody?
+ Can hybrid assays be used as an alternative or complement to LBAs for the analysis of conjugated-antibody?
+ Can ADC bioanalysis be supported in a LC–MS based laboratory without full LBA capabilities?
+ What is the ideal ADC bioanalytical strategy from early Discovery to late clinical stage?

Wang J, Gu H, Liu A *et al*. *Bioanalysis*, (2016) 8(13), 1383–1401

A model ADC BA case study

ADC BA case study



Bristol-Myers Squibb ADC

+ Targeting solid tumors
+ Random lysine conjugation
+ Microtubule polymerization inhibitor payload
+ Cathepsin B cleavable dipeptide linker
+ Potential soluble target interference
+ Conjugated payload inactivated by metabolism

Myler H, Rangan VS, Wang J *et al*. An integrated multiplatform bioanalytical strategy for antibody–drug conjugates: a novel case study. *Bioanalysis*, (2015) 7(13), 1569–1582

Myler H, Rangan VS, Kozhich A *et al*. Validation of an integrated series of ligand-binding assays for the quantitative determination of antibody–drug conjugates in biological matrices. *Bioanalysis*, (2016) 8(6), 519–531

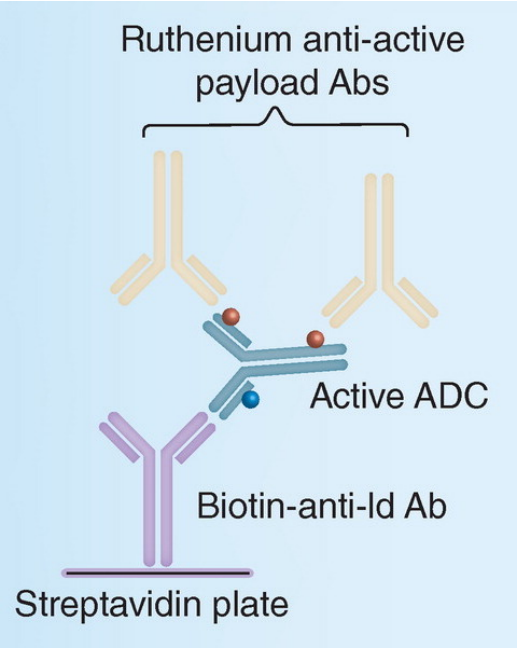
Wang J, Gu H, Liu A *et al*. Antibody–drug conjugate bioanalysis using LB-LC–MS/MS hybrid assays: strategies, methodology and correlation to ligand-binding assays. *Bioanalysis*, (2016) 8(13), 1383–1401

ADC BA case study

Program involved data from 15 different assays to evaluate ADME, safety and efficacy of the ADC

Assays needed to evaluate PK

+ **Total antibody**
+ **Active ADC**
+ **Total ADC**
+ **Antibody-conjugated payload**
+ **Unconjugated payload**

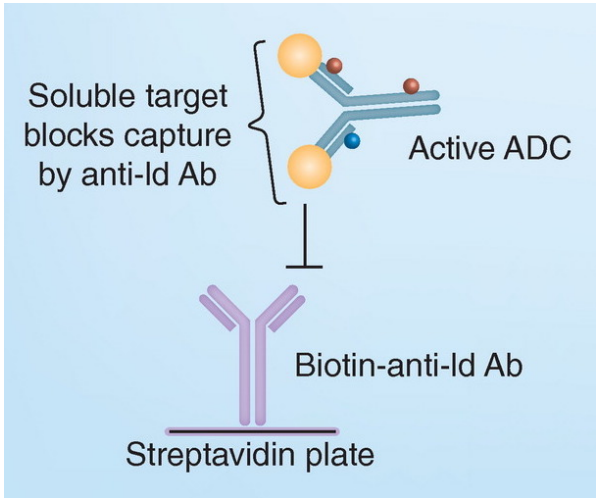


Myler H, Rangan VS, Wang J *et al*. *Bioanalysis*, (2015) 7(13), 1569–1582

ADC BA case study—nonclinical

LBAs

+ Technically/operationally harmonized ELISAs using MULTI-ARRAY® microplate (MSD) platform
+ Cynomolgus monkey and Sprague–Dawley rat serum



