Novel Platform Using LC-MS and Ligand Binding Assays for Characterization and PK analysis of ADCs

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THE DEVELOPMENT OF LARGE MOLECULES

Recombinant proteins

Therapeutic Antibodies

Novel scaffolds

Biosimilars/ Biobetters

Antibody - Drug Conjugates

THE CYTOTOXIC DRUG

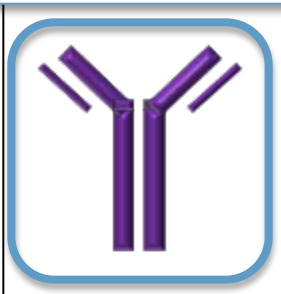
Cytotoxic drugs

very efficient but unspecific –

Off-target effects

Drug	Mechanism
Doxorubicin derivatives	Inhibit DNA religation, leading to DNA double-strand breaks
Maytansinoids; Auristatins	Prevent tubulin polymerization
Calicheamicins	Cause double-strand DNA breaks
CC-1065	Induces adenine alkylation
Duocarmycins	Break down adenine-specific molecules in the DNA structure
Anthracyclines	Inhibit DNA and RNA synthesis by intercalating between base pairs, preventing replications

THE MONOCLONAL ANTIBODY



Therapeutic antibodies in oncology –

Very specific/targeted,

But efficacy was lower than expected

THE LINKER

Criteria for linker in ADC:

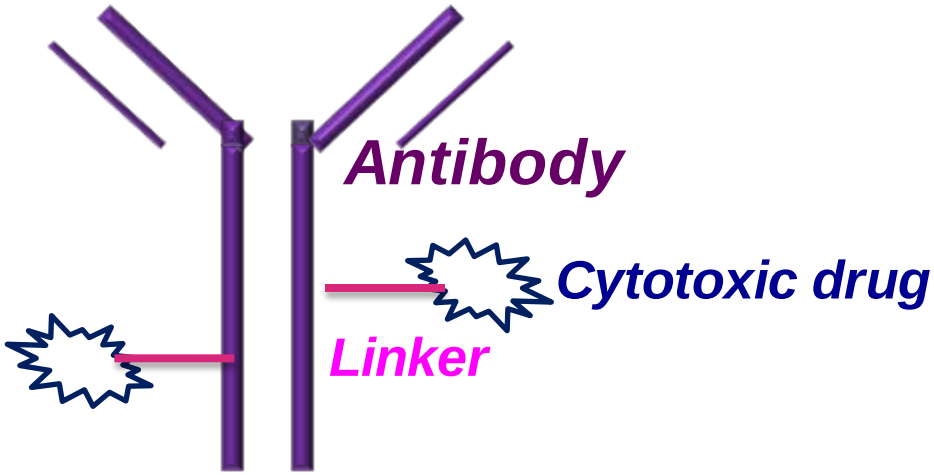
- *Stable in blood*
- *Able to release effector drug at target*

EXAMPLES CURRENT LINKERS

Linker	Release Mechanism
Hydrazone	degradation in acidic compartments within the cytoplasm
Peptide	enzymatic hydrolyzed by lysosomal proteases such as cathepsin B
Disulfide	Cleavable through disulfide exchange with an intracellular thiol, such as glutathione
Thioether	Nonreducible and designed for intracellular proteolytic degradation
Hydrophilic	improve activity against multidrug resistant cells and carry a higher maytansinoid load
DNA alkylator	DNA-specific binding that increases reactivity, deactivates the cytotoxic, and reactivates only after the cytotoxic is cleaved

