ABZENA

10th APS International PharmSci Conference
Greenwich 2019

AN OVERVIEW OF THE ADC DESIGN AND DEVELOPMENT LANDSCAPE

10th APS International PharmSci 2019 Conference, University of Greenwich, London 11 - 13 September 2019

Session: **2.4b, Review session: Antibody-drug conjugates**

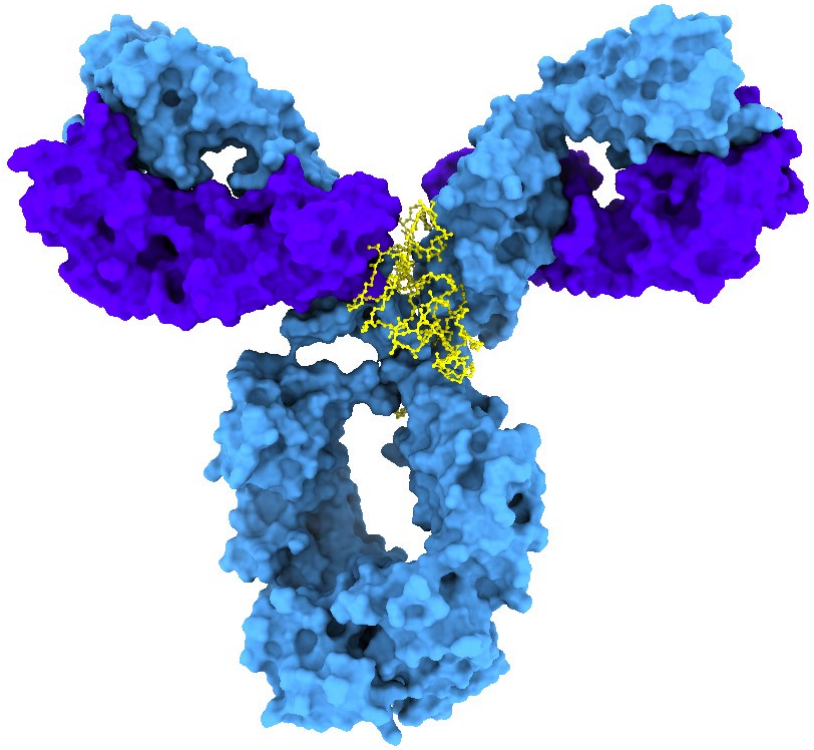
Mark Frigerio, VP Design and Development, ABZENA

An overview of the ADC design and development landscape

- Introduction to the field
- Key components of ADC architecture
- How different companies have approached the challenges faced during development
- Clinical landscape and progress
- How drug developability can build the best product by design
- Summary and future development

ABZENA

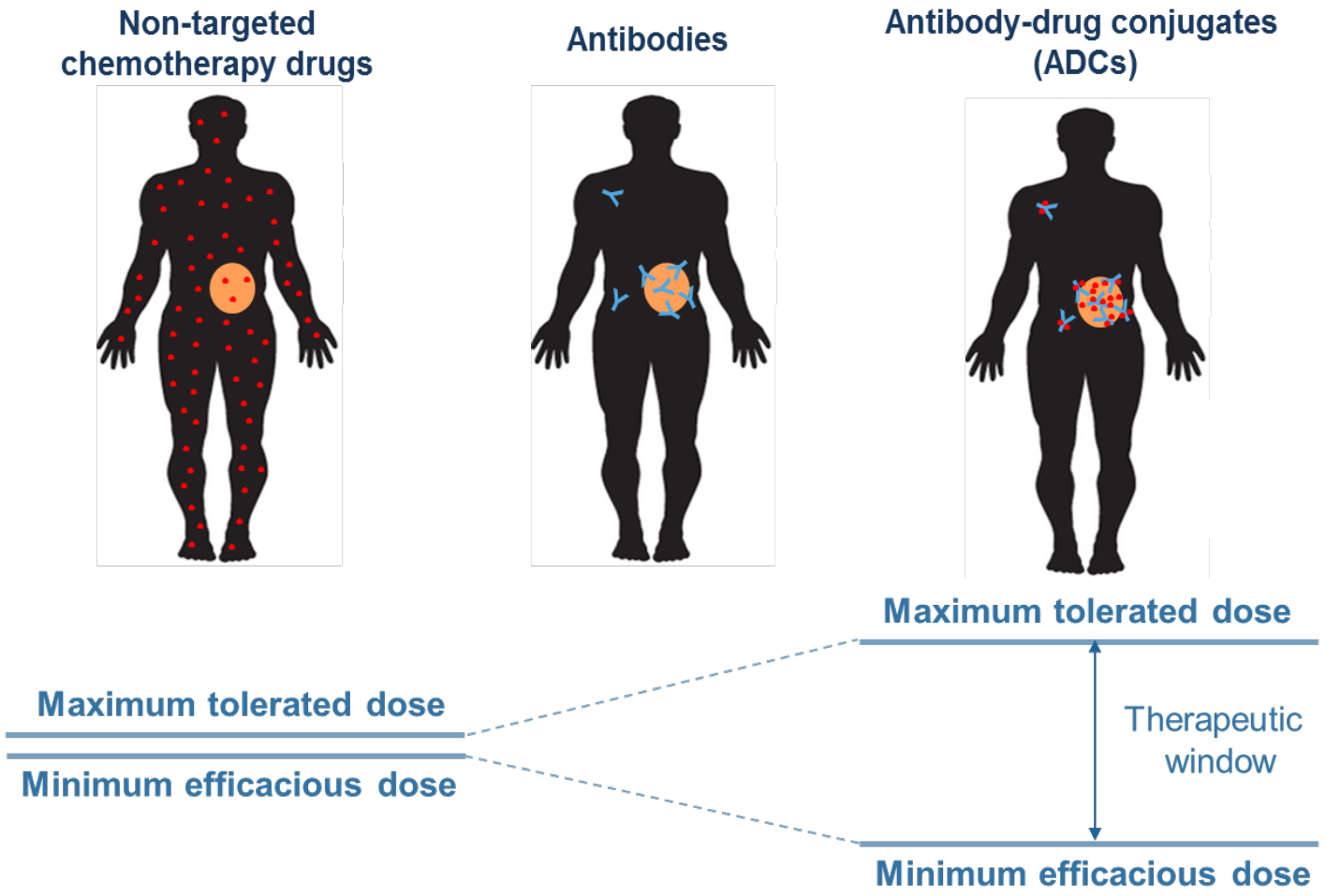
What is an Antibody Drug Conjugate (ADC)?



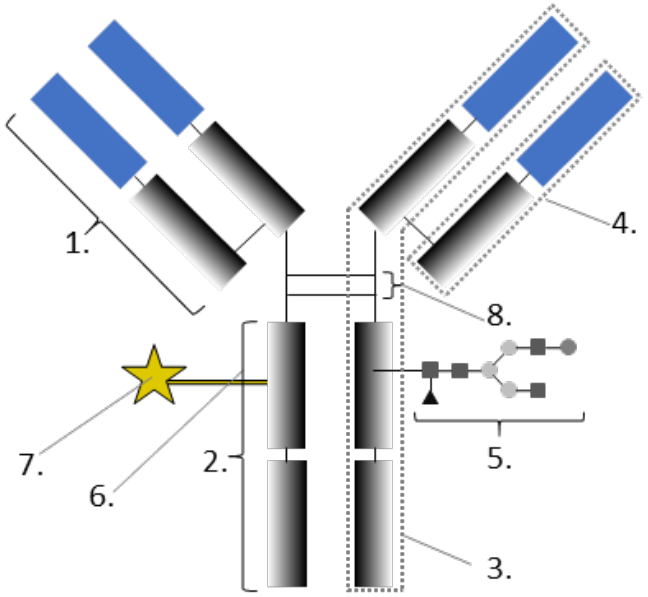
- Antibody
- Linker
- Cytotoxic payload

ABZENA

Targeted delivery of drugs using ADCs



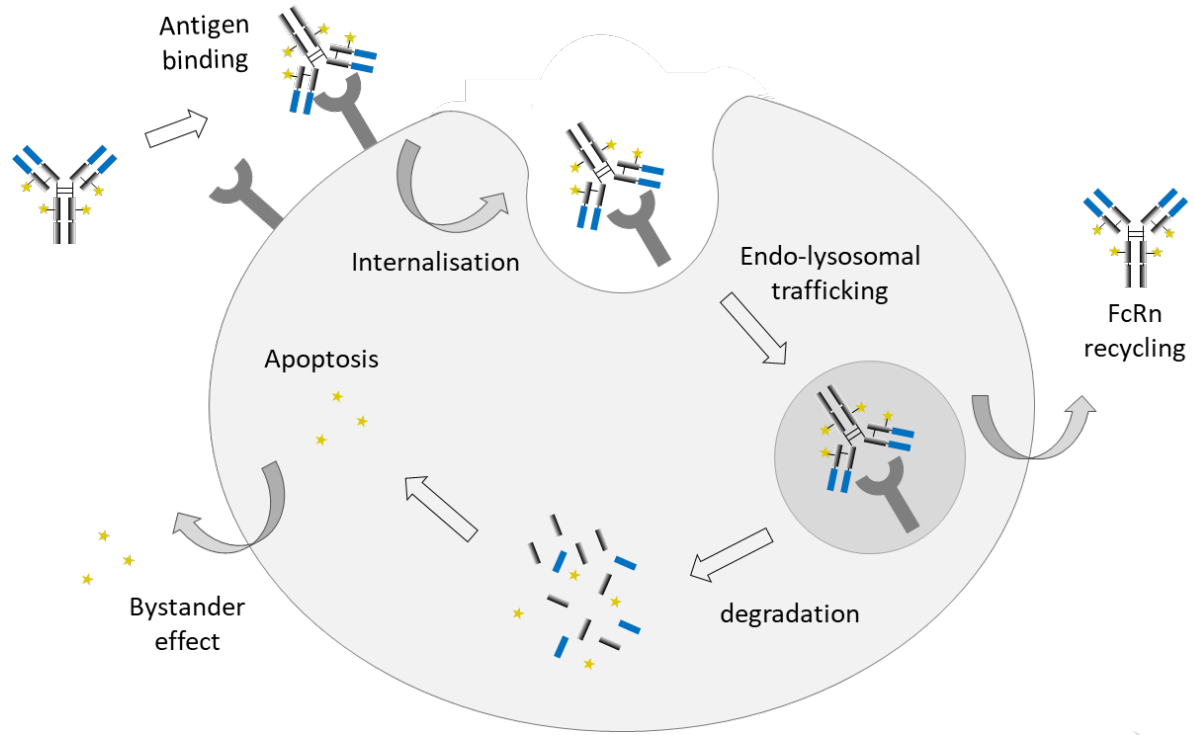
Understanding the key mechanistic requirements of an ADC is critical to successful design and development



1. Fab fragment
2. Fc portion (CH2 and CH3 domains)
3. Heavy chain
4. Light chain
5. Glycosylation site/glycoform
6. Linker
7. Payload
8. Hinge region

Simple concept - complex products

- Multiple components and multi-step mechanism of action
- Specific requirements for each step / component. For example, stable in circulation but drug released in tumour cells



What are the key components of an ADC?

Targeting protein

- Targets antigen with high affinity and with and low expression in healthy tissue
- Minimal non-specific binding
- Maintains binding, stability, internalisation and PK when conjugated to linker-drug

Conjugating unit

- Electrophile capable of reacting with lysines and native/engineered cysteines or residues OR
- Functionality to react with engineered peptides OR
- Site-specific moiety capable of rebridging disulfide

Polymer

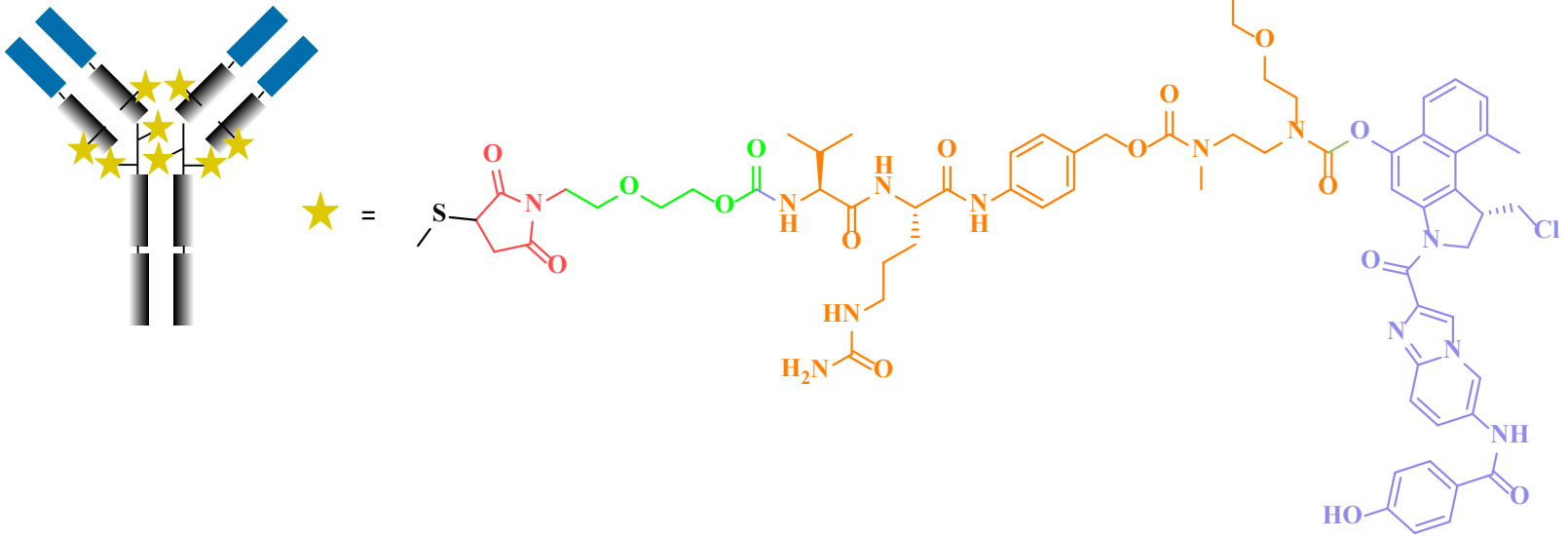
- Used to enhance ADC PK
- Enhances aqueous solubility
- Modulates payload release through shielding and distance from mAb
- Examples include – Amino acids, alkyl chains and polymers including PEG

Release linker

- Cleavable or non-cleavable
- Sufficiently stable during ADC synthesis/storage and in systemic circulation
- Efficient intracellular drug release – Driven by pH, enzymes, reactive thiols, antibody catabolism

Payload

- High potency (nM-pM)
- Defined MOA e.g. DNA Alkylator/tubulin inhibitor
- Suitable functional handle to attach to linker
- Examples include auristatins, maytansinoids, PBD dimers, duocarmycins

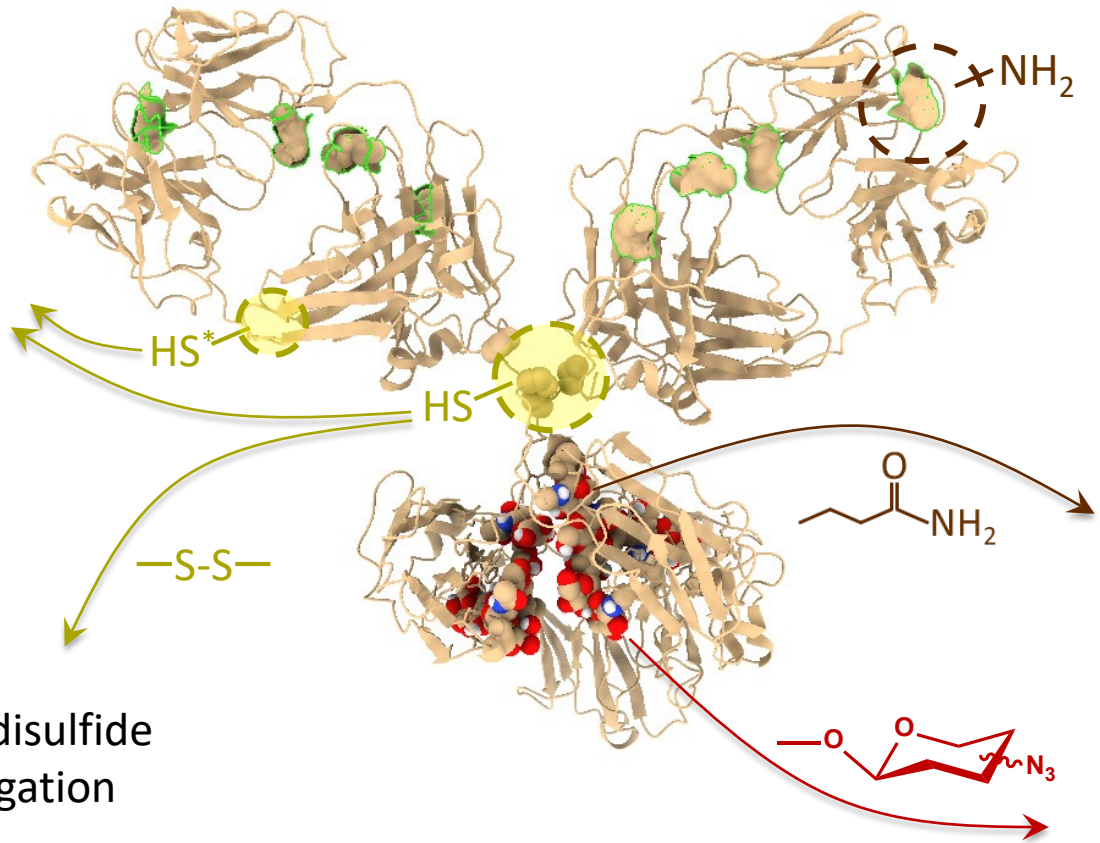


Main challenges faced in ADC development

ADC challenge	Reasons	Efficacy	Toxicity
Antigen	Antigen heterogeneity (tumour, metastases) Insufficient expression in tumour Expression on healthy cells	↓ ↓	 ↑
Payload MOA	Resistance of tumour cell to payload MOA	↓	
Heterogeneity of drug-antibody ratio (DAR)	Naked antibody – competitive inhibitor Low DAR – Insufficient drug delivered High DAR – Fast clearance	↓ ↓ ↓	 ↑
ADC **instability**	Systemic release of drug Disarming of ADC Fragmentation of antibody	 ↓ ↓	↑ ↑
Suboptimal PK	High DAR (fast clearance) Immunogenicity (fast clearance)	↓ ↓	↑ ↑