Reagents

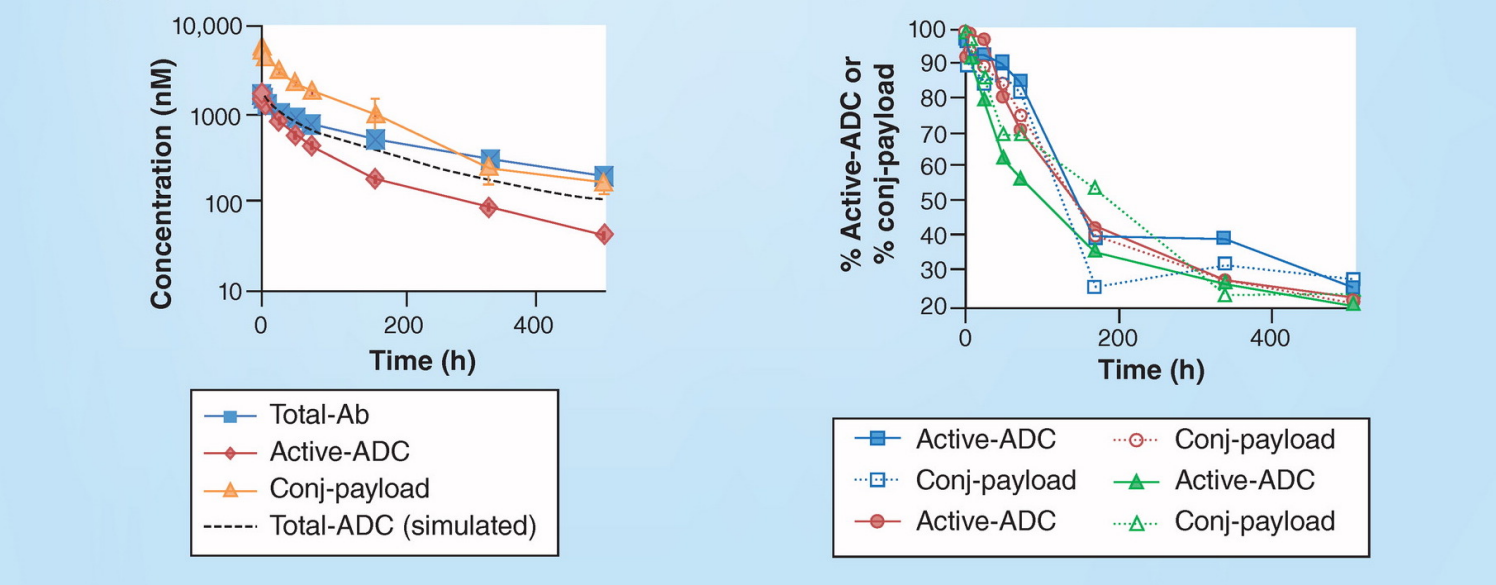
+ Capture mAb (biotinylated): mouse anti-idiotype
+ Detection mAbs (ruthenylated):
  + **Total antibody** (T-Ab): mouse anti-human IgG Fc
  + **Active ADC** (A-ADC): mouse anti-payload (active only)
  + **Total ADC** (T-ADC): mouse anti-payload (active and inactive)

Myler H, Rangan VS, Wang J *et al*. *Bioanalysis*, (2015) 7(13), 1569–1582

What does the LBA data show?

Biotransformation and integrated monkey TK profiles

+ T-Ab > T-ADC indicates deconjugation
+ T-ADC > A-ADC indicates (payload) metabolism
+ T-Ab > A-ADC indicates deconjugation + metabolism