Release mechanism linker – drug will impact in vivo behavior

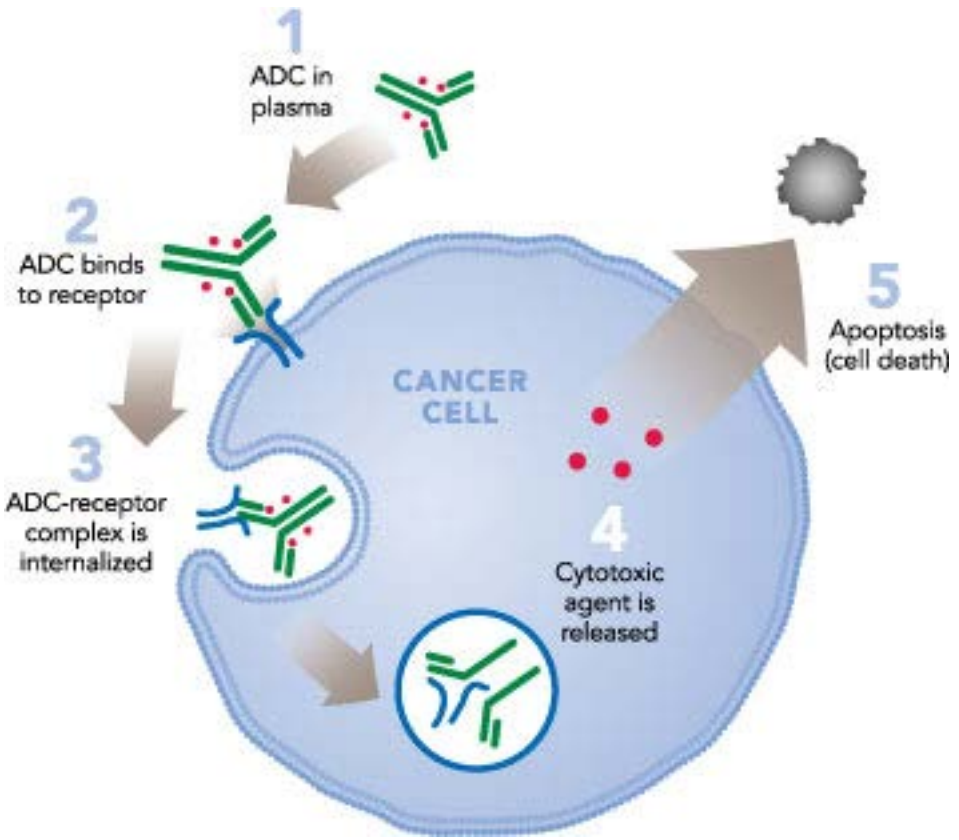
ANTIBODY-DRUG CONJUGATE



Combining the specificity of a mAb

with the efficacy of a cytotoxic drug

BASIC MECHANISM ADCS



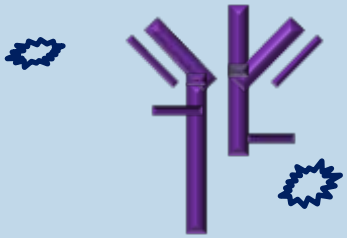
http://www.seattlegenetics.com/adc_technology

OFF-TARGET EFFECTS

Target dependent toxicity of normal cells (e.g. Her2)

Target independent toxicity via Mannose binding receptor (glycosylation mAb)

Premature



release of drug

BIOANALYSIS

Why is bioanalysis

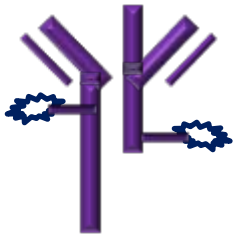
assessment of ADCs challenging?

BIOANALYSIS

- *Heterogeneity – more than one linker attached to mAb – varying drug-to-antibody ratio (DAR)*

- *Biotransformation – dynamic in vivo behavior*

WHAT TO EXPECT IN VIVO?



ADC

mAb

free drug

mAb + linker

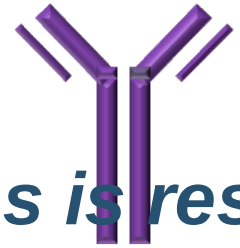
metabolites

linker

drug + linker

WHAT TO EXPECT IN VIVO?