ABZENA

Multiple conjugation options can be evaluated to identify suitable functional attachment as part of the design stage



Native and engineered* cysteine conjugation

Native lysine conjugation

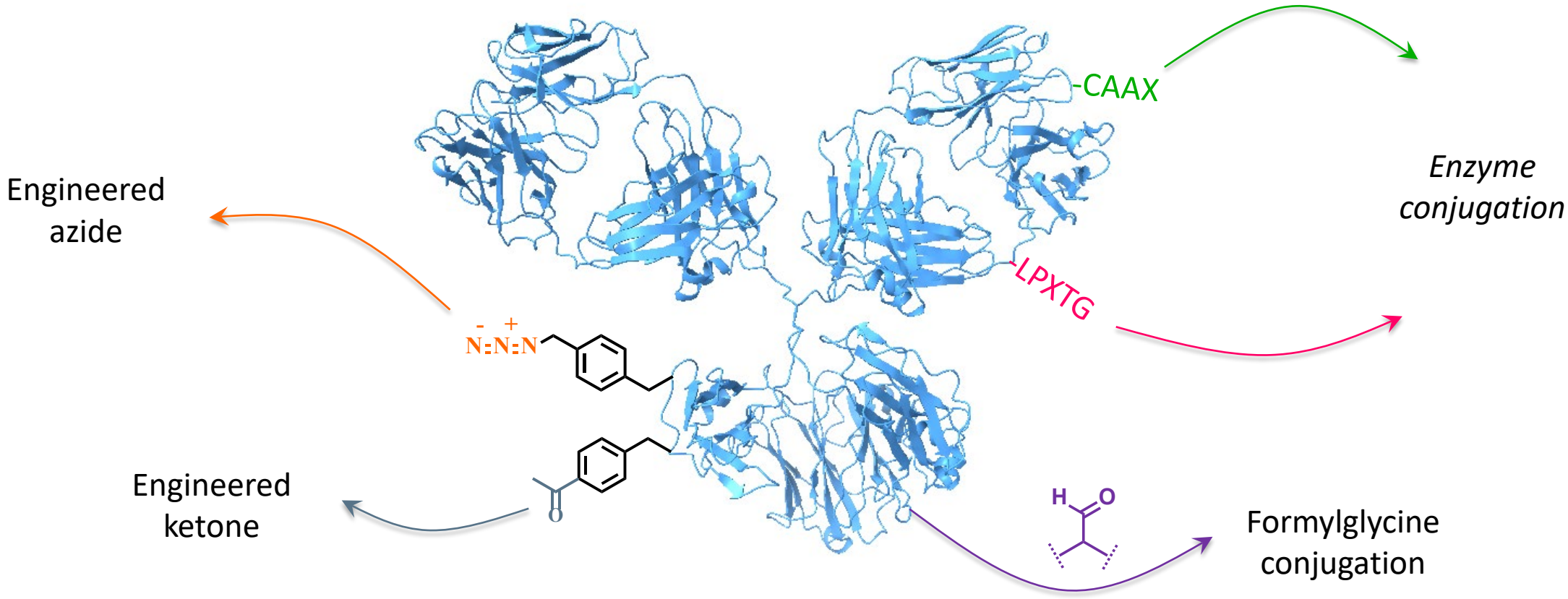
Transglutaminase conjugation

Native disulfide conjugation

Glycan conjugation

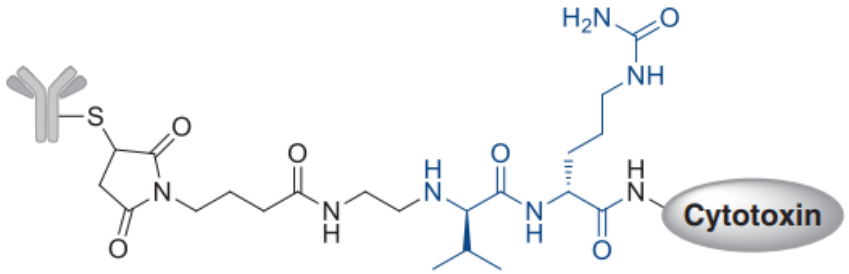
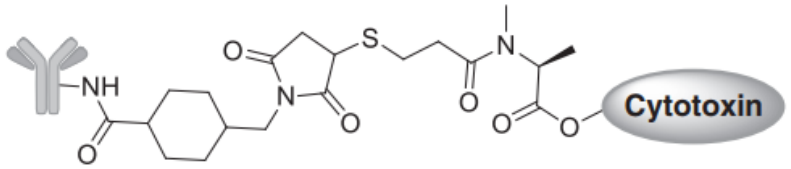
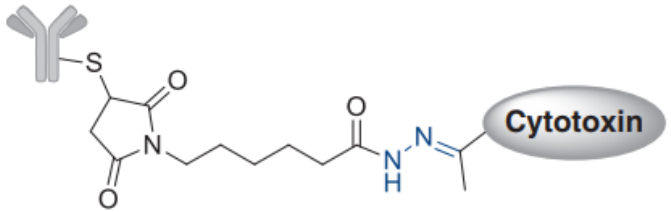
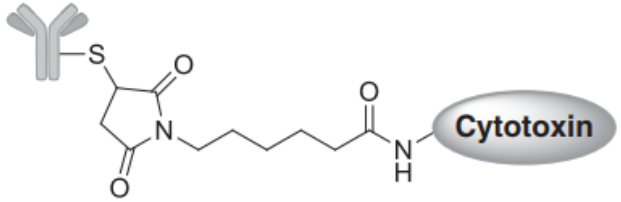
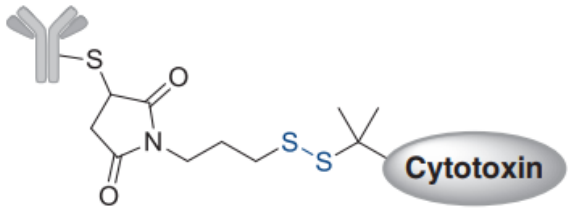
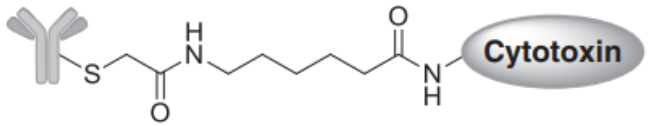
ABZENA

Multiple conjugation options can be evaluated to identify suitable functional attachment as part of the design stage



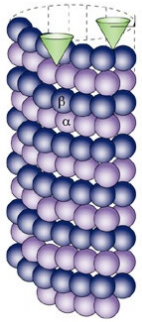
Early ADC included use of cleavable or non-cleavable linkers

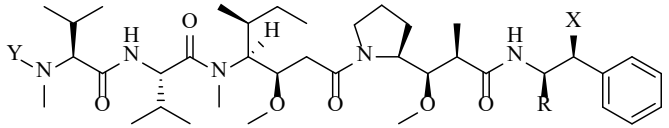
Examples of ADC drug linkers

Cleavable linkers	Noncleavable linkers
 Valine-Citrulline (protease sensitive)	 *N*-Maleimidomethylcyclohexane-1-carboxylate (MCC)
 Hydrazone (acid-sensitive)	 Maleimidocaproyl
 Disulfide (glutathione-sensitive)	 Mercaptoacetamidocaproyl

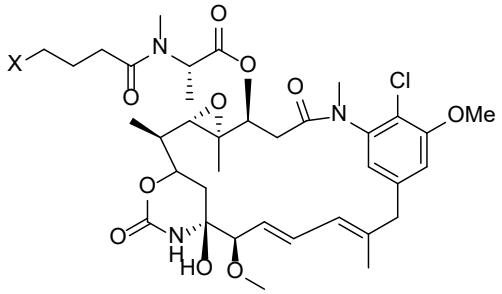
Payloads for ADC development are typically highly potent

Anti-mitotic





Auristatins

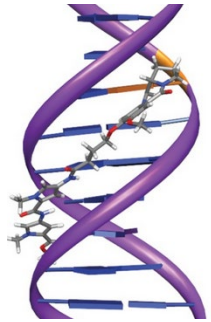


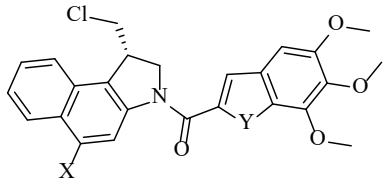
Maytansinoids

Calicheamicins

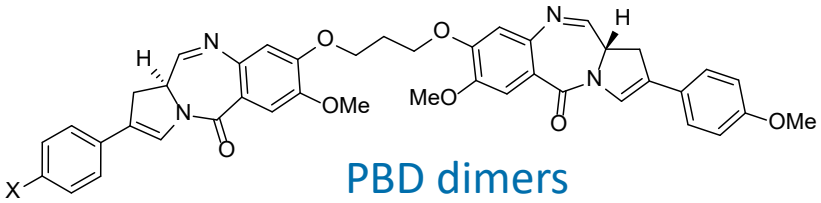
Tubulysins

DNA-interacting

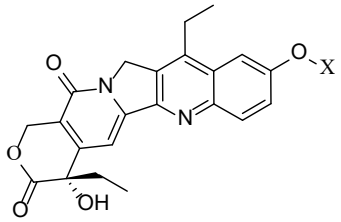




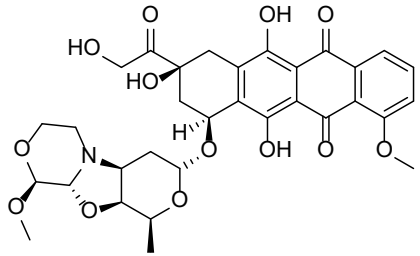
Duocarmycins



PBD dimers



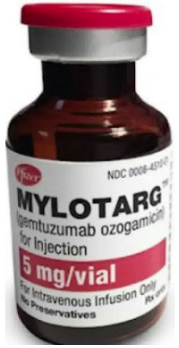
Camptothecins

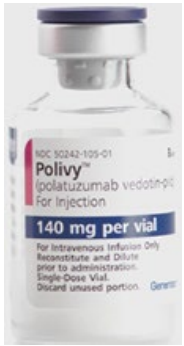


Anthracyclines

ABZENA

Marketed ADCs have clinically relevant efficacy but are still associated with toxicity



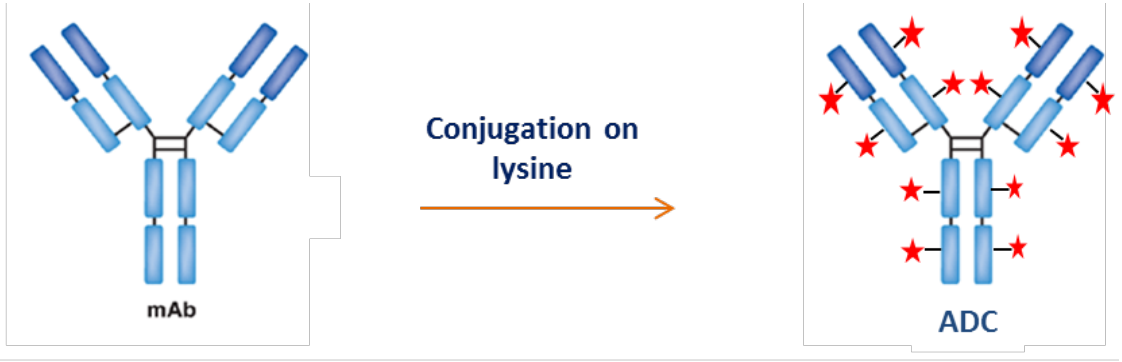


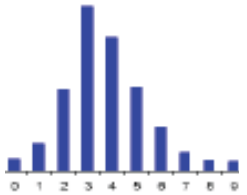
	Mylotarg®	Adcetris®	Kadcyla®	Besponsa®	Polivy™
Release date	2000-10; 2017	2011	2013	2017	2019
Target	CD33	CD30	HER2	CD22	CD79b
mAb isotype	IgG4	IgG1	IgG1	IgG4	IgG1
Toxin	Calicheamicin	MMAE	DM1	Calicheamicin	MMAE
Conjugation site	Lysine	Cysteine	Lysine	Lysine	Cysteine
Release mechanism	Hydrazone + disulfide	Dipeptidic	Non-cleavable	Hydrazone + disulfide	Dipeptidic
Clinical dose	~0.1 mg/kg	1.8 mg/kg	3.6 mg/kg	0.02 mg/kg	1.8 mg/kg
Clinical toxicities	Veno-occlusive disease Neutropenia Thrombocytopenia	Neutropenia	Thrombocytopenia	Veno-occlusive disease Neutropenia Thrombocytopenia	Neutropenia Thrombocytopenia

ABZENA

Approved ADCs: Conjugation approaches used

Conjugation to lysine residues



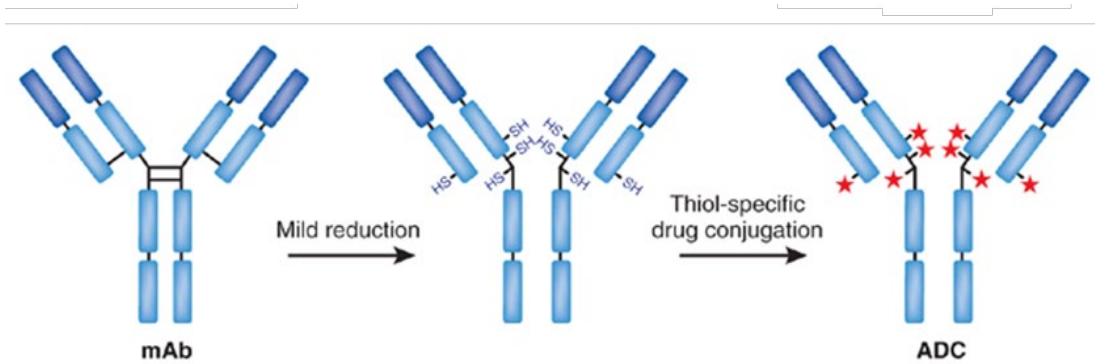


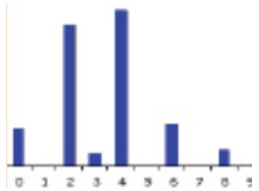
Kadcyla® (Immunogen / Roche-Genentech)

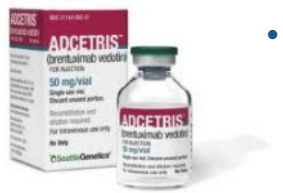
FDA approved Feb 2013 for breast cancer

- 5.8 months longer survival than standard therapy
- Significantly longer PFS

Conjugation to interchain cysteine residues







Adcetris® (Seattle Genetics / Takeda-Millenium)

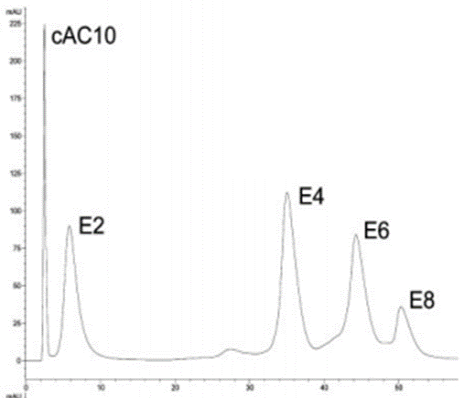
FDA approved Aug 2011 for Hodgkin lymphoma

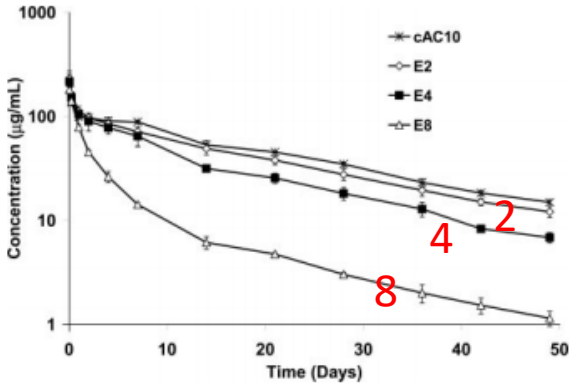
- 75% ORR in HL patients
- 1/3 responders had complete remission

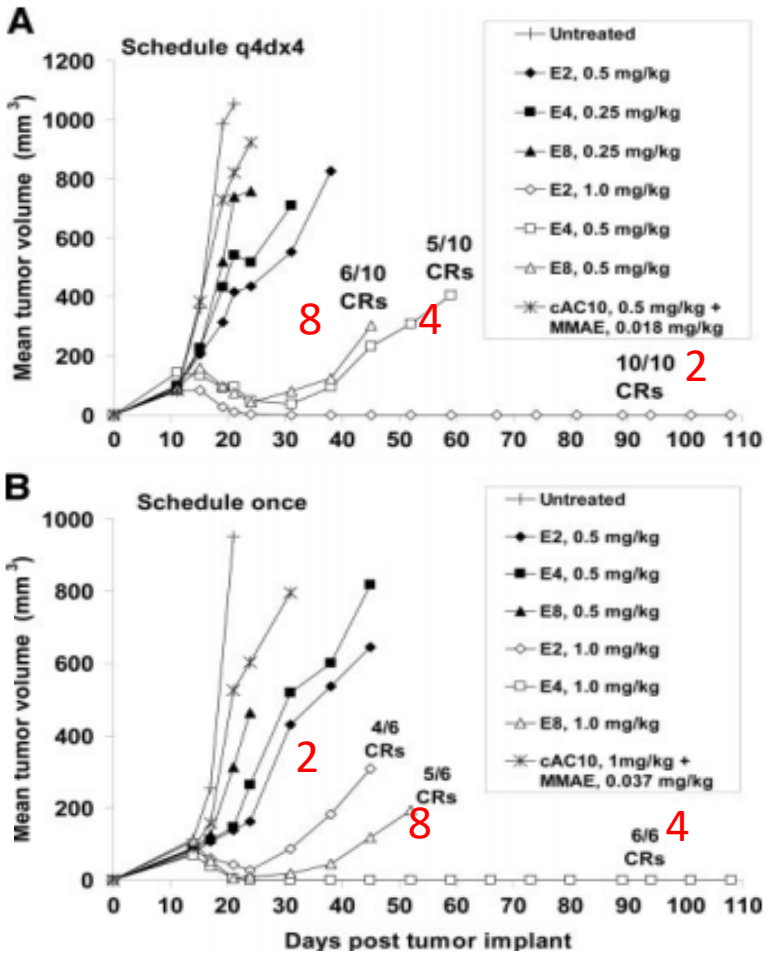
ABZENA

Seattle Genetics: Drug-antibody ratio (DAR) matters

- DAR 2, 4 and 8 purified by HIC from heterogeneous mixture
- Correlation between DAR and PK
- Highly loaded DAR species cleared faster
- Highly loaded DAR species had lower efficacy
- Differences between single-dose and repeat dose studies (cumulative effects?)