Myler H, Rangan VS, Wang J *et al*. *Bioanalysis*, (2015) 7(13), 1569–1582

ADC BA case study—clinical

LBAs

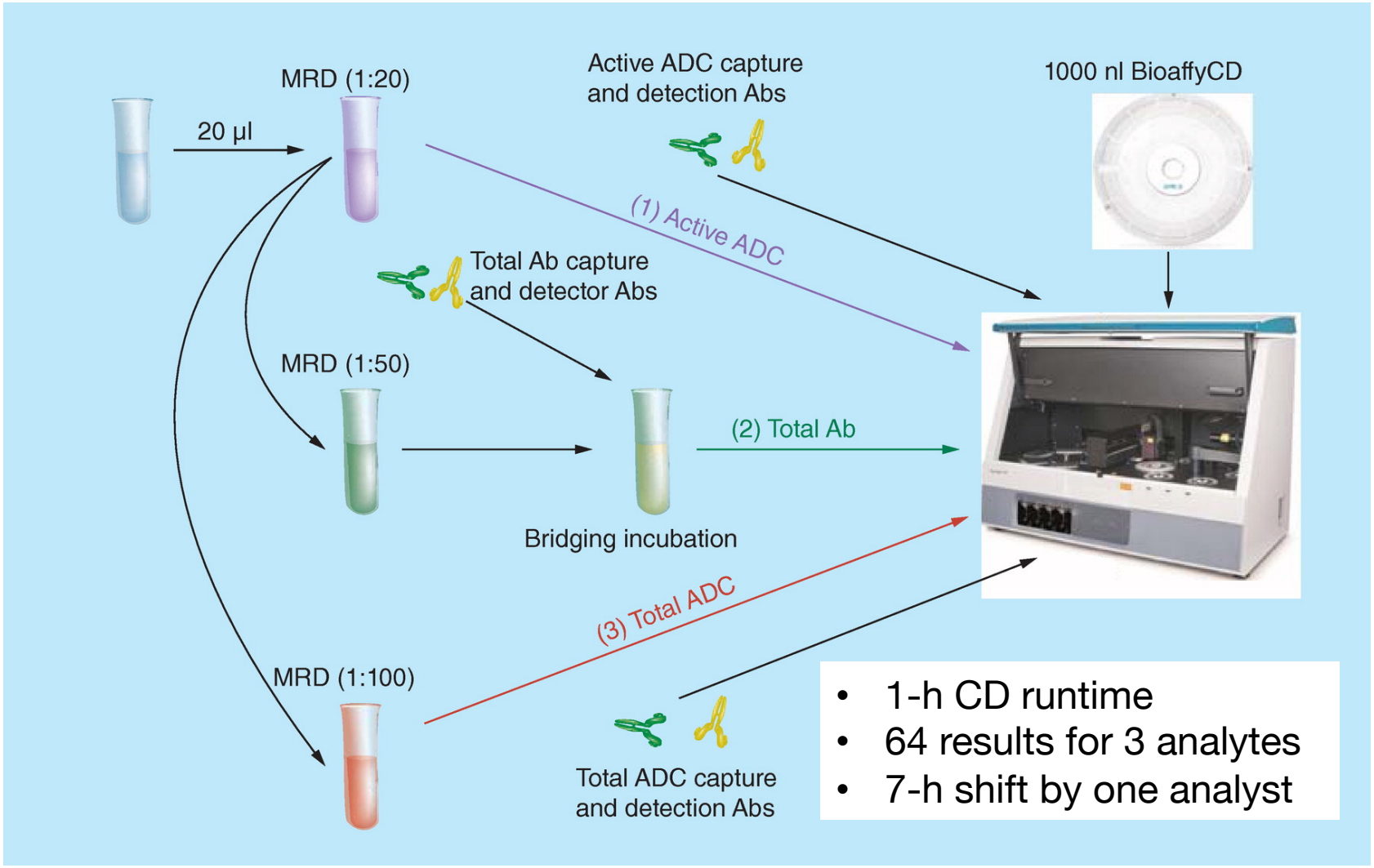
+ Transferred and optimized to use Bioaffy 1000 microfluidic CDs containing streptavidin-coated columns with a Gyrolab™ xP Workstation
+ Human serum

Reagents

+ Capture mAb (biotinylated): mouse anti-idiotype
+ Detection mAbs (Alexa-647 conjugated):
  + **Total antibody** (T-Ab): *same* mouse anti-idiotype
  + **Active ADC** (A-ADC): mouse anti-payload (active only)
  + **Total ADC** (T-ADC): mouse anti-payload (active and inactive)

Myler H, Rangan VS, Kozhich A *et al*. *Bioanalysis*, (2016) 8(6), 519–531

Single multi-analyte PK Gyrolab™ method



Myler H, Rangan VS, Kozhich A *et al*. *Bioanalysis*, (2016) 8(6), 519–531

ADC BA case study

Hybrid LB/LC-MS/MS & LC-MS/MS payload assays

+ Utilized stable isotope-labeled payload SIL-IS
+ Same UPLC separation conditions and SRM detection transitions using AB Sciex Triple Quad API-5500 system