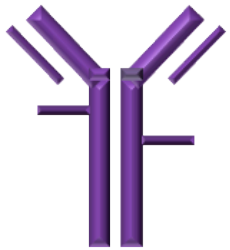
ADC



mAb

free drug

Which of these species is responsible for safety and efficacy?



mAb + linker

metabolites

linker

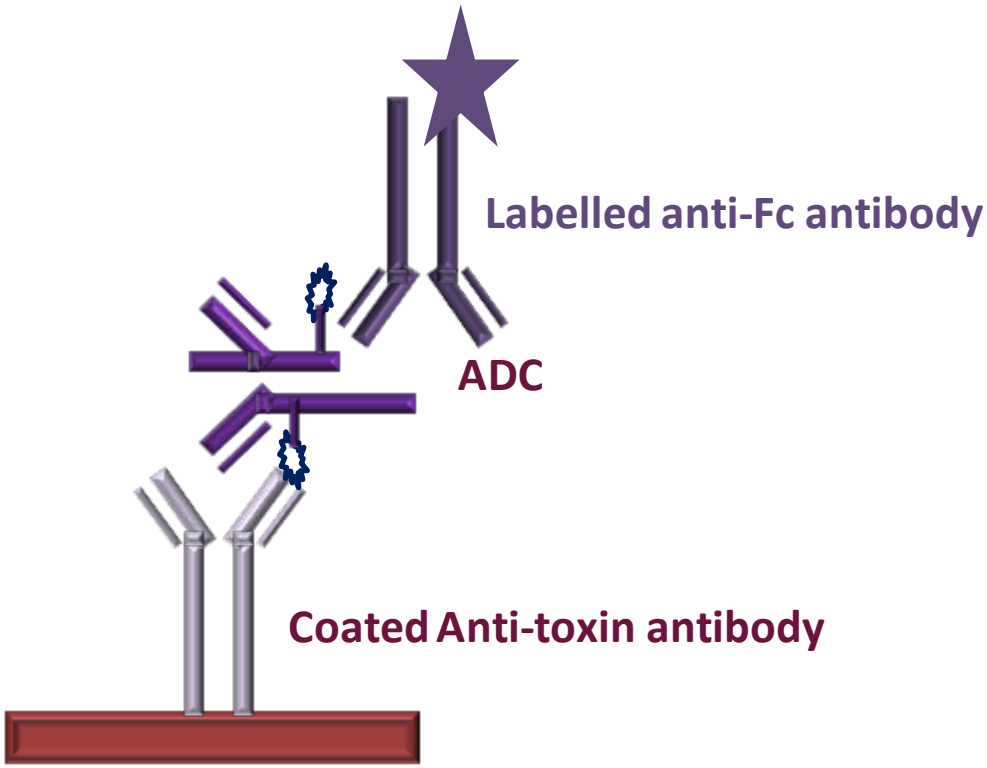
drug + linker

BIOANALYSIS

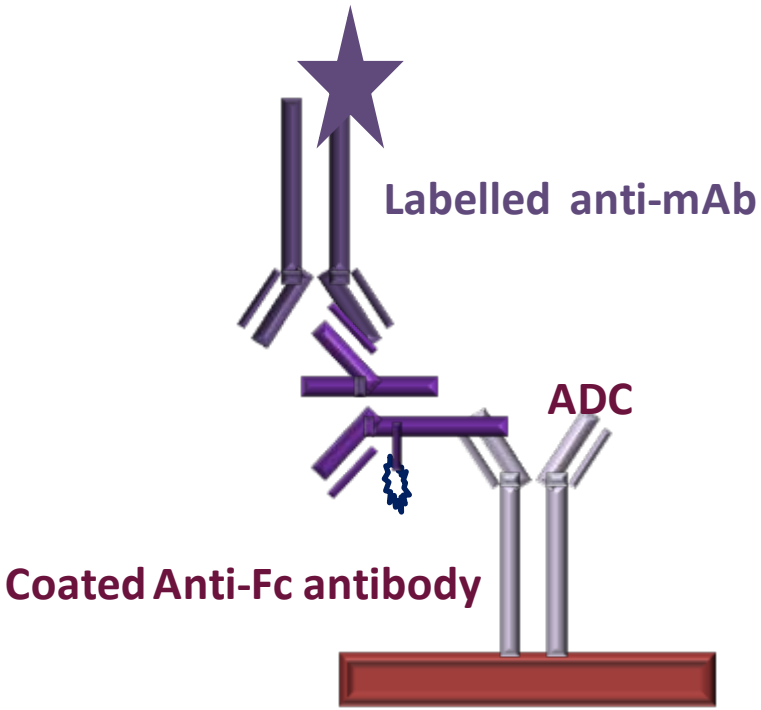
Typical approach to measure ADCs in matrix is multi-disciplinary:

Linker	Definition	Assay type
Total antibody	DAR ≥ 0	LBA
Conjugated antibody	DAR ≥ 1	LBA
Antibody-conjugated drug	Total small molecule drug conjugated to antibody	Affinity LC-MS/MS, LBA
Free drug	Unconjugated drug in circulation	LC-MS/MS
Total drug	Conjugated & unconjugated drug	LC-MS/MS

CONJUGATED AB DAR ≥ 1



TOTAL MAB DAR ≥ 0

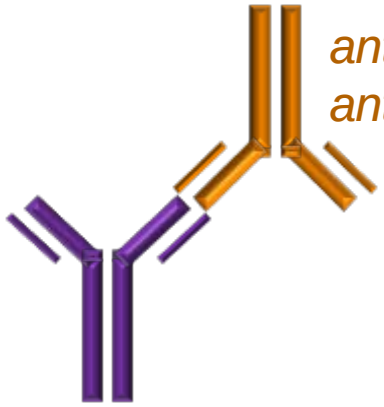


IMPROVING SPECIFICITY ASSAY

Use of anti-idiotypic reagents

Anti-idiotypic = structure directed against the idiotopes

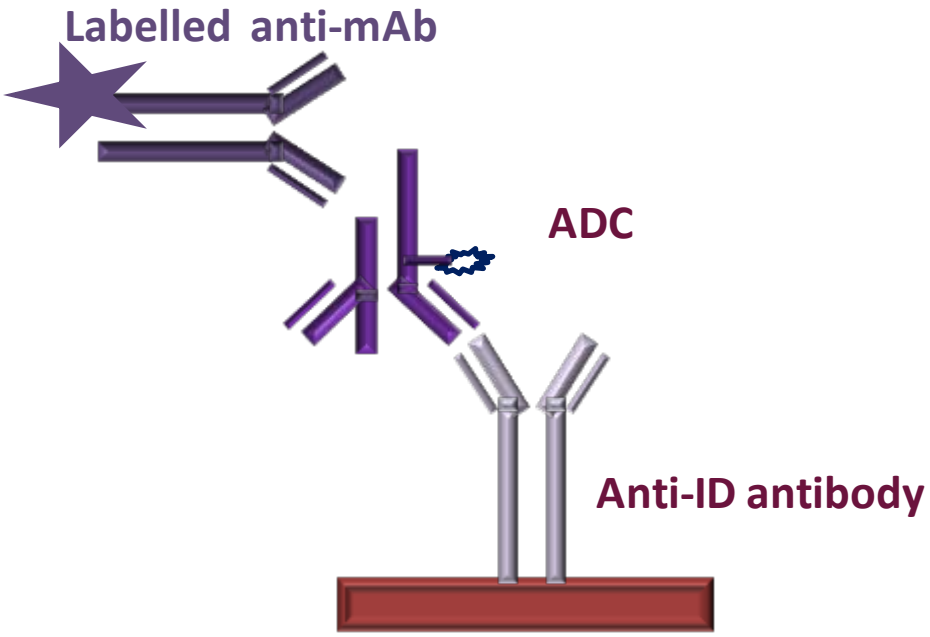
Idiotopes: : unique set of antigen determinants