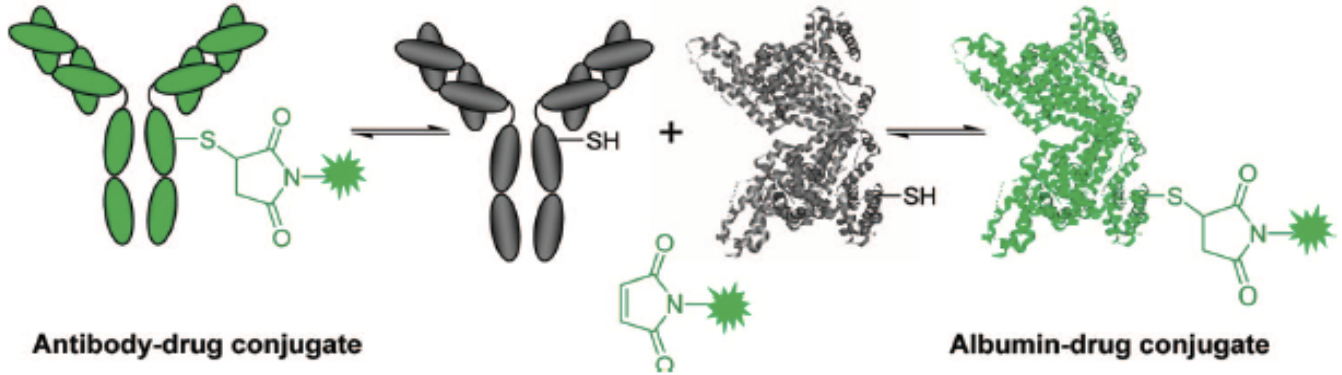




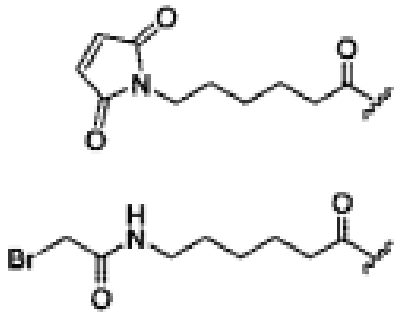
Hamblett *et al.*, Clin Cancer Res. 2004. 10, 7063

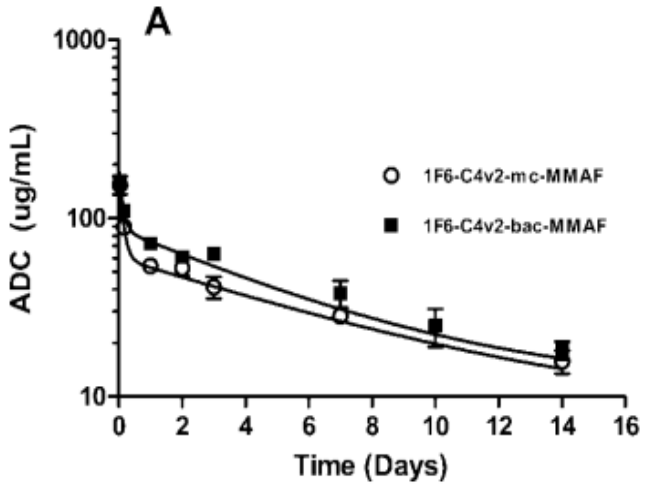
ABZENA

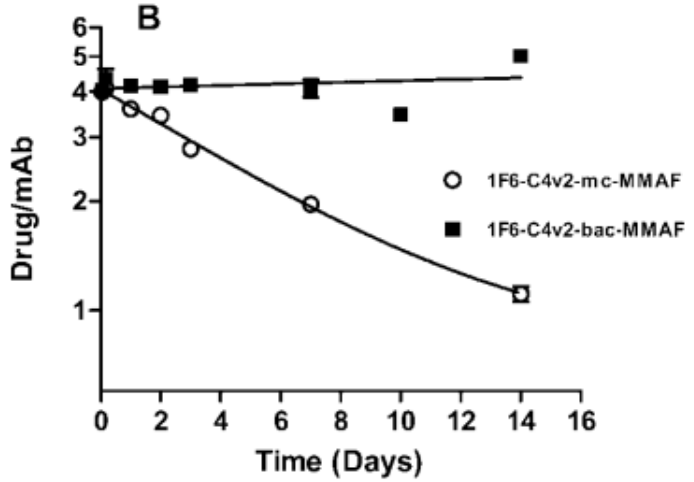
Seattle Genetics: Maleimide instability can lead to conjugation of drug to albumin



Proposed mechanism of drug transfer from an antibody to albumin through the thioether fragmentation reaction.





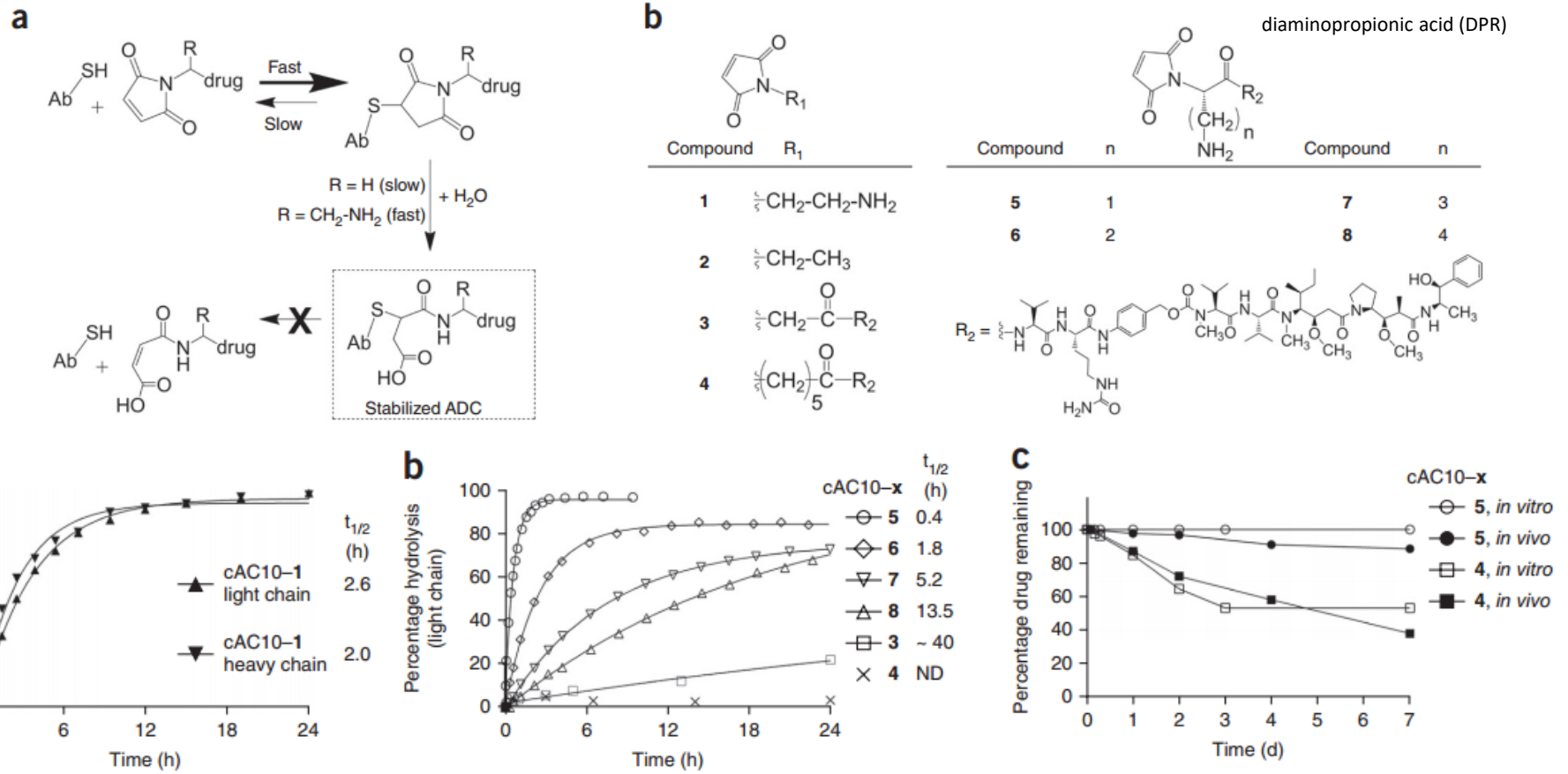


- Bromoacetamide proposed as alternative chemistry

Alley *et al.*, 2008. Bioconjugate Chem. 19: 759-765

ABZENA

Seattle Genetics: self-stabilizing maleimide

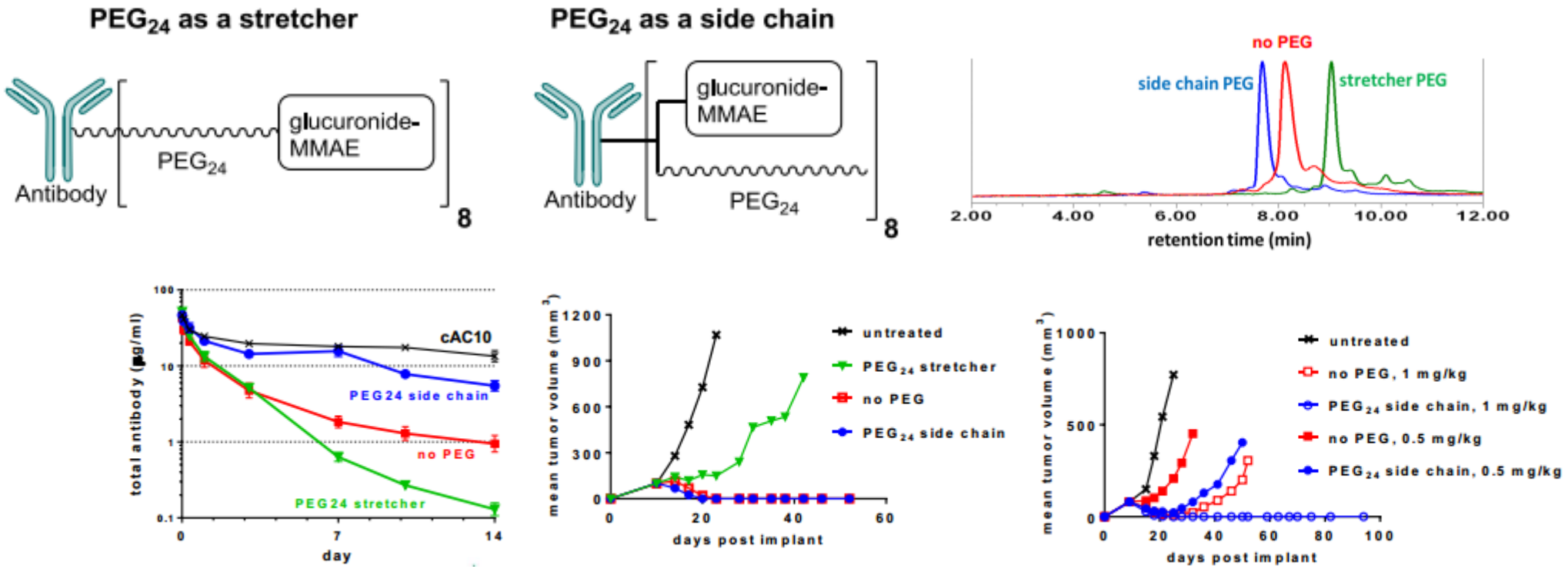


- The rate of hydrolysis depends on the distance between the succinimide ring and the amine
- ADC stability depends on rate of ring opening

Lyon *et al.,* Nature Biotechnology, 2014. 32, 10

ABZENA

Seattle Genetics: Effect of drug-linker hydrophobicity

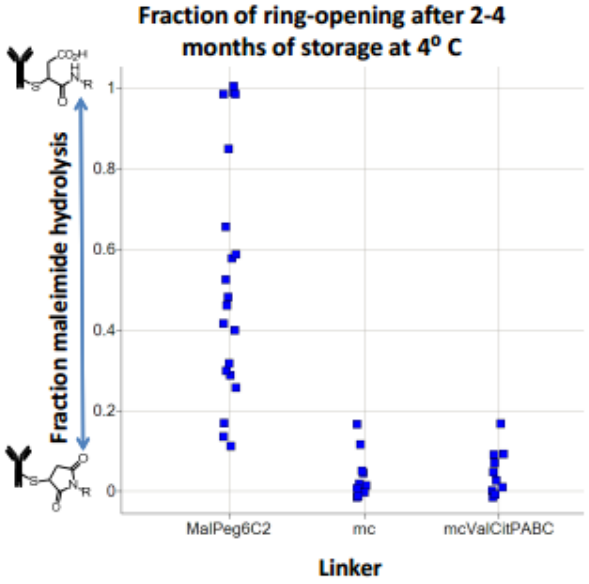


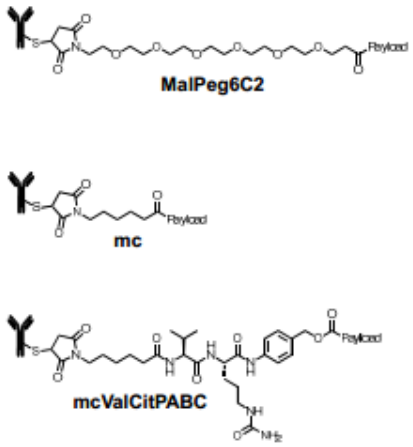
- PEG configuration influences apparent hydrophobicity and PK
- In vivo efficacy correlates with PK profile
- PEG can mask the hydrophobicity of the drug

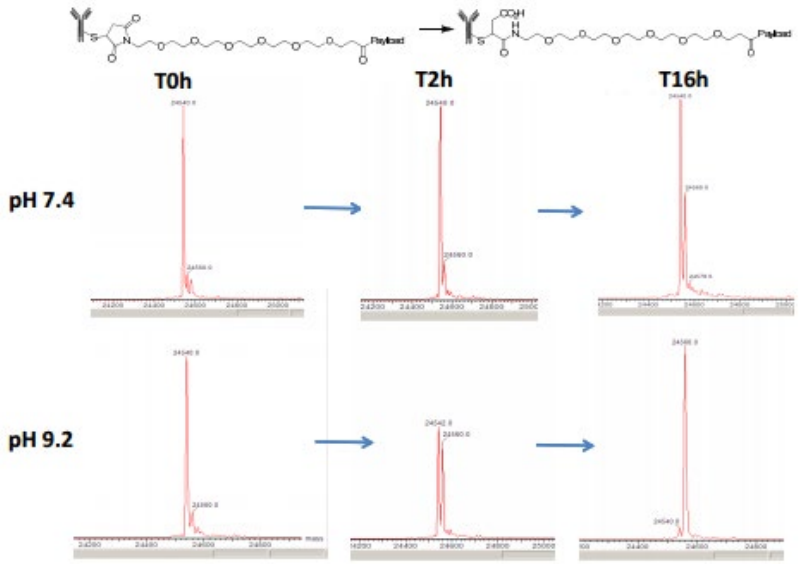
Robert Lyon, Seattle Genetics. 2014. PEGS Boston

ABZENA

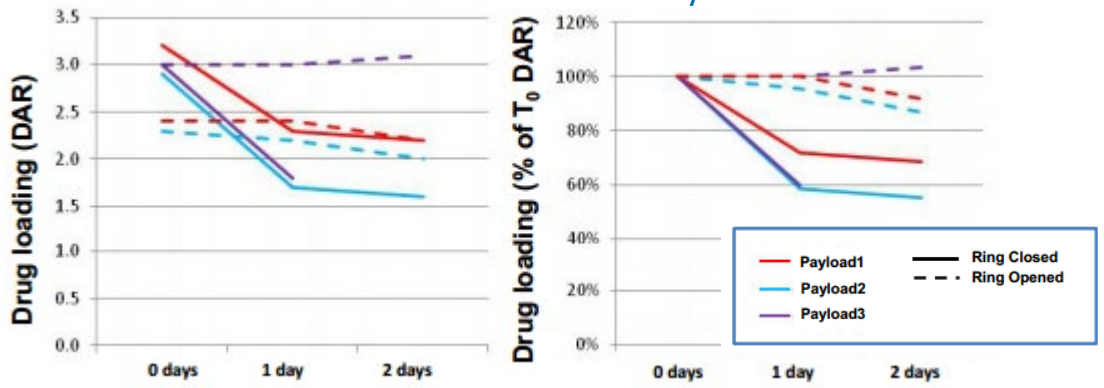
Pfizer: Forcing ring opening for improved maleimide stability