Sample preparation

+ **Antibody-conjugated payload** (conj-payload)
  + Immunocapture with *same* biotinylated mouse anti-ID mAb and streptavidin (SA-W) cartridges or Protein A or G cartridges (Agilent AssayMAP Bravo)
  + Elute and release payload with cathepsin B
+ **Unconjugated payload** (unconj-payload)
  + Protein precipitation with acetonitrile

Myler H, Rangan VS, Wang J *et al*. *Bioanalysis*, (2015) 7(13), 1569–1582

Wang J, Gu H, Liu A *et al*. Antibody–drug conjugate bioanalysis using LB-LC–MS/MS hybrid assays: strategies, methodology and correlation to ligand-binding assays. *Bioanalysis*, (2016) 8(13), 1383–1401

ADC BA case study

Hybrid LB/LC-MS/MS antibody assays

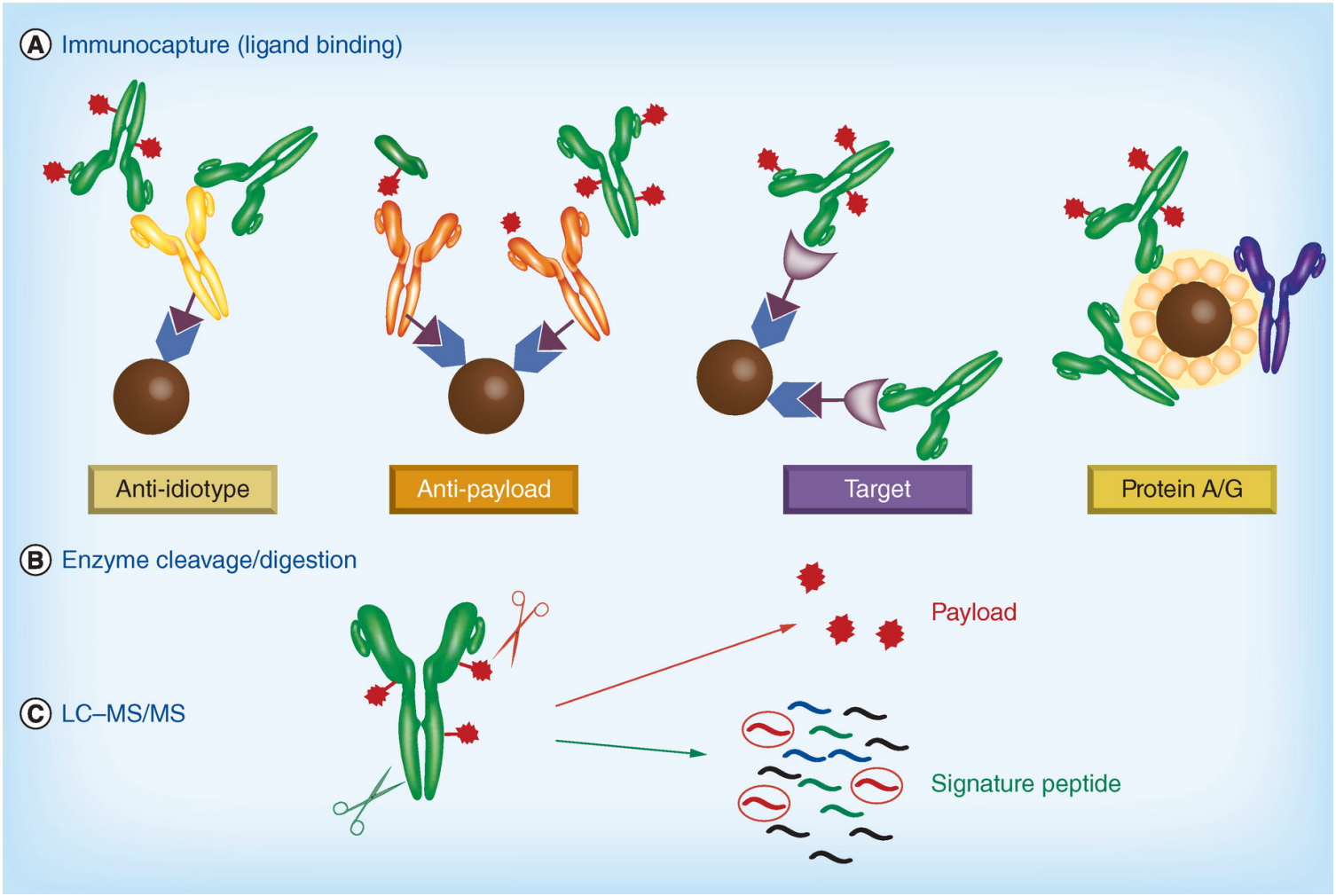
+ Utilized stable isotope-labeled signature peptide (SIL-IS)
+ UPLC separation and SRM detection of CDR signature peptide (w/o Lys) using AB Sciex Triple Quad API-6500 system