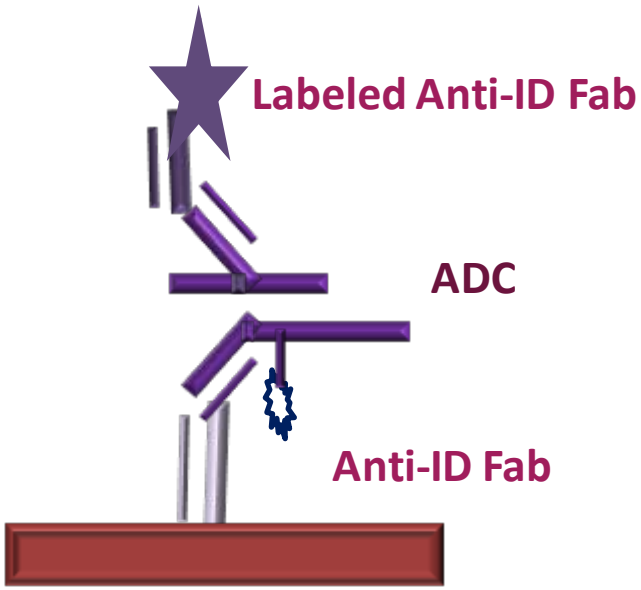
anti-idiotypic antibody

anti-idiotypic Fab fragment

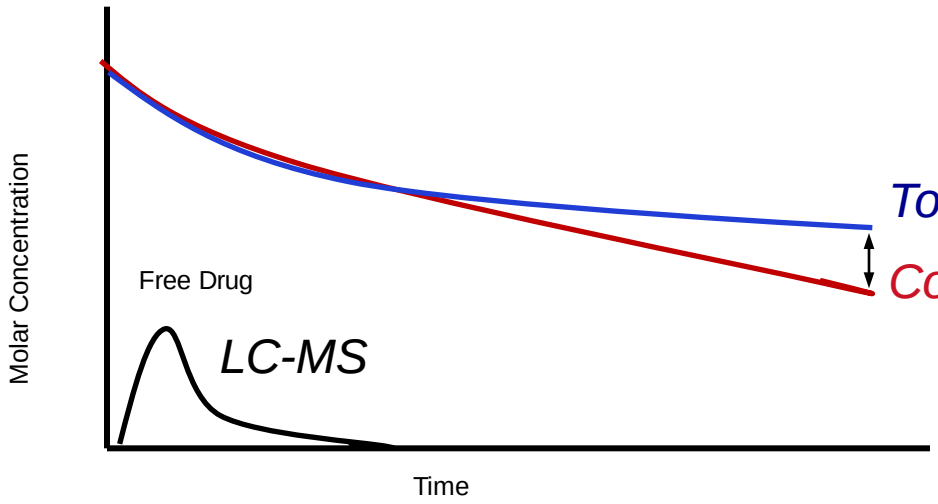
TOTAL MAB WITH ANTI-IDS



TOTAL MAB WITH ANTI-IDS



MULTI-DISCIPLINARY APPROACH



Total antibody - Conjugated antibody = deconjugated Ab

Information gained: average ADC in vivo fate/ no DAR distribution

BIOANALYSIS

Problem ligand binding assay:

- *Reference standard may not be appropriate for quantification of analytes in vivo*
- *Possible solution: calibrator with most anticipated DAR form (engineered homogenous ADC)*
- *High payload (drug) may interfere with assay (steric hindrance)*

BIOANALYSIS

Problem LC-MS

- *Measurement based on fixed mass (e.g. intact drug alone)*
- *Only a fraction of the cytotoxic drug may be measured*