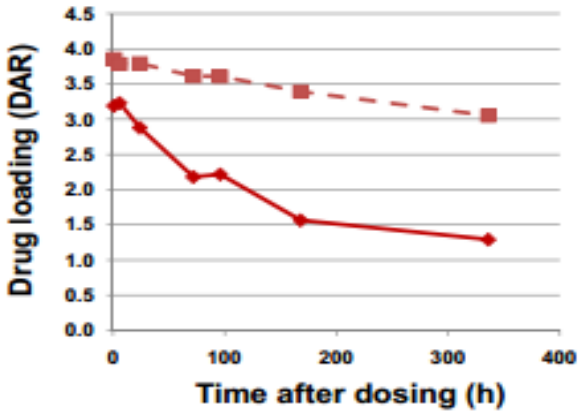




In vitro serum stability



In vivo stability

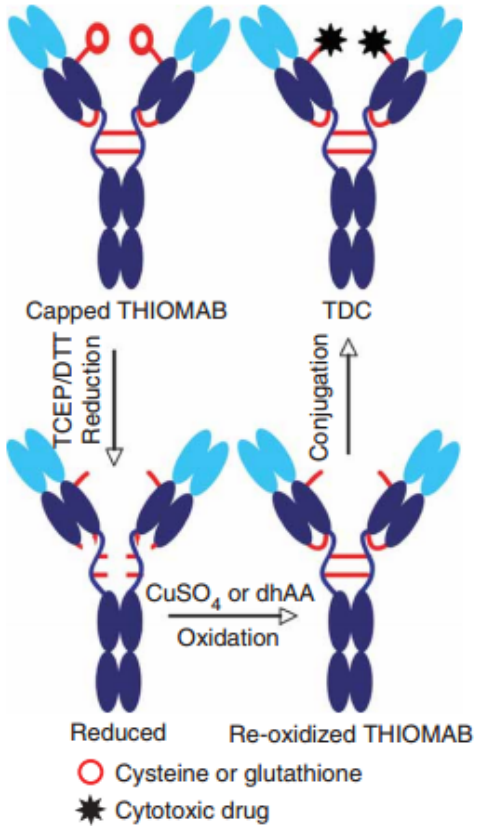


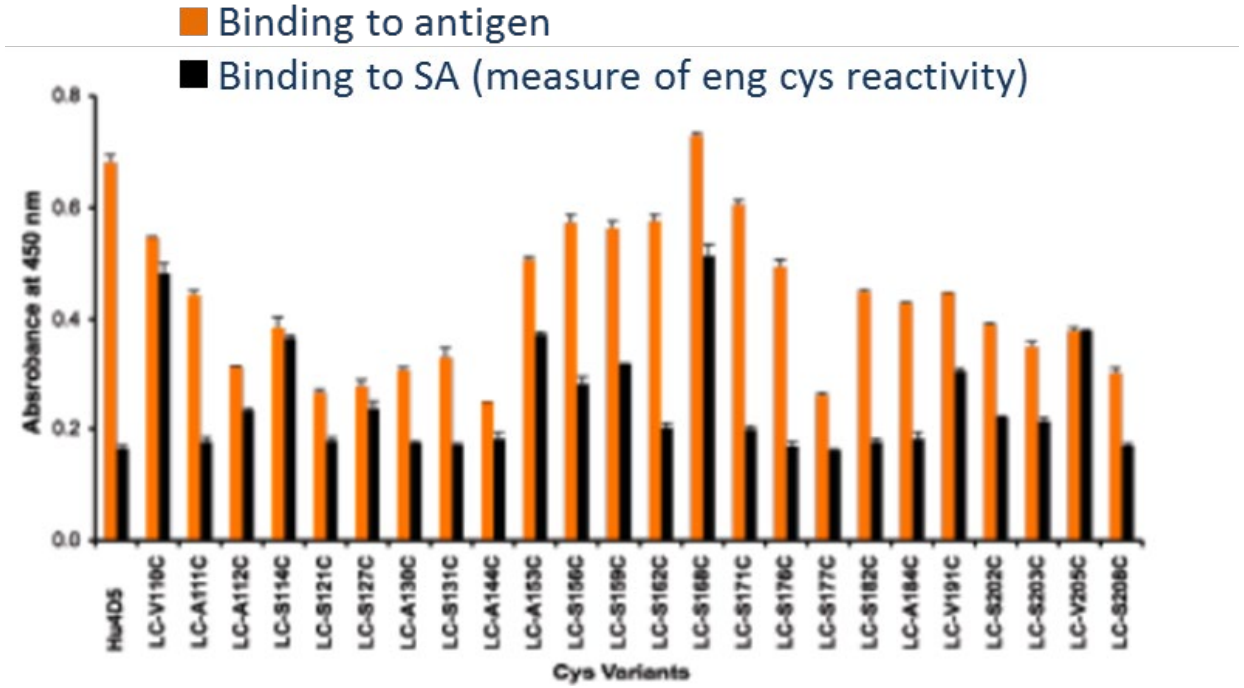
Tumey *et al.,* Bioconjugate Chem., 2014. 25, 1871

ABZENA

Genentech: Attempts to control DAR by engineering cysteines into the antibody sequence, named THIOMABS

- Approach based on engineering-in cysteine residues as conjugation sites
- Location of engineered cysteine residues affects reactivity and antigen binding
- Conjugation require reduction for uncapping of engineered cysteine residue

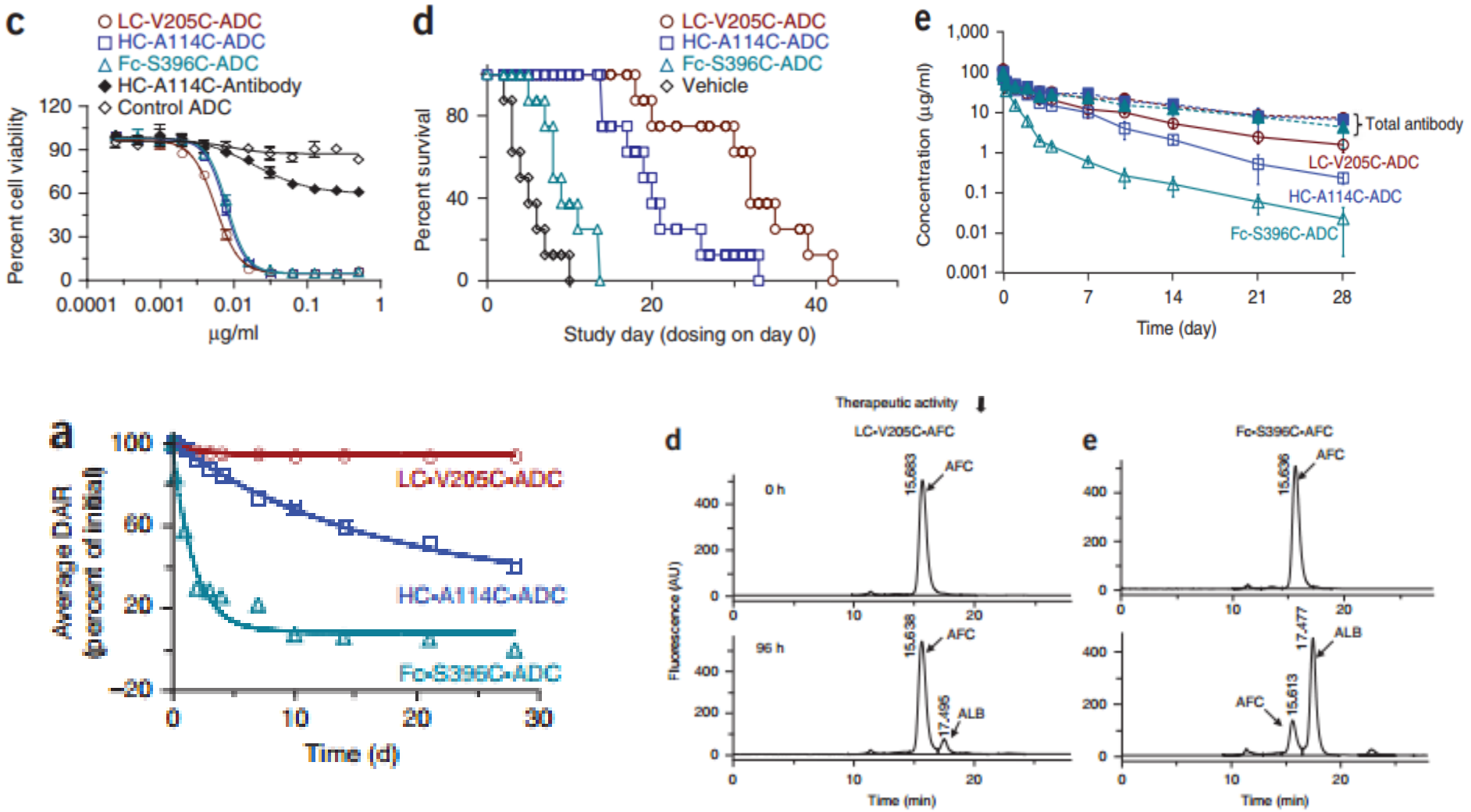




Junutula *et al.,* 2008. Nature Biotechnology 26(8)

ABZENA

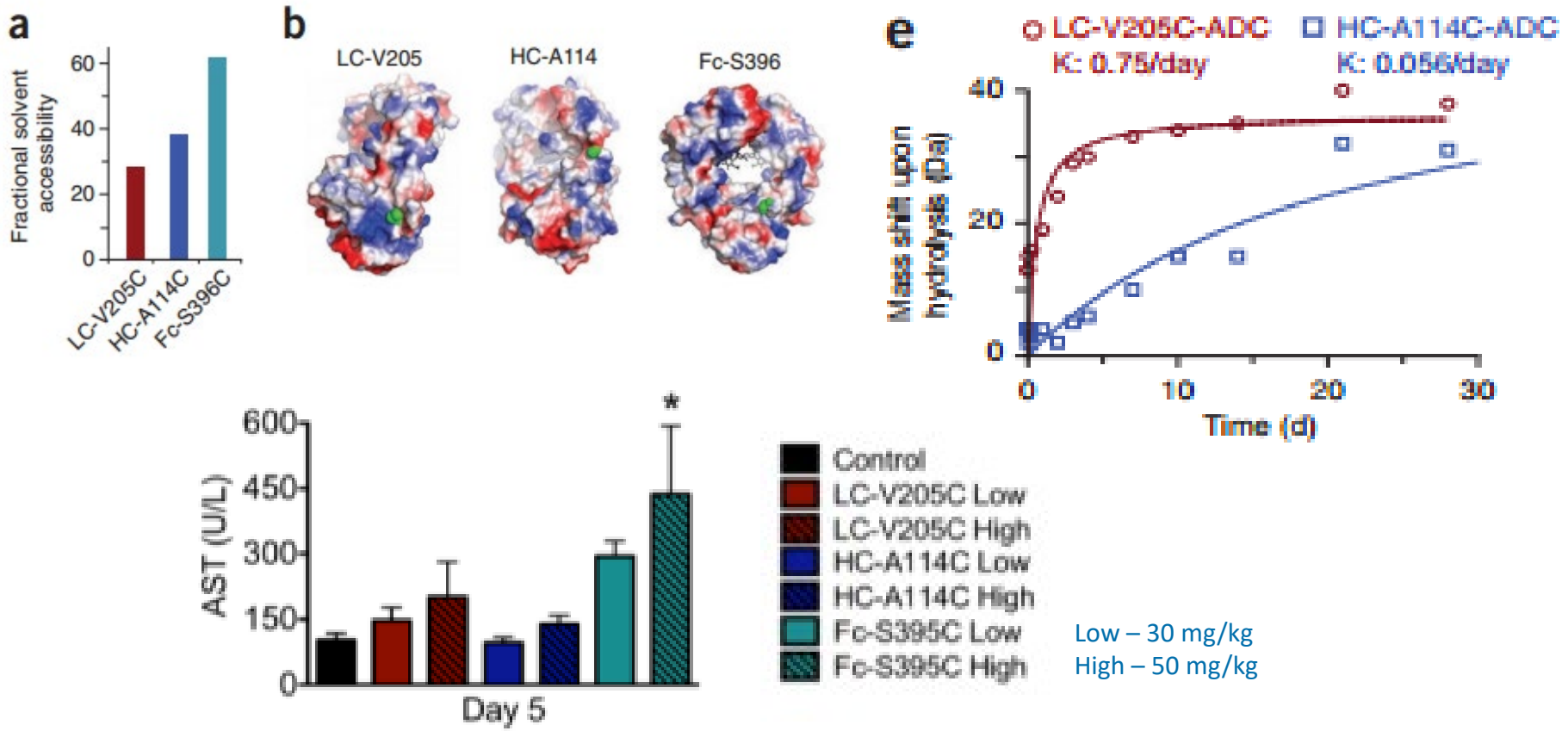
Genentech: Location of the engineered cysteine matters



Shen *et al.*, 2012. Nature biotechnology. 30(2)

ABZENA

Genentech: Location of the engineered cysteine matters



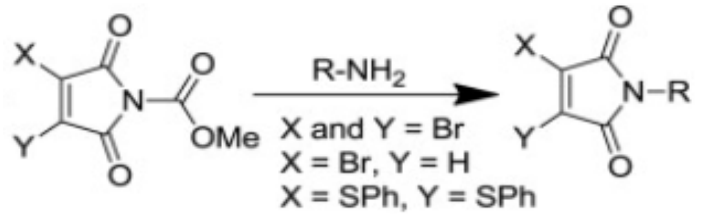
- The rate of succinimide ring hydrolysis and stability of ThioMAB variant impacts the PK, efficacy and tolerability profile of the ThioMAB ADC

Shen *et al.*, 2012. Nature biotechnology. 30(2)

ABZENA

ThioLogics: Disulfide-bridging based on maleimide chemistry

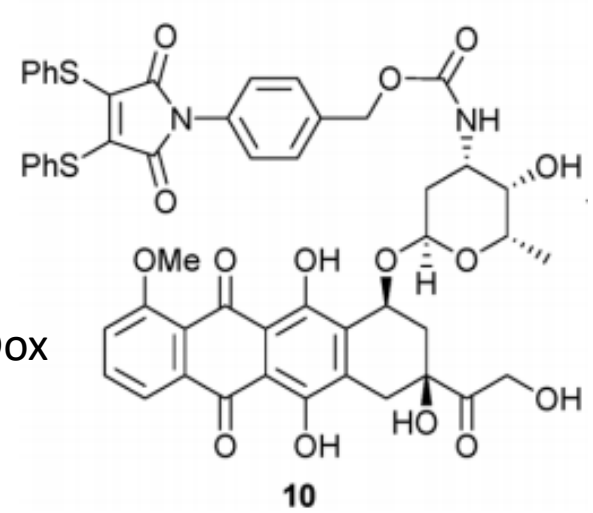
- Disulfide bridging approach
- Bromomaleimides, thiomaleimides
- Maleimide-based chemistry
- No data on efficiency of conjugation, stability, PK, efficacy, safety

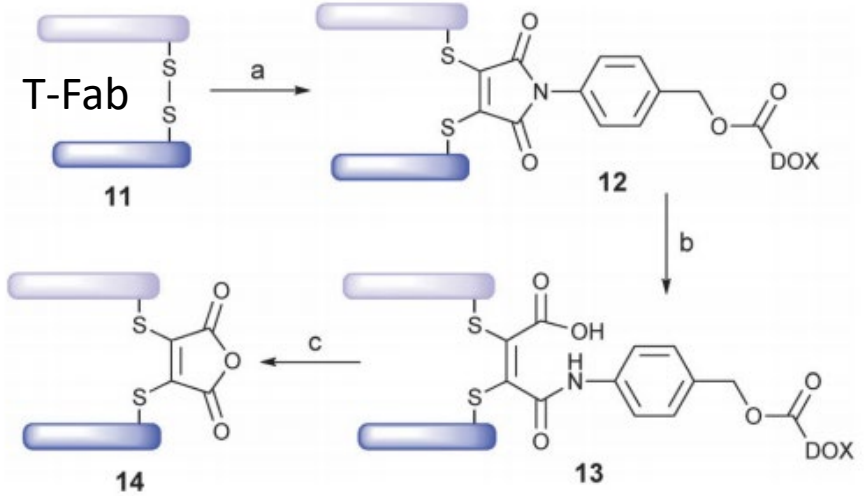


Castaneda *et al.*, 2013. Tetrahedron Letters. 54(27): 3493–3495

Acid-cleavable thiomaleamic acid linker

Dox





Scheme 4 Assembly/cleavage study of Fab ADC **13**: (a) TCEP, pH 8.0, 37 °C, 1.5 h, then **10**, 37 °C, 1 h; (b) pH 7.4, 20 h; (c) pH 4.5, 72 h.