Sample preparation

+ **Total antibody & conjugated antibody**
  + Cartridge-based: IC with biotinylated mouse anti-ID or anti-payload mAb and streptavidin (SA-W) cartridges or anti-payload mAb and Protein A cartridges (Agilent AssayMAP Bravo)
  + Bead-based: IC with biotinylated mouse anti-ID or anti-payload mAb and MyOne streptavidin T1 magnetic beads or anti-payload mAb and Protein G beads (Dynabeads)
  + Elute and/or digest with trypsin

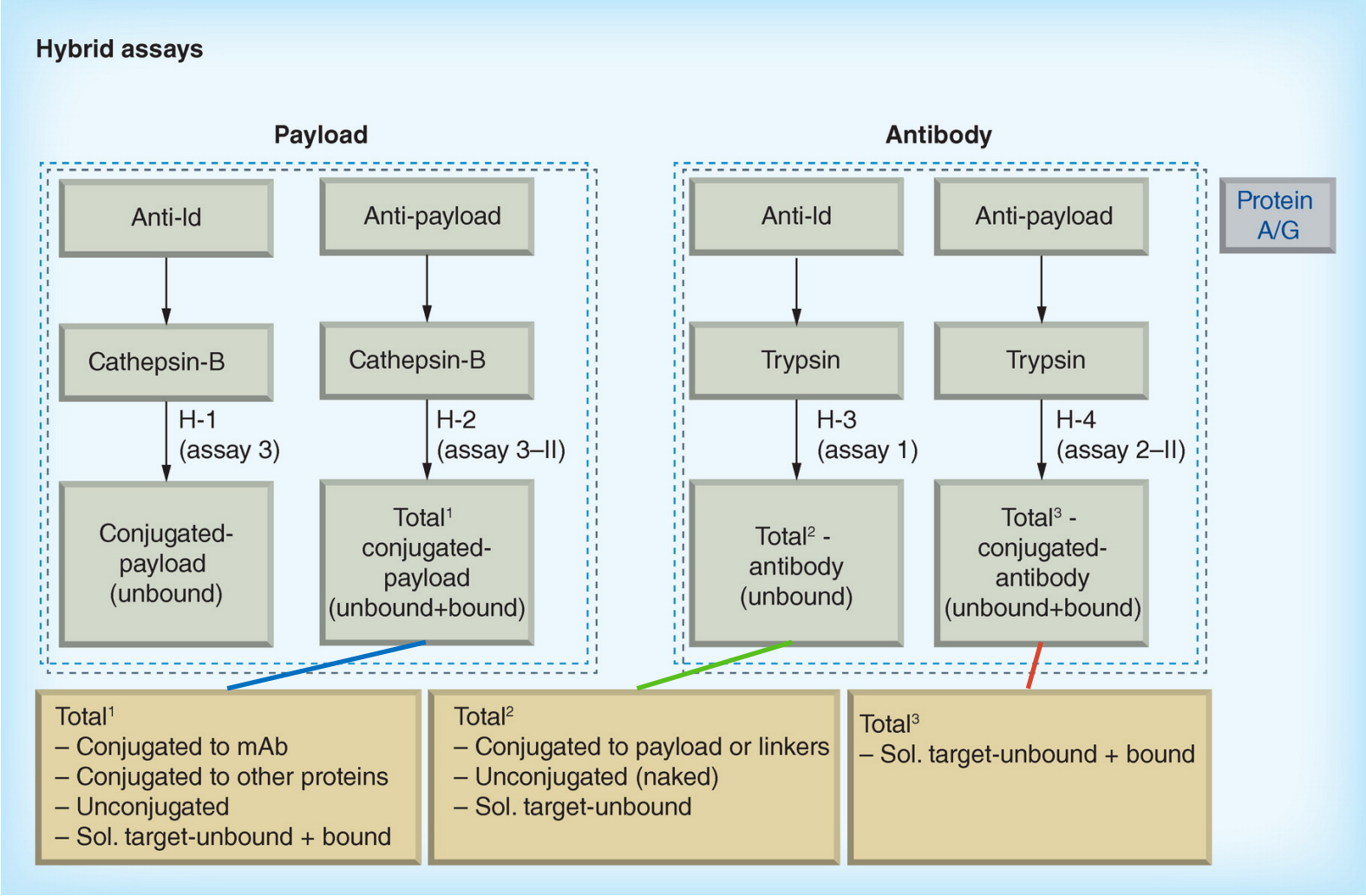
Wang J, Gu H, Liu A *et al*. *Bioanalysis*, (2016) 8(13), 1383–1401