due to interaction with matrix components

or linker partially attached to drug – most dominant form of

released drug may not be measured

- *Free drug concentration normally very low (~ 1%)*

NOVEL BIOANALYSIS APPROACH

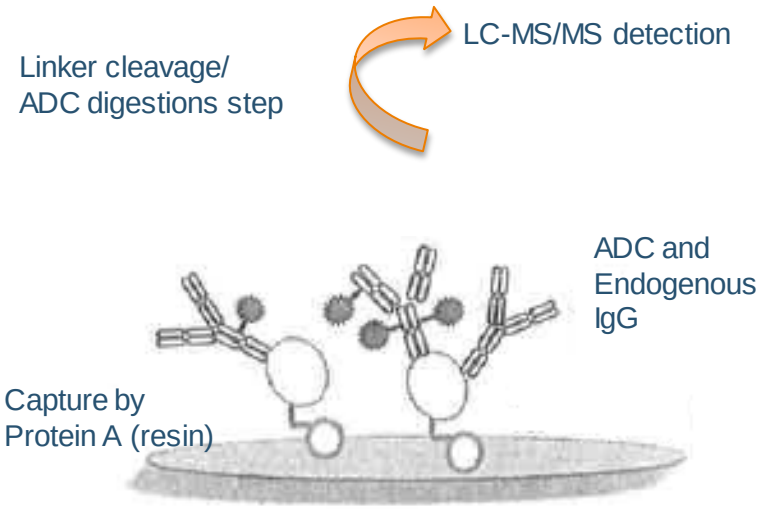
Combine LC-MS with immuno pull down/ affinity capture

- ***Opportunity to measure ADCs and their biotransformation in vivo over time***

GENERIC CAPTURE & LC-MS/MS

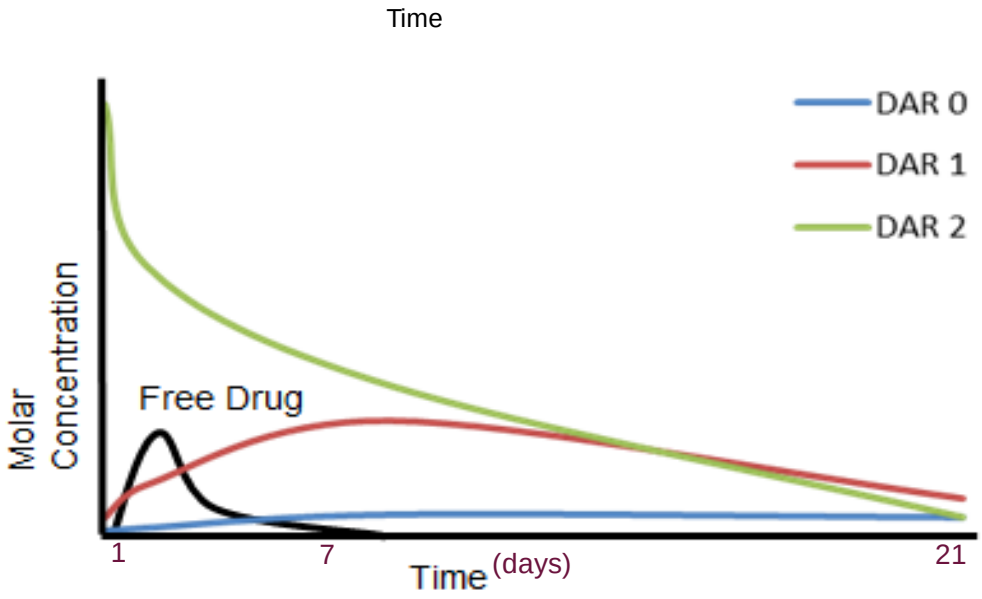
Generic: capture antibody (unspecific with Protein A/G),