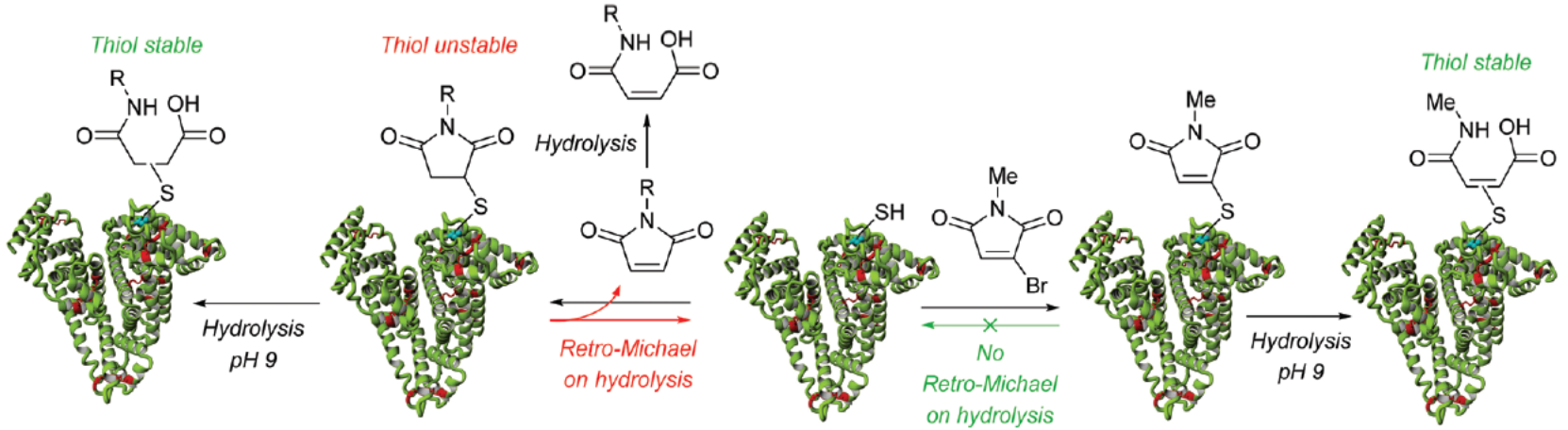
Castaneda *et al.*, 2013. Chem. Commun. 49

ABZENA

ThioLogics: stabilising maleimide linker chemistry through hydrolysis

- Cysteine 34 on albumin reacts with maleimide, however with a bromo leaving group present, this limits the retro-Michael reaction of the conjugate

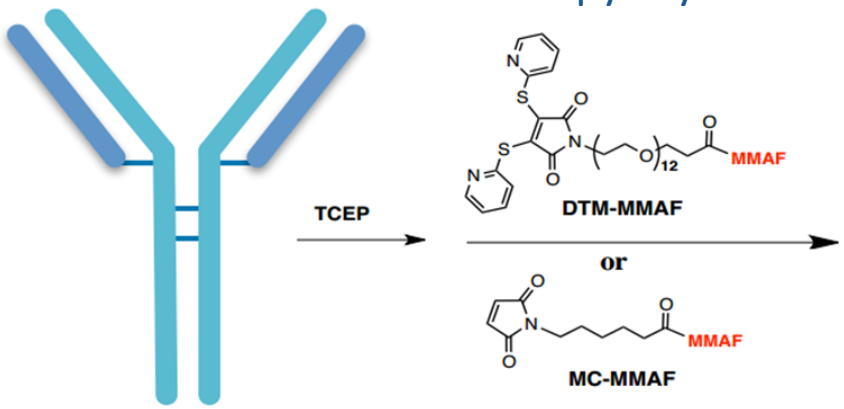
- Hydrolysis is still required but can be performed at basic pH to lock the protein conjugate and limit the de-conjugation reactions



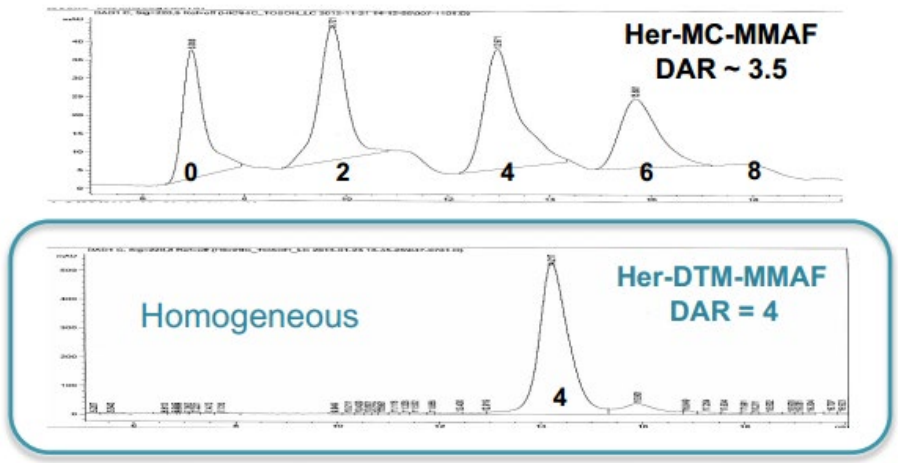
Smith *et al*., Org. and Biomol. Chem. 2013, 49

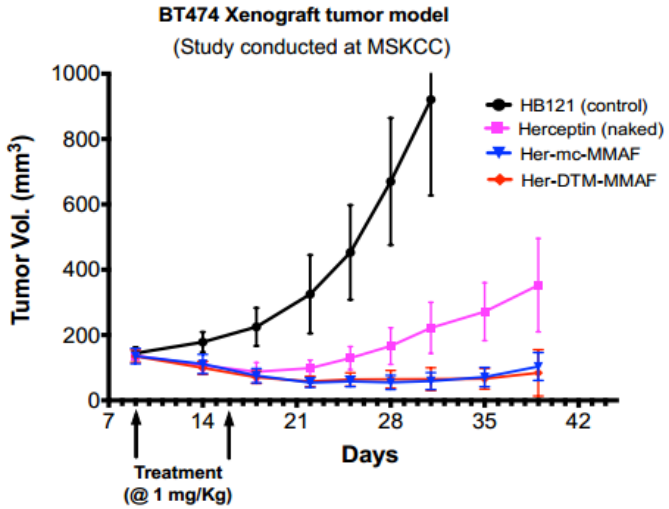
Igenica: Disulfide-bridging based on maleimide chemistry

Dithiopyridylmaleimide (DTM-MMAF)



1) **Her-MC-MMAF** (conventional)
2) **Her-DTM-MMAF** (bifunctional)
3) **HB121-MC-MMAF** (isotype control)

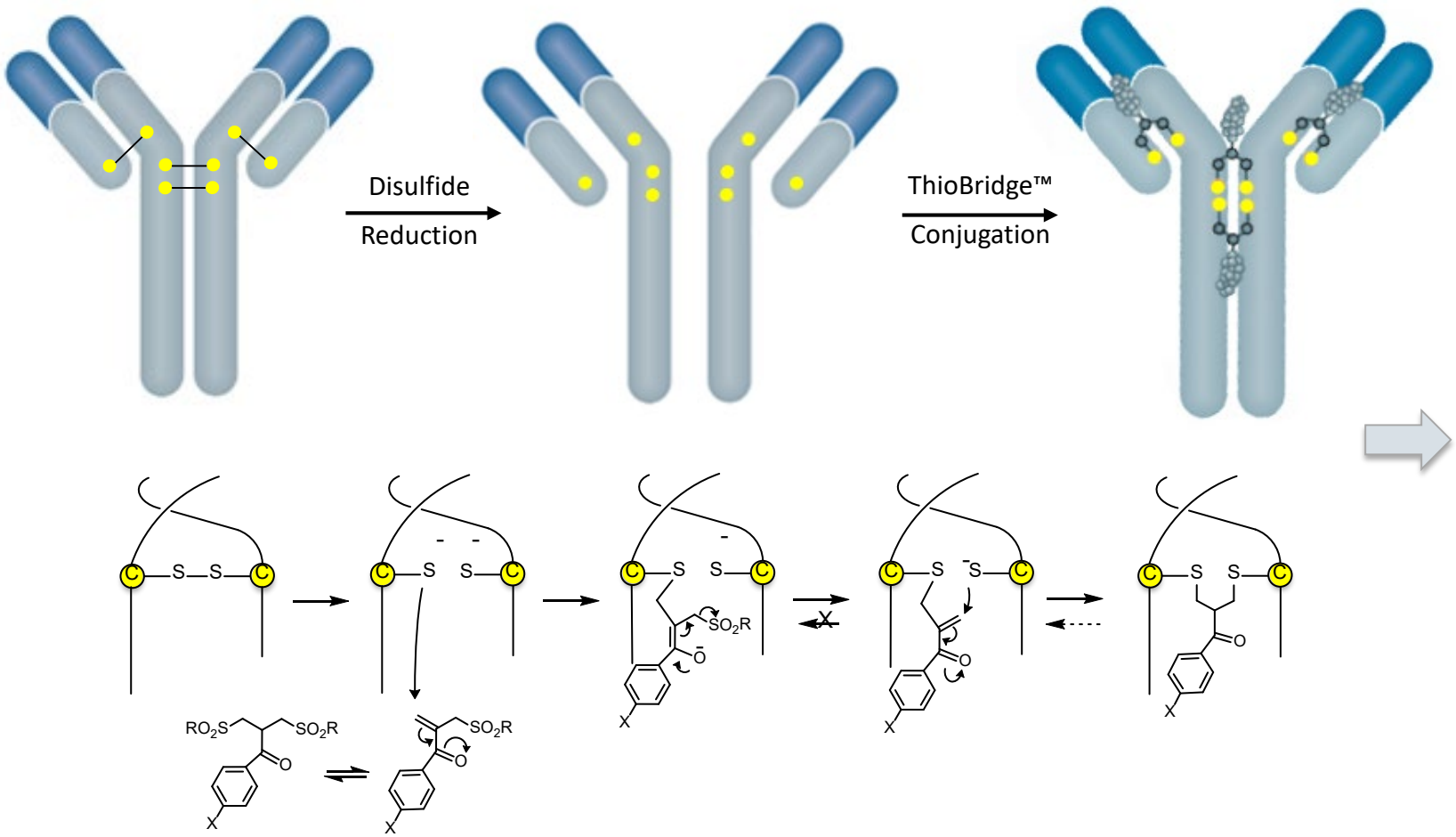


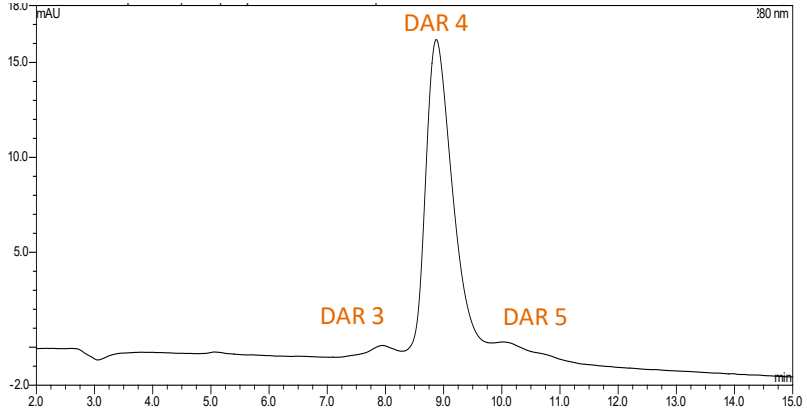


David Jackson, 2013. World ADC World Summit: San Francisco
T. Migone, 2014. World ADC World Summit: Frankfurt

ABZENA

ThioBridge® site specific conjugation at accessible disulfides via an addition/elimination reaction mechanism



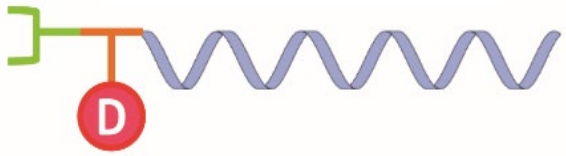


ABZENA

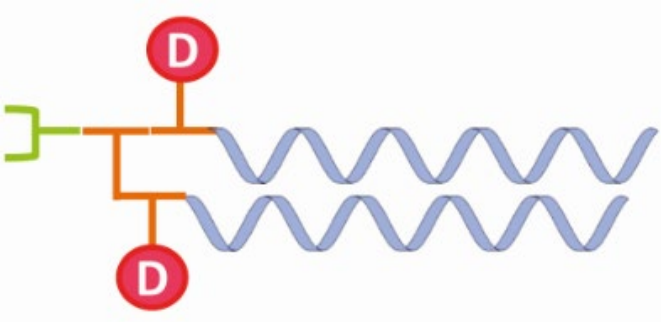
ADCs using the ThioBridge® linker demonstrates architectural design and the impact on ADC success

ThioBridge®
Format 1, DAR 4

ThioBridge®
Format 2, DAR 4

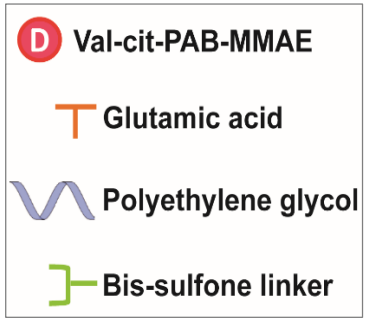


ThioBridge®
Format 5, DAR 8



ThioBridge®
Format 3, DAR 4

ThioBridge®
Format 4, DAR 4



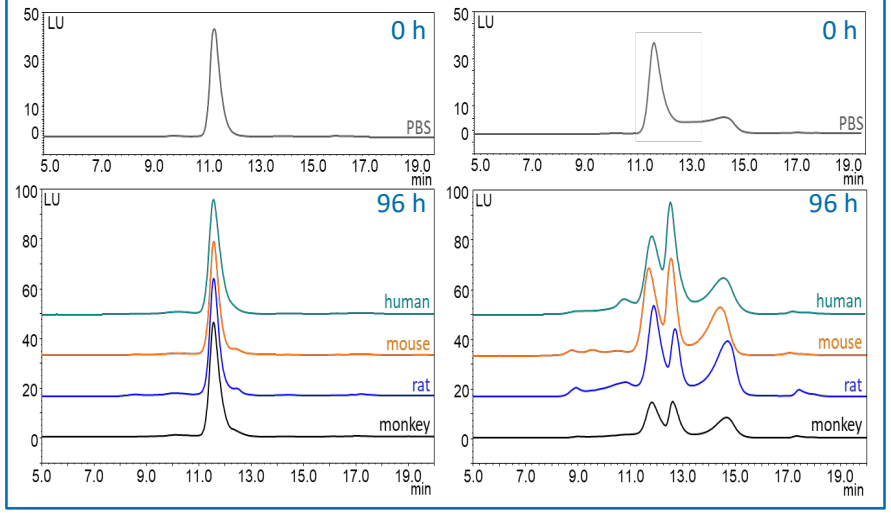
Reagent architecture influences the properties of the ADC

ABZENA

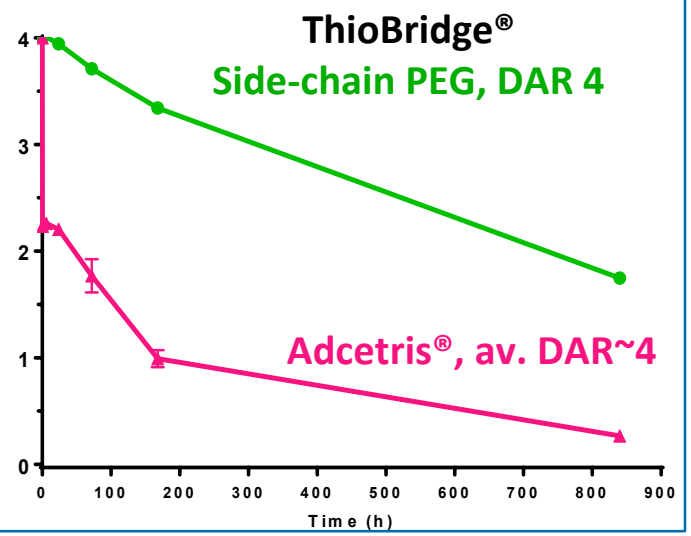
ThioBridge® linker demonstrates high ex vivo and in vivo stability with overall positive impact on ADC efficacy

ThioBridge®
Side-chain PEG, DAR 4

Maleimide conjugate, av. DAR 4

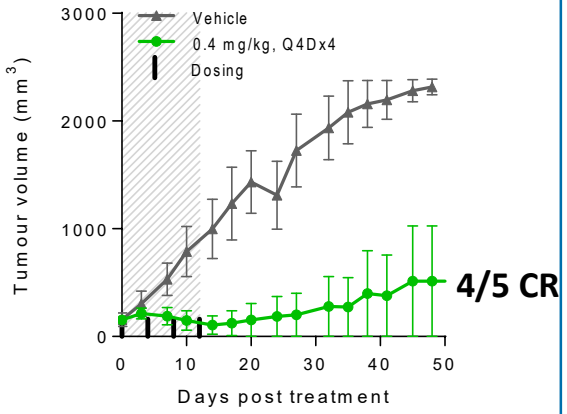


Change in average DAR in PK study (Affinity capture then HIC)