Procedure of hybrid LB/LC–MS/MS assays (from Fig. 2)



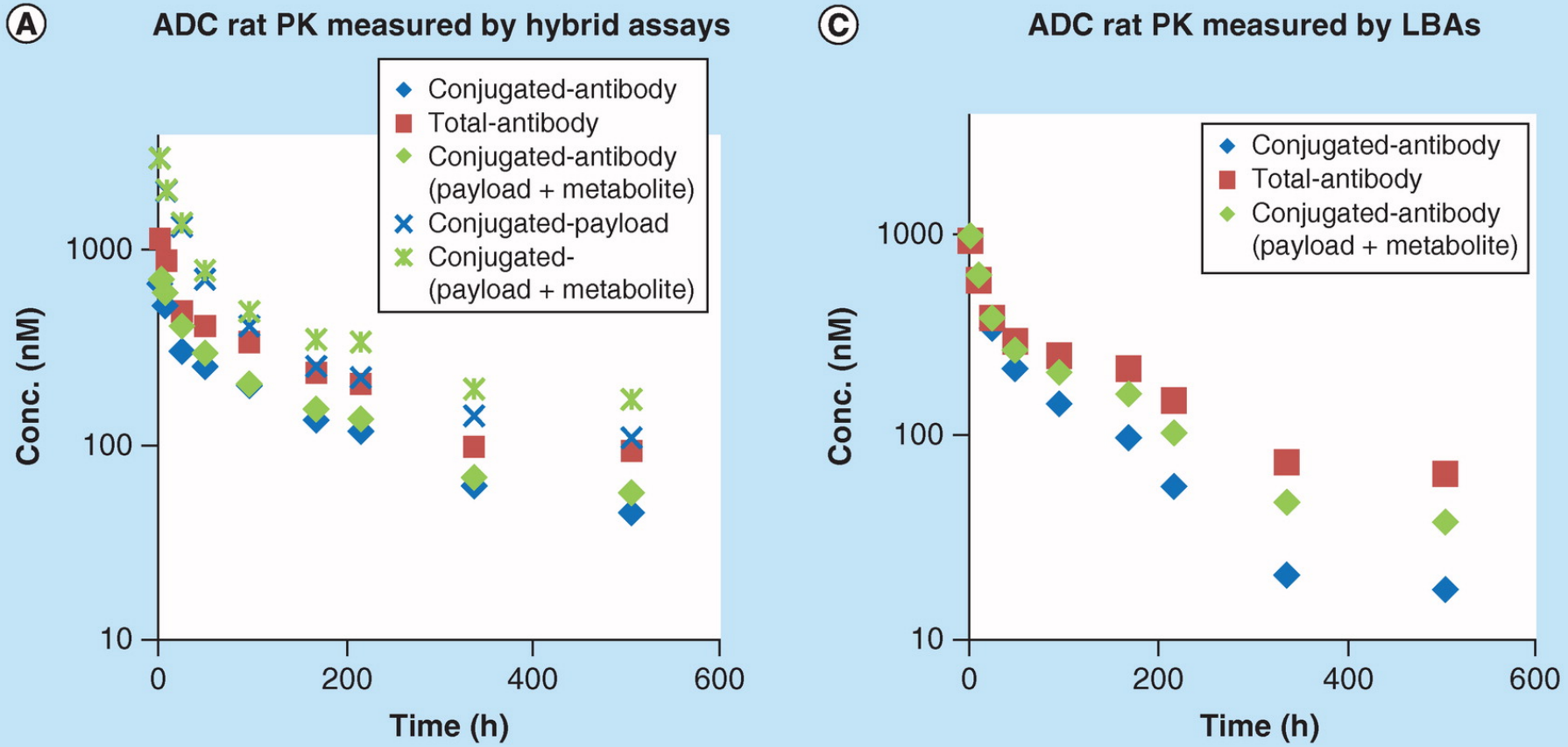
Wang J, Gu H, Liu A *et al*. *Bioanalysis*, (2016) 8(13), 1383–1401

Hybrid assays developed with combinations of different capture reagents and enzymes (from Fig. 3)



Wang J, Gu H, Liu A *et al*. *Bioanalysis*, (2016) 8(13), 1383–1401

Hybrid LB/LC-MS and LBAs in ADC rat PK (from Fig. 6)



Wang J, Gu H, Liu A *et al*. *Bioanalysis*, (2016) 8(13), 1383–1401

LBA or LC-MS?