cleave drug/digest and measure ***on peptide level***

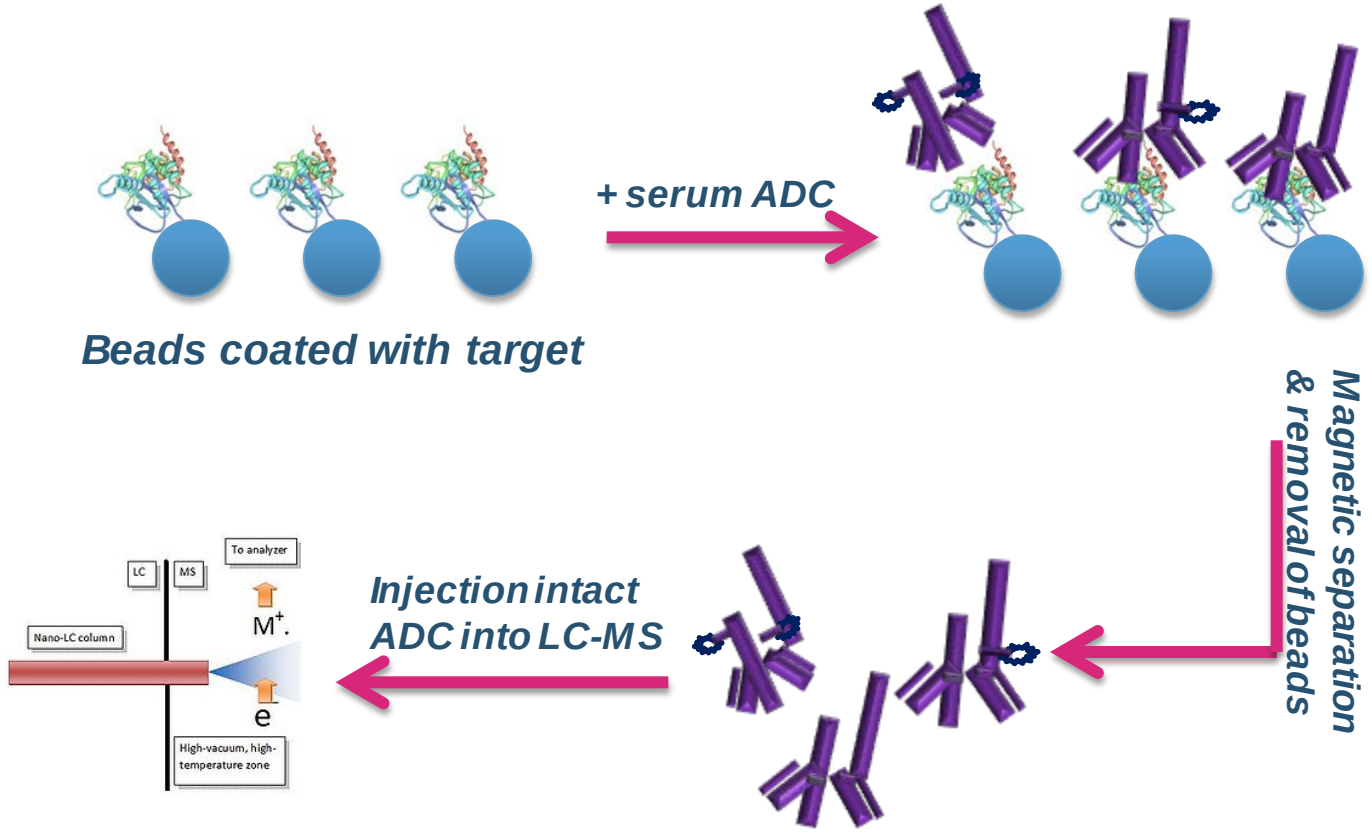


Still no DAR distribution!

GOAL – MEASURE DAR DISTRIBUTION

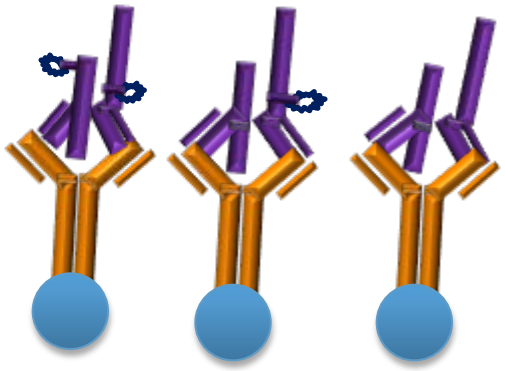


STEP 1: SPECIFIC AFFINITY CAPTURE

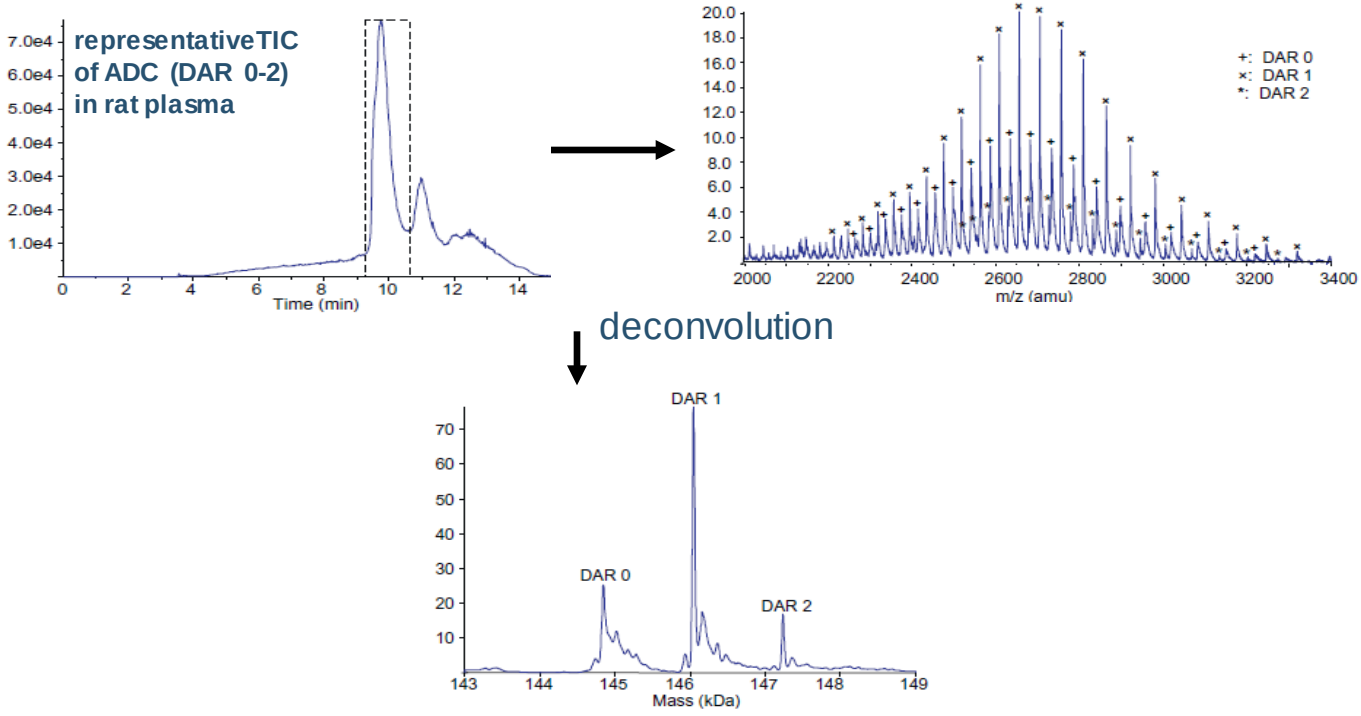


IMPROVING SPECIFICITY

Beads coated with anti-idiotypic antibodies



STEP 2: LC-MS OF INTACT ADC

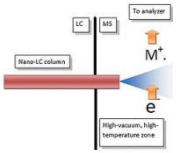


WHICH APPROACH AT WHICH STEP?

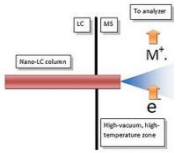
Average DAR

LBA with Generic reagents

LBA with Anti-idiotypic reagents

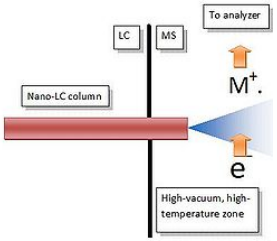


LC-MS Free drug



Generic capture/ digest & LC-MS

DAR distribution



Affinity Capture (nano) LC-MS

R&D → Preclinics → Clinics

THANK YOU FOR YOUR ATTENTION