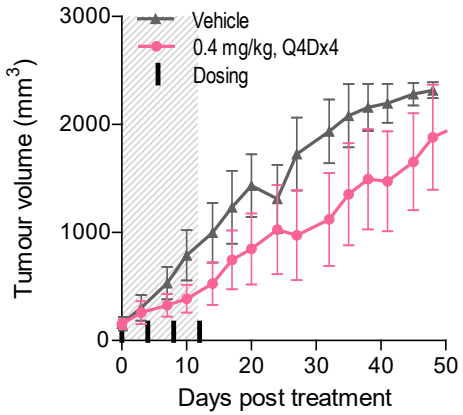


ThioBridge®
Side-chain PEG, DAR 4

ThioBridge®
Side-chain PEG, DAR 4

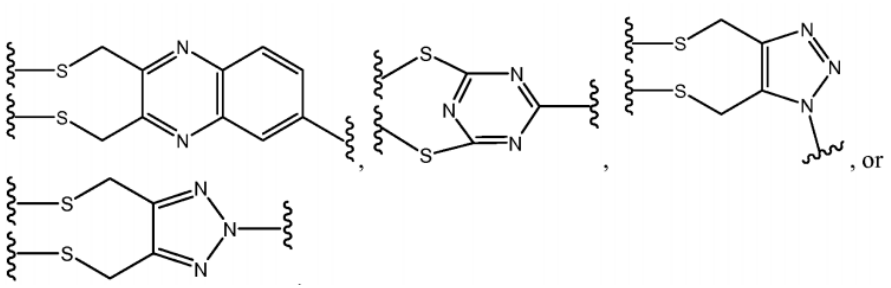


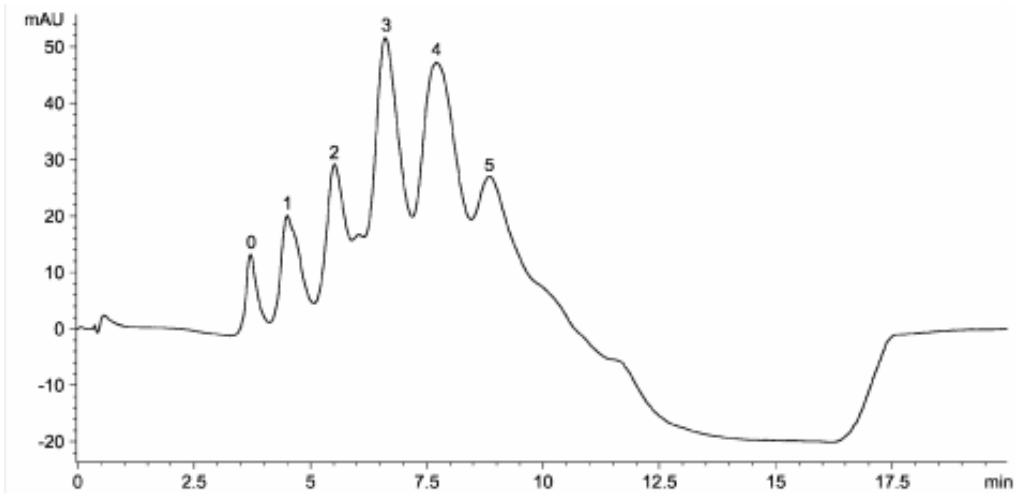
Adcetris®



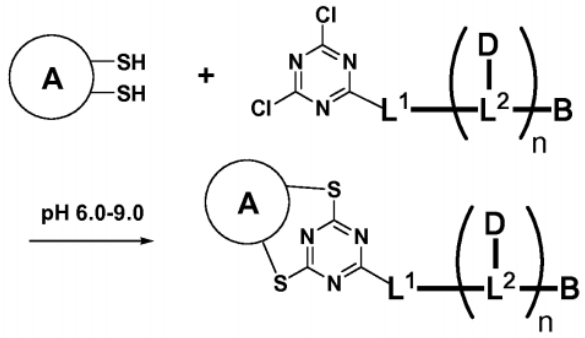
Concortis: C-Lock™ conjugation at interchain disulfides

- Disulfide bridging approach

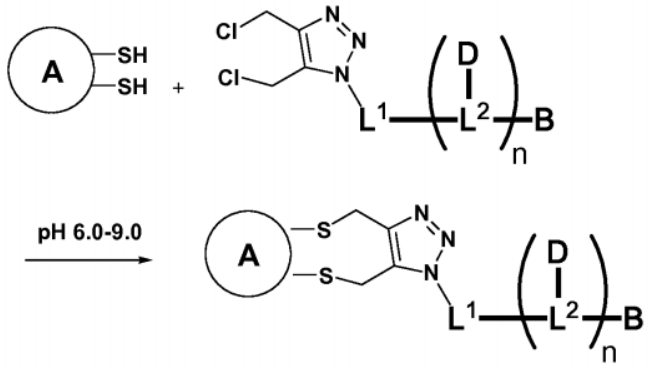




Triazine one step method



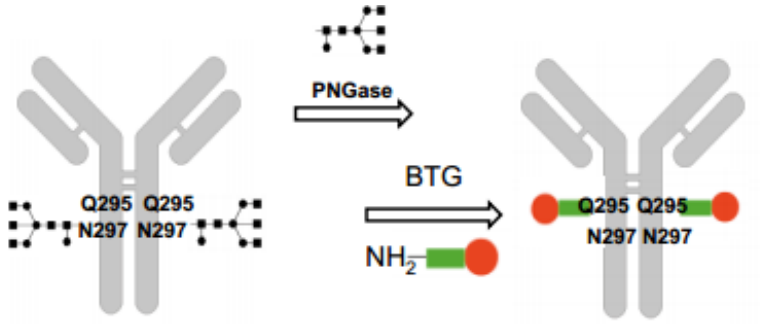
Triazole one step method

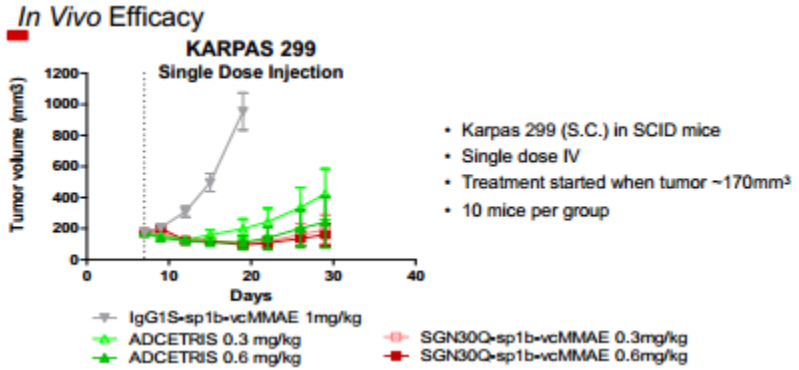


David Miao, Concortis, 2013 World ADC Summit San Francisco
WO 2013/ 173391 A1

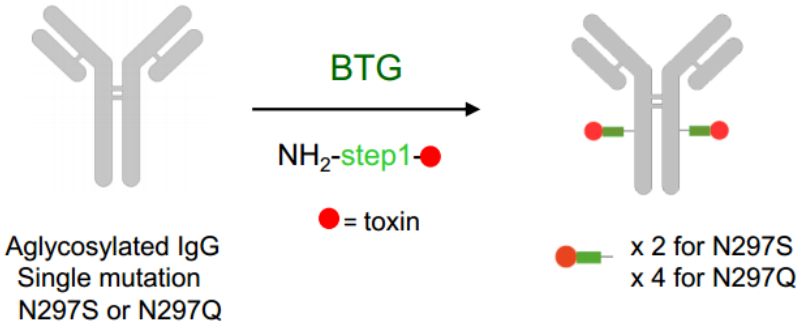
ABZENA

Innate Pharma: Bacterial transglutaminase (BTG) catalysed linker conjugation

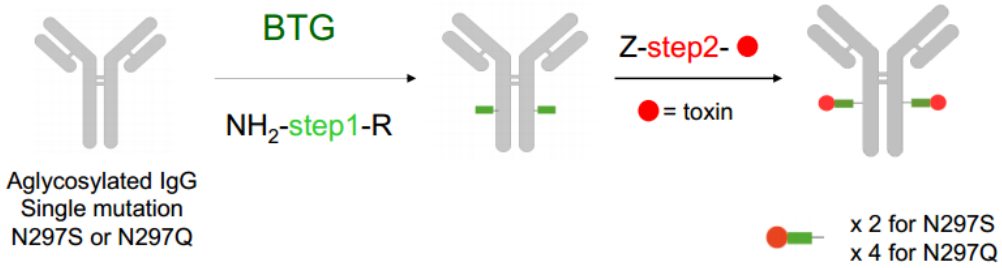




1 step conjugation (20 eq reagent)



2 step conjugation

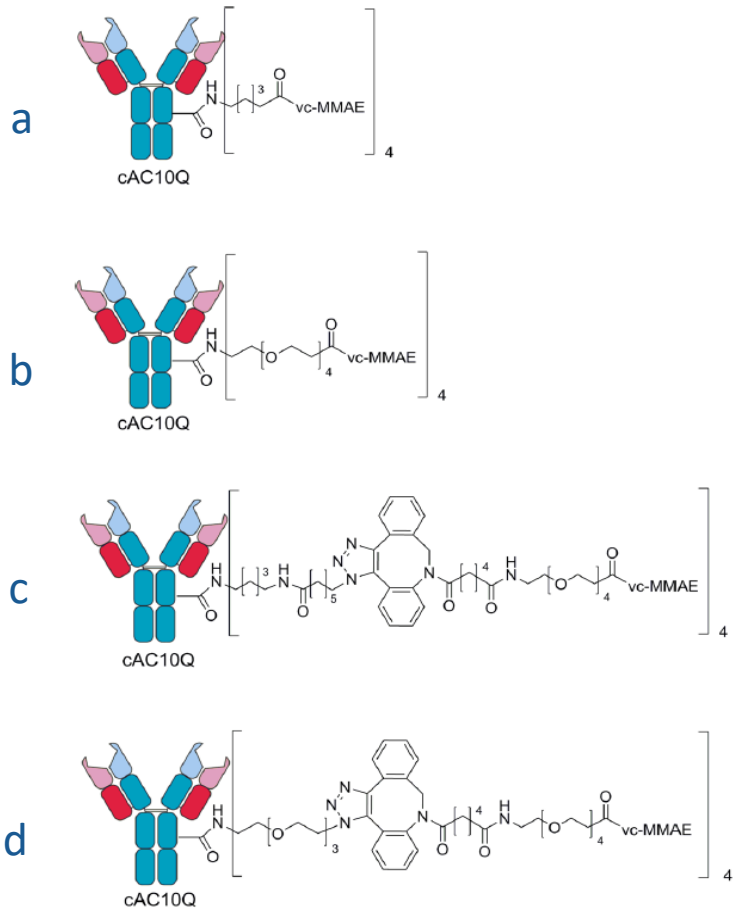


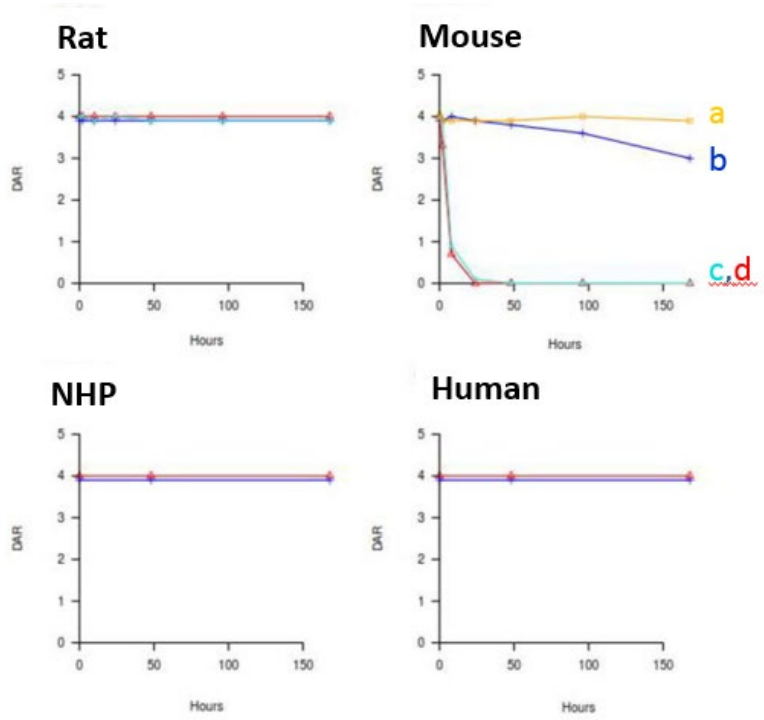
- Requires mAb degycosylation
- Enzyme sensitive to organic solvent concentration
- 1 step process required 20 eq reagent per site
- 2 step process: 10 eq first step / 1.5 – 2 eq second step

L'Hospice *et al*, Mol. Pharmaceutics, 2015, 12, 1872

ABZENA

Innate Pharma: Linker chemistry directly correlates to species-specific stability





- Mouse serum loss of drug was more pronounced with the longer chemical linker

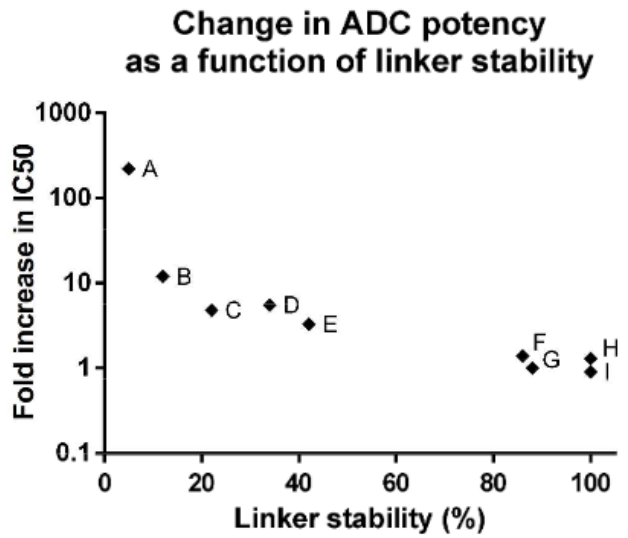
ABZENA

Same linker chemistry but different location is affected by different serum stabilities

- Data from Pavel Strop's group at Rinat have also showed that location of the same cleavable linker demonstrates a range of serum stabilities
- Mouse instability is particularly pronounced and relates to the in vivo stability

Site	Position	Payload	Mouse plasma stability (%)	Rat plasma stability (%)	Cyno plasma stability (%)	Human plasma stability (%)	Mouse in vivo stability (%)
A	LC 200-202	C6-VC-PABC-Aur0101	5	94	99	99	0
B	HC 160	C6-VC-PABC-Aur0101	12	99	100	100	-
C	HC 135	C6-VC-PABC-Aur0101	22	97	98	96	-
D	HC C-terminus	C6-VC-PABC-Aur0101	34	97	99	100	-
E	HC 190-192	C6-VC-PABC-Aur0101	42	97	97	100	0
F	LC C-terminus	C6-VC-PABC-Aur0101	86	99	100	98	-
G	N297A	C6-VC-PABC-Aur0101	88	100	99	100	-
H	N297Q	C6-VC-PABC-Aur0101	100	100	100	100	-
I	HC 294-297	C6-VC-PABC-Aur0101	100	100	100	100	57

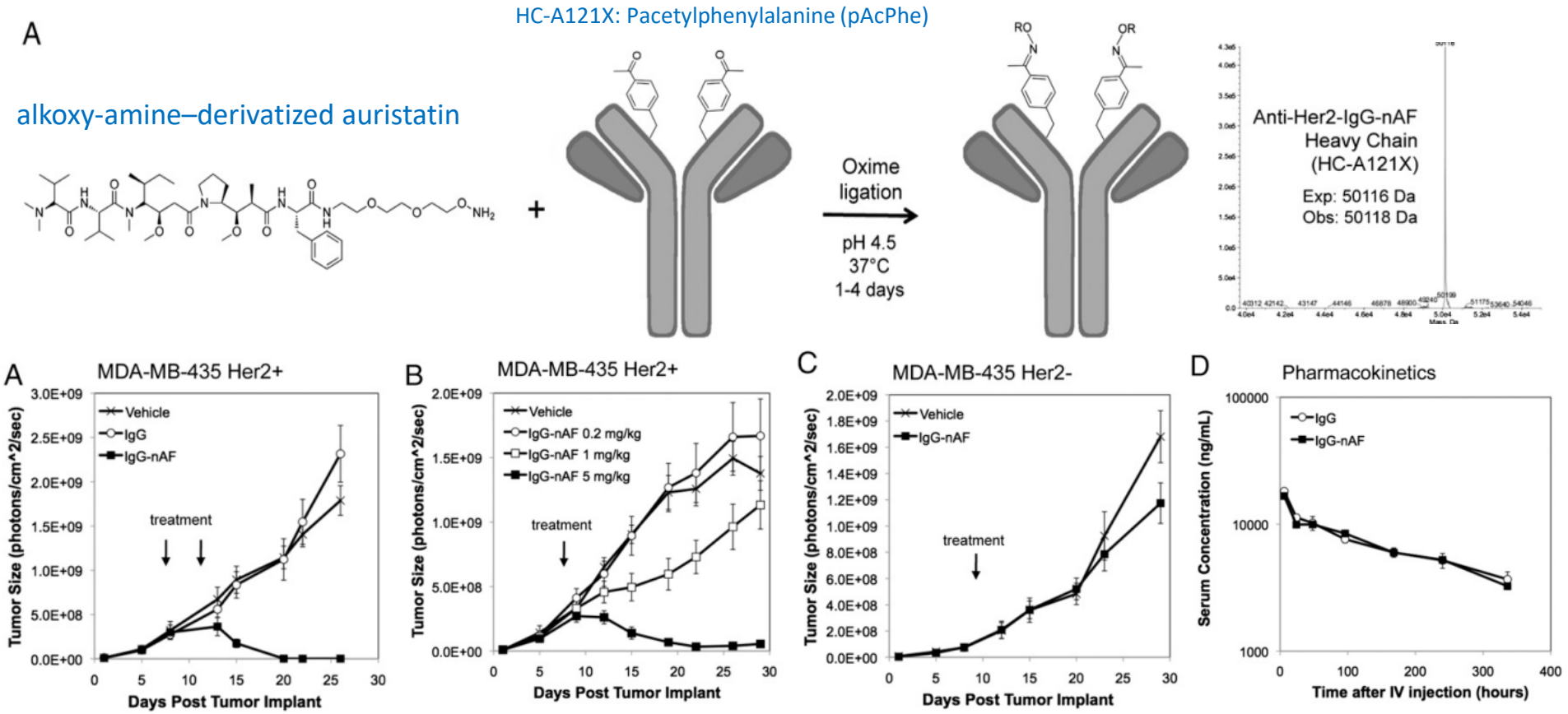
- This qualitative correlation between the linker stability and ADC activity can be plotted



Dorywalska *et al*, Bioconjugate Chem., 2015, 26, 650

ABZENA

Ambrx: Controlling DAR by engineering in non-natural amino acids (NNAAs)



- Fab conjugation: 30-fold excess of reagent, 1-2d at 37°C
- IgG conjugation: 20-fold excess of reagent, 4d at 37°C

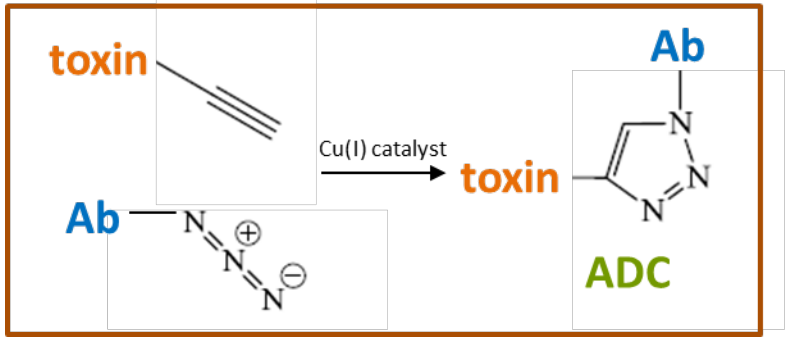
Axup *et al.,* 2012. PNAS 109(40): 16101-16106

ABZENA

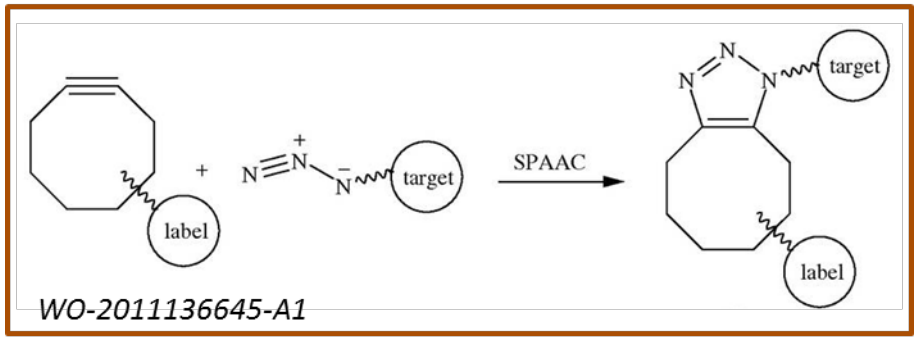
Allozyne: Controlling DAR by engineering in azido non-natural amino acids (NNAAs)

- AzAbs are mAbs with site-specifically engineered azide handles designed for bioconjugation
- Azide handles are incorporated into the Ab in the form of a NNAA at a specific “stop” codon (VIGENÈRE platform)
- Azide-Alkyne “Click” chemistry
- Cycloaddition can be carried out using both metal-catalysed and metal-free reactions

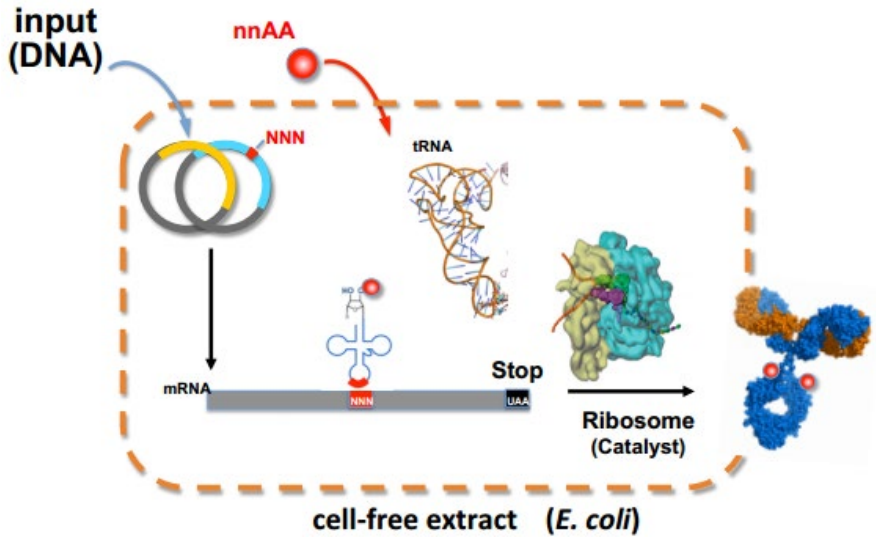
copper-catalysed reaction (CuAAC)

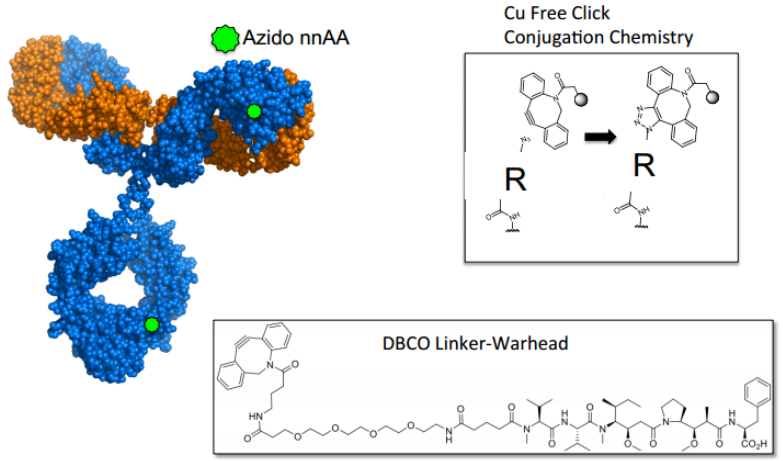


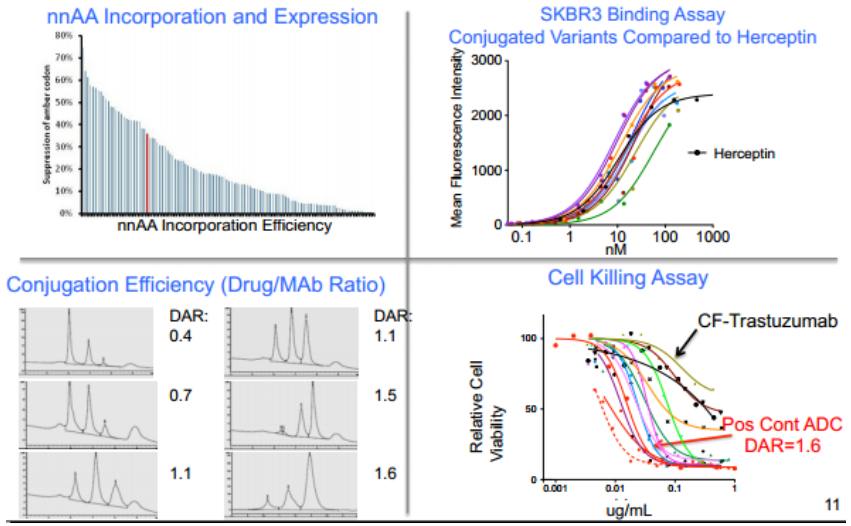
Strain-promoted metal-free reaction (SPAAC)



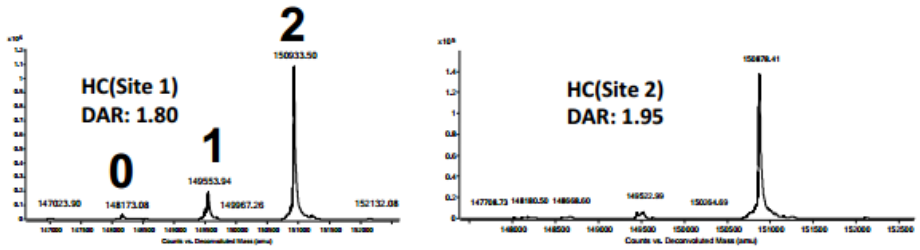
Sutro: Controlling DAR by engineering in azido non-natural amino acids (NNAAs)







Top sites: nearly complete conversion in 4 h, 2.5 molar excess reagent

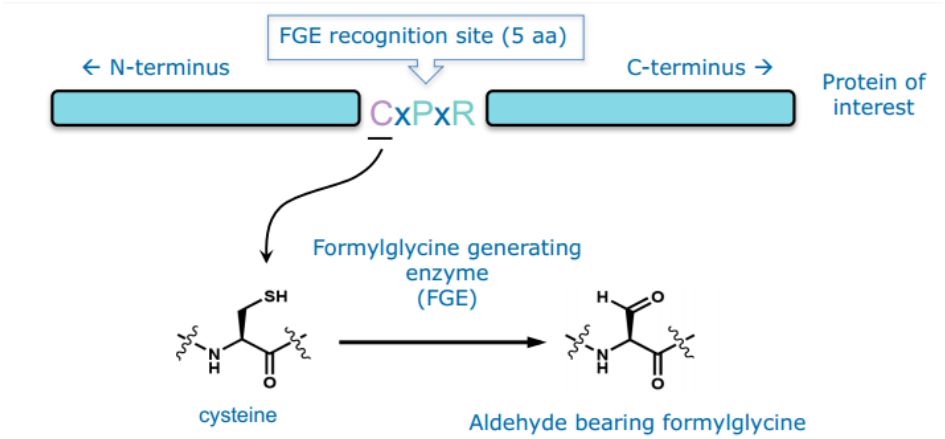


- Specialized expression system
- Lacks mAb glycosylation

Aaron Sato, Sutro, 2014. Bioconjugates San Francisco

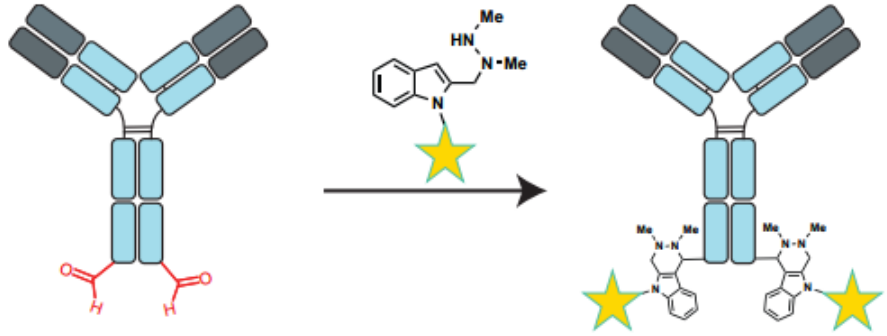
ABZENA

Catalent: Controlling DAR by engineering in enzyme recognition sequence - SMARTag™ Technology

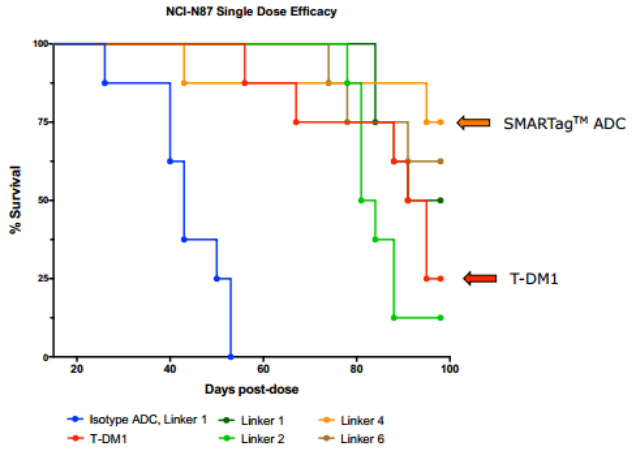


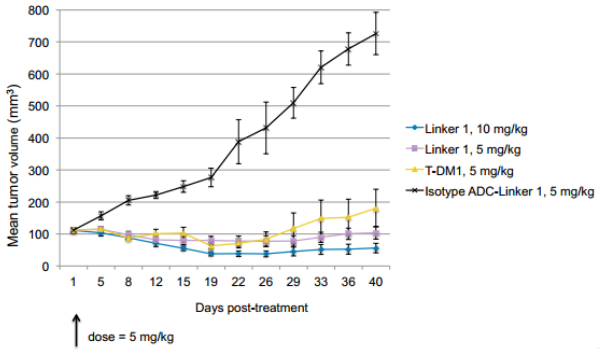
FGE is highly selective for Cys in CxPxR sequence to generate an aldehyde tag (SMARTag™)

Pictet-Spengler ligation



C-C bond Formation Using Proprietary HIPS Chemistry

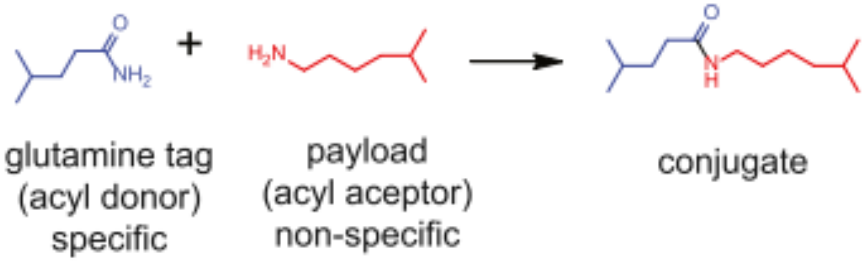


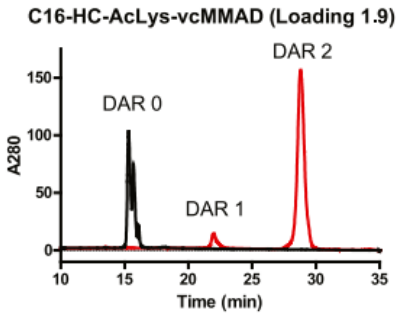


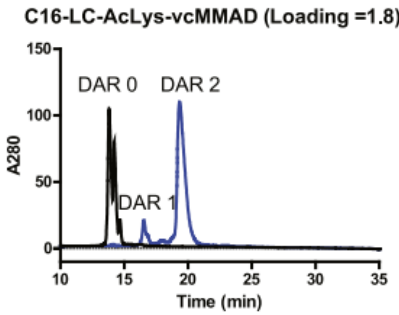
David Rabuka, Redwood / Catalent, 2014. World ADC Summit Frankfurt

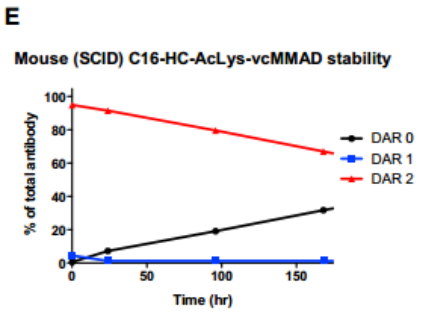
ABZENA

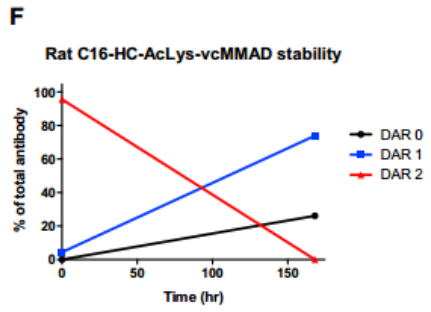
Pfizer: Controlling DAR by engineering in transglutaminase (TG) recognition sequence – LLQG Tag

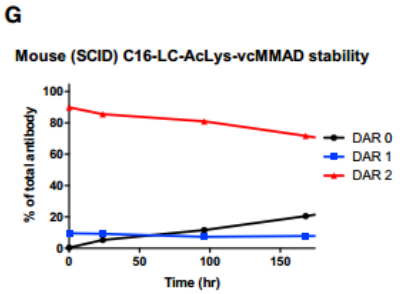


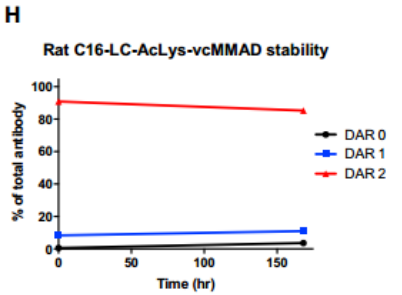










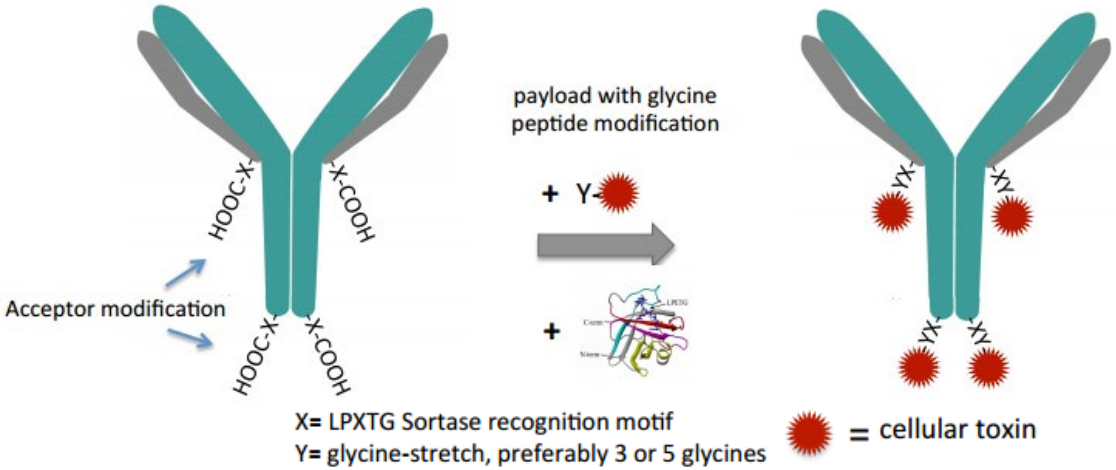


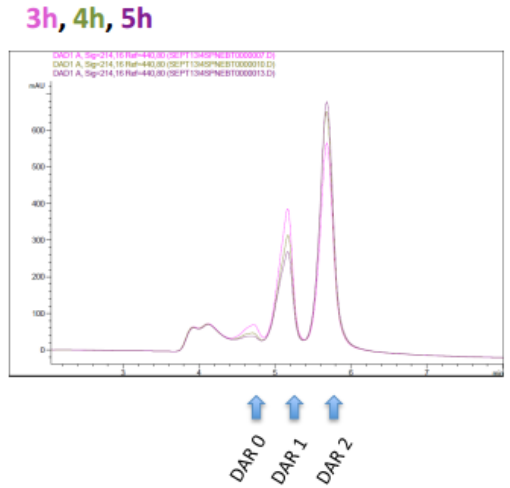
- Efficiency of conjugation and stability depends on the location of the LLQG tag (vc-PAB instability)
- 5-10 fold-excess of reagent
- Enzyme sensitive to organic solvent – challenging for hydrophobic drugs

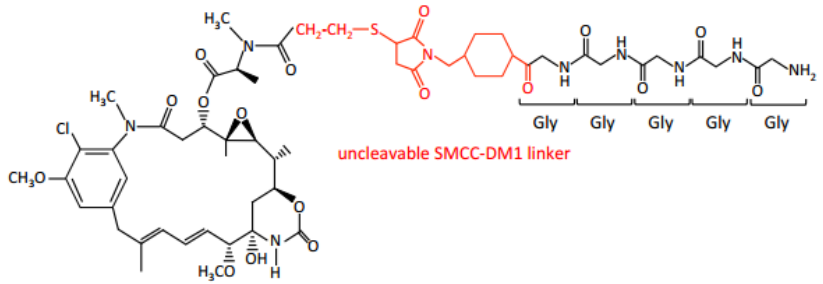
Strop et *al.*, 2013. Chemistry&Biology 20:161-167

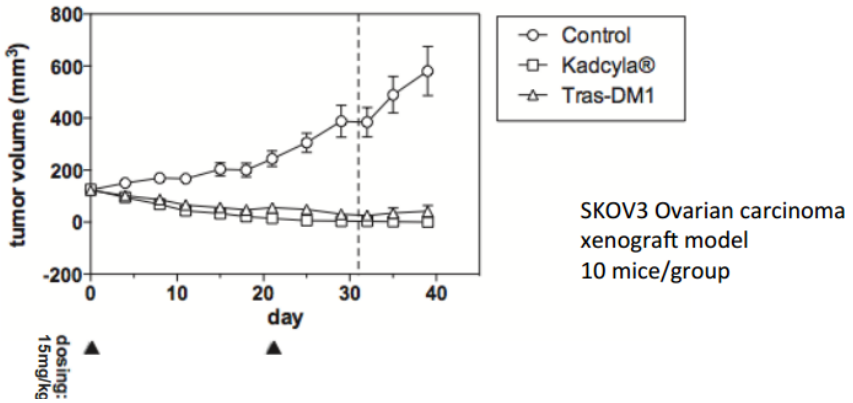
ABZENA

NBE Therapeutics: SMAC™ Technology (Sortase Mediated Antibody Conjugation)









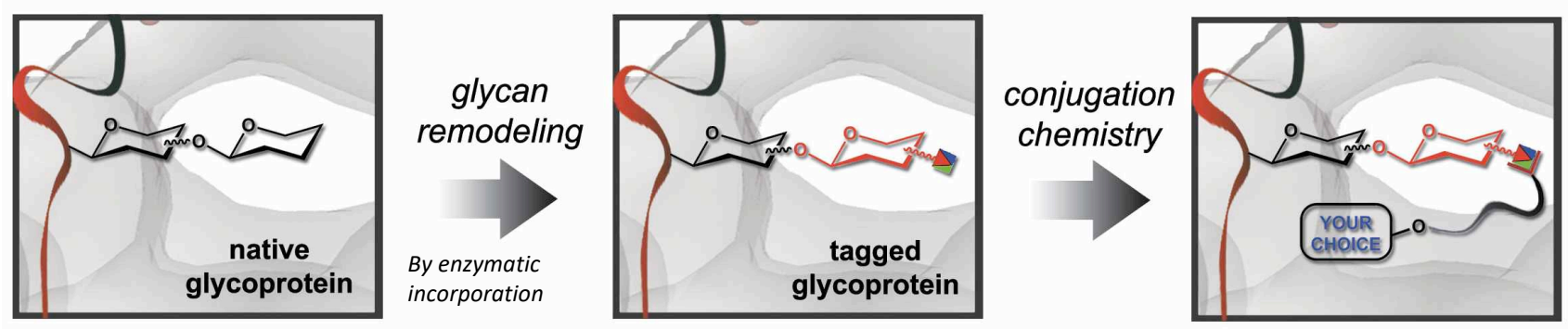
SKOV3 Ovarian carcinoma
xenograft model
10 mice/group

- Ligation between C-terminal LPXTG sortase B recognition motif and glycin-stretch of peptide modified payload
- Requires engineering

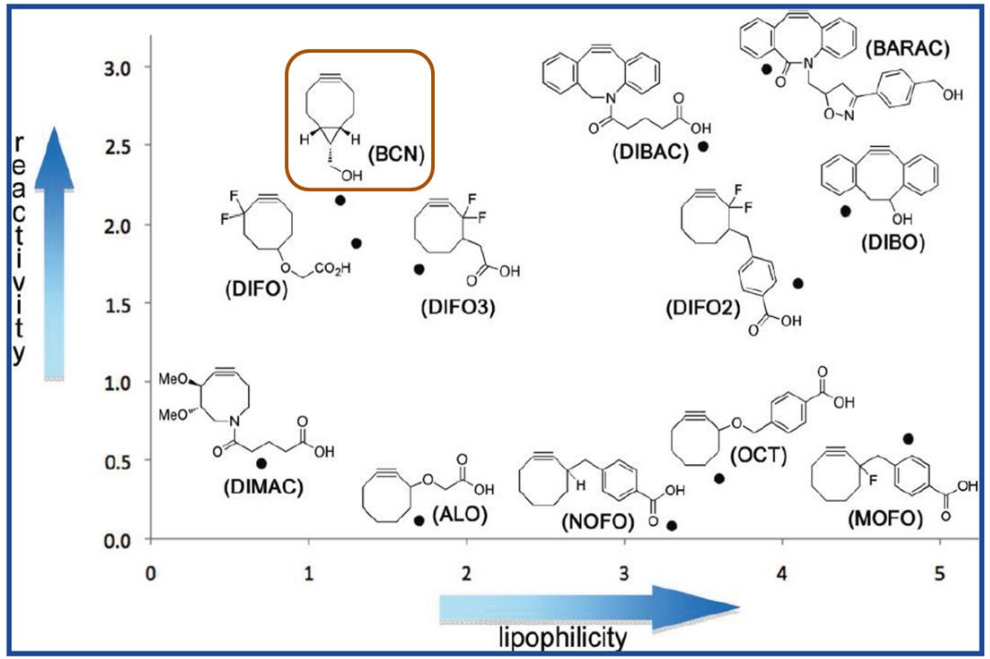
Ulf Gewunder, NBE Therapeutics, 2014. PEGS Boston

ABZENA

SynAffix: Enzyme-catalysed post-recombinant glycan remodelling followed by chemical conjugation



- Post-recombinant mAb modification
- Two steps of conjugation required:
  - Enzymatic tagging (conserved glycan in the Fc fragment) or engineering of one (or more) alternative glycosylation site(s)
  - Chemical conjugation:
  - Strain-promoted alkyne-azide cycloaddition (SPAAC) between cyclooctyne and azide.
  - BCN is 100-fold more reactive than plain cyclooctyne

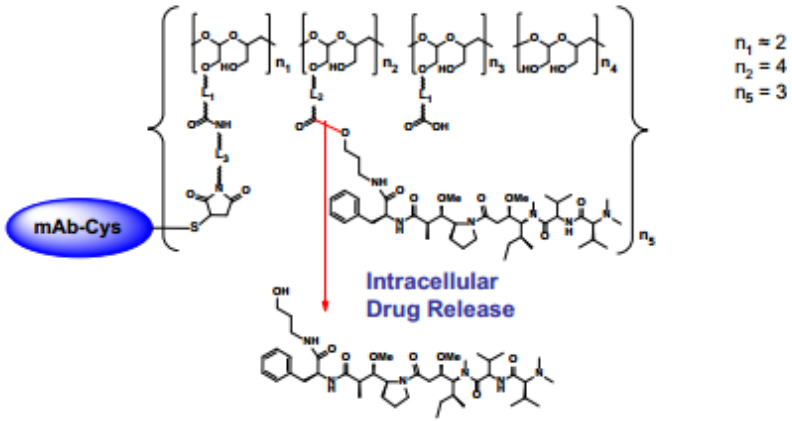


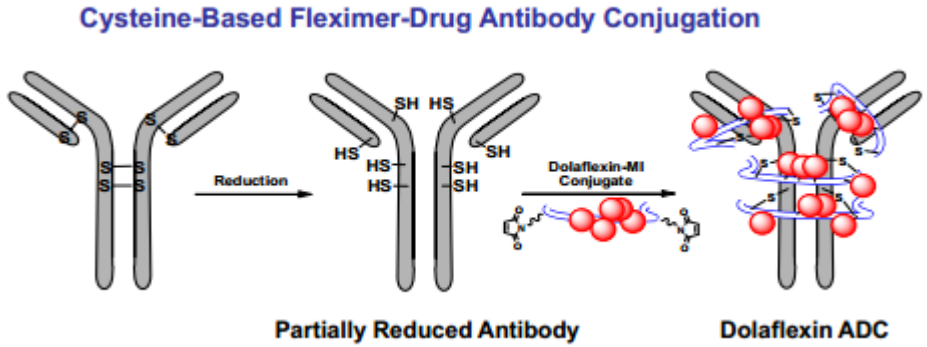
Debets *et al.*, Acc. of Chem. Res., 2011. 44, 805

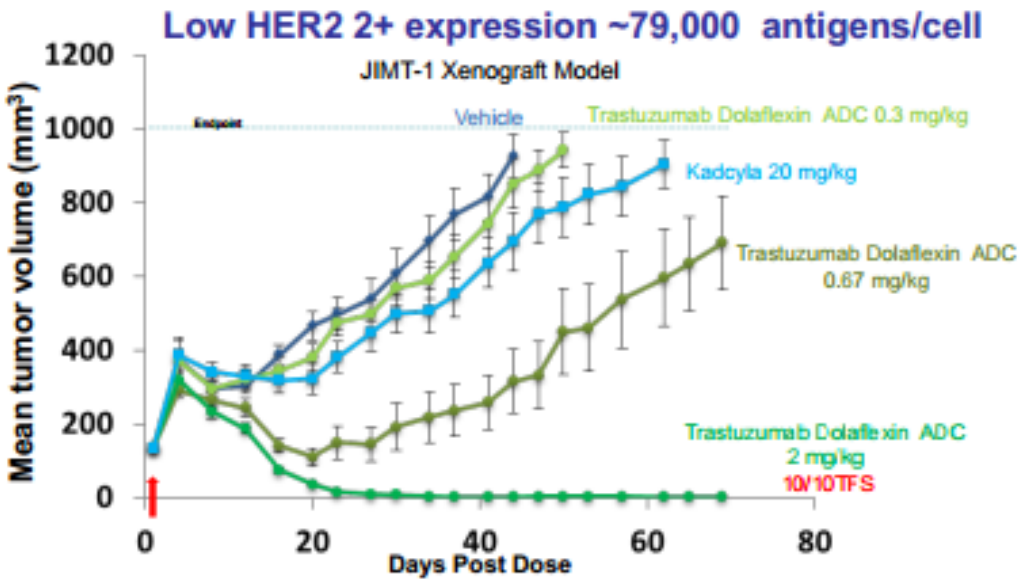
ABZENA

Mersana: Fleximer™ technology for high DAR ADC production

Dolaflexin ADC Structure and Drug Release





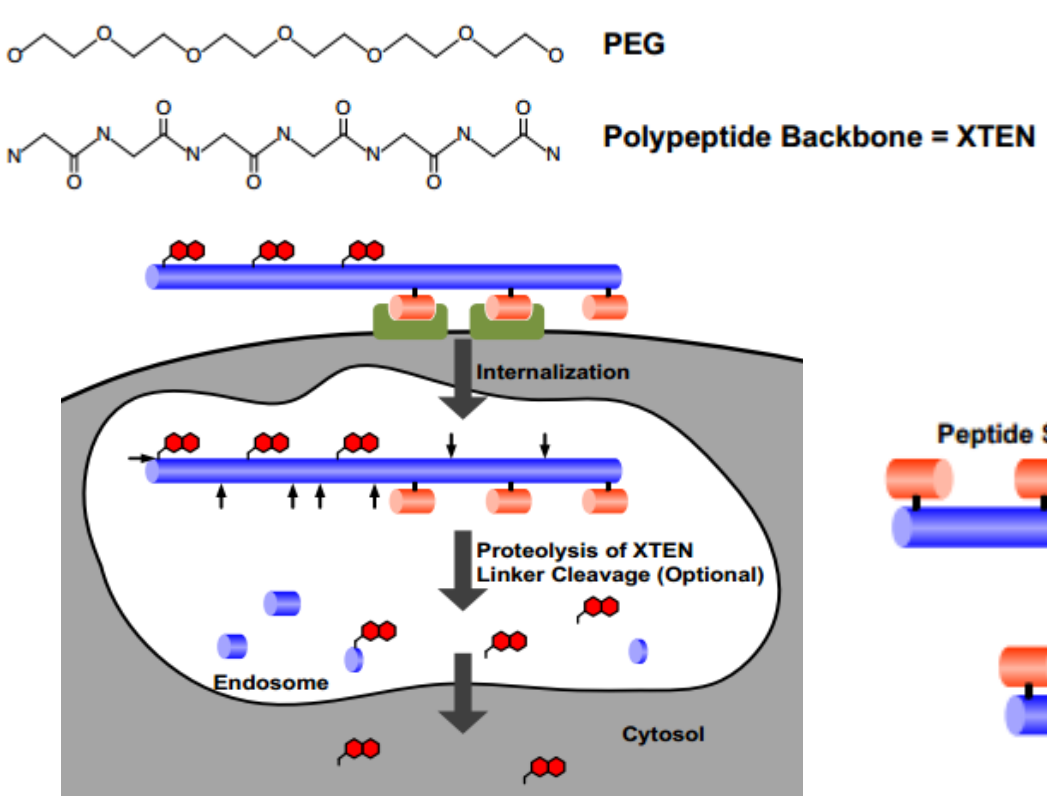


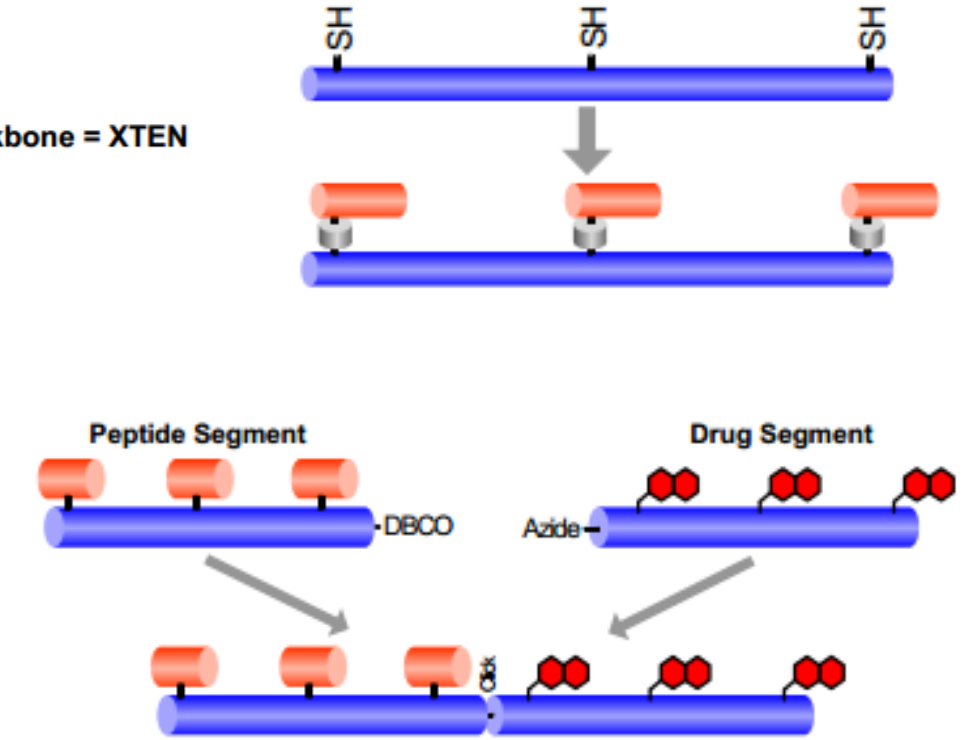
- Doloflexin – 10 kDa Fleximer
- 6-8 drugs per Fleximer
- 3-4 Fleximer chains per antibody
- DAR ~20
- Asana ASN004 (DAR15) advancing into the clinic

Yurkovetskiy et al., 2014. Poster #2645, AACR San Diego

ABZENA

Amunix: XTEN™ technology for high DAR ADC production



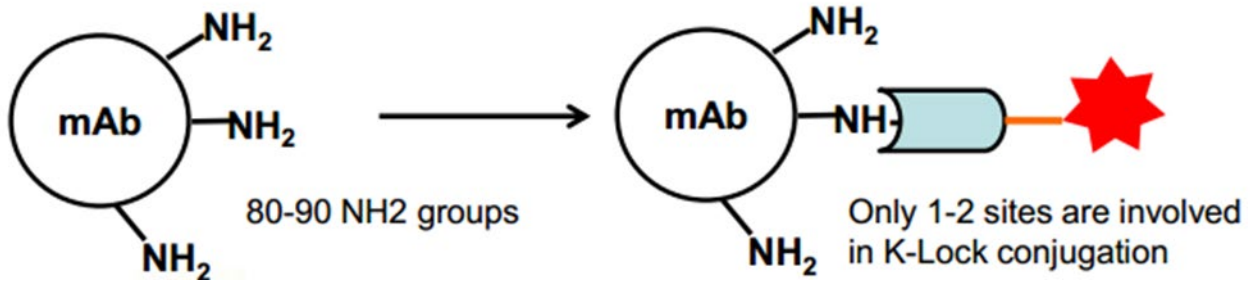


- Genetically encoded polypeptide that mimics the polymer properties of PEG
- Can be engineered to incorporate payload attachment sites
- XTEN polymer produced in E. coli