ADCs bioanalysis

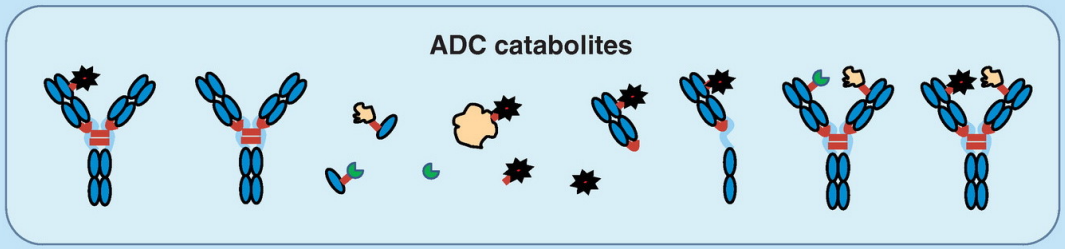
- LBA and LC-MS technologies are both essential to fully support comprehensive ADC development
- LBAs and hybrid assays can be complementary or alternatives—*often a matter of preference*
- One suggested strategy (Wang *et al*):
  - Early discovery: generic protein A or G capture conjugated-payload (LC-MS) assay and generic capture total-antibody in LBA or hybrid format for screening and candidate selection
  - Late discovery/early development: DAR-insensitive conjugated-antibody in LBA or hybrid format and both conjugated-antibody and conjugated-payload measured
  - Late development: continuation with only one of the antibody-conjugate assays

Recent efficiency gains in ADC bioanalysis

using LC-MS-based *multiplex* assays

+ **Conjugated & unconjugated payload**
  + From a single aliquot, isolate into two separate fractions
  + Cleave conjugated payload in one and analyze both fractions
+ **Total antibody & conjugated payload**
  + Generic or specific immunocapture
  + Digestion/payload release
  + Analyze Ab peptide(s) and payload together
+ **Total probody, total antibody, & conjugated payload**
  + Generic or specific immunocapture
  + Digestion/payload release
  + Analyze Ab and other peptide(s) and payload together

Hybrid LB/LC-HRMS for ADC biotransformation



May use radiolabeled or cold studies
Apply to plasma/serum, bile, urine, or tissue

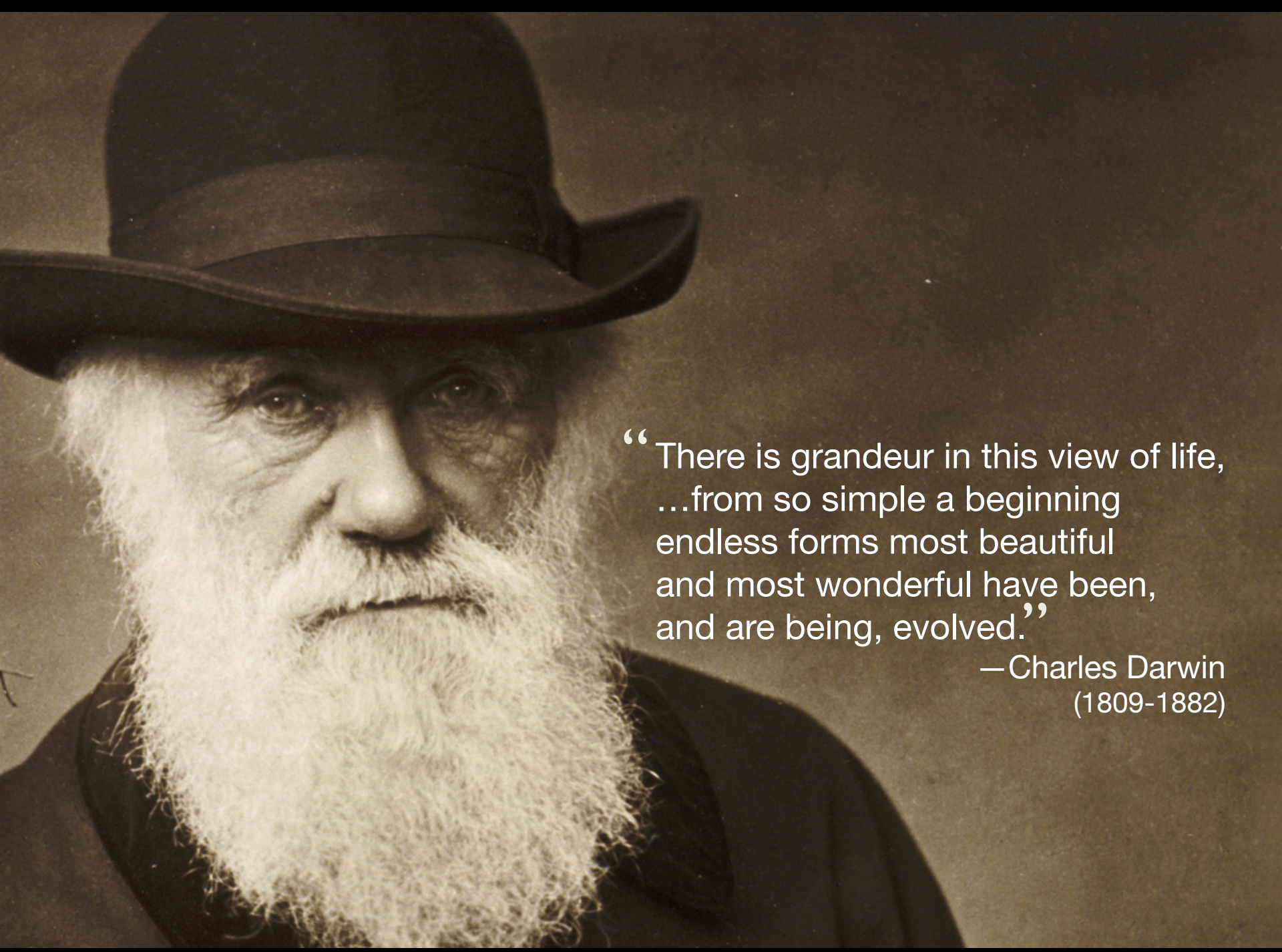
Immunocapture → Large molecule bioanalytical strategy

High level sample clean-up (e.g., PPT/LLE/SPE) → Small molecule bioanalytical strategy

Method	Analyte	Information
No digest + direct LC–MS	Intact protein	• Molecular mass; • DAR distribution; • Average drug load; • Detection of significant mass shifts due to stability/catabolism
Partial digest, direct LC–MS	Protein sub-units (e.g., Fab/Fc, LC/HC chain)	• Large catabolite structural characterization; • Better resolution for smaller mass shifts due to stability/catabolism
Trypsin digest; peptide map (LC–MS & MS/MS)	Peptides	• Identification and localization of low molecular mass catabolic products at the peptide level

Method	Analyte	Information
LC + online radioactivity detection	All radio-label containing catabolites (drug or antibody depending on probe preparation)	• Catabolite profile; • Number and relative quantitation of catabolites
LC–MS and LC–MS/MS	Small molecule metabolites/ catabolites (e.g., drug and linker-drug)	• Metabolite/catabolite structural ID; • Utilize current data processing tools for ID

Saad OM *et al*. *Bioanalysis*, (2015) 7(13), 1583–1604


“There is grandeur in this view of life,
…from so simple a beginning
endless forms most beautiful
and most wonderful have been,
and are being, evolved.”
—Charles Darwin
(1809-1882)

Final thoughts

- **Multi-domain biotherapeutic modalities, including ADCs, are rapidly evolving**
- Critically assessing exposure, safety, efficacy, stability and biotransformation is becoming more complicated
- Bioanalysis strategies must evolve in parallel to meet both scientific and cost effectiveness needs
- Decisions between LBA or hybrid LC-MS platforms are based on many factors:
  - case-by-case, fit-for-purpose considerations
  - strength/experience of particular bioanalytical labs
  - sponsor's bioanalytical philosophy
  - technical benefits vs. costs

One size does not fit all—will harmonization be possible?

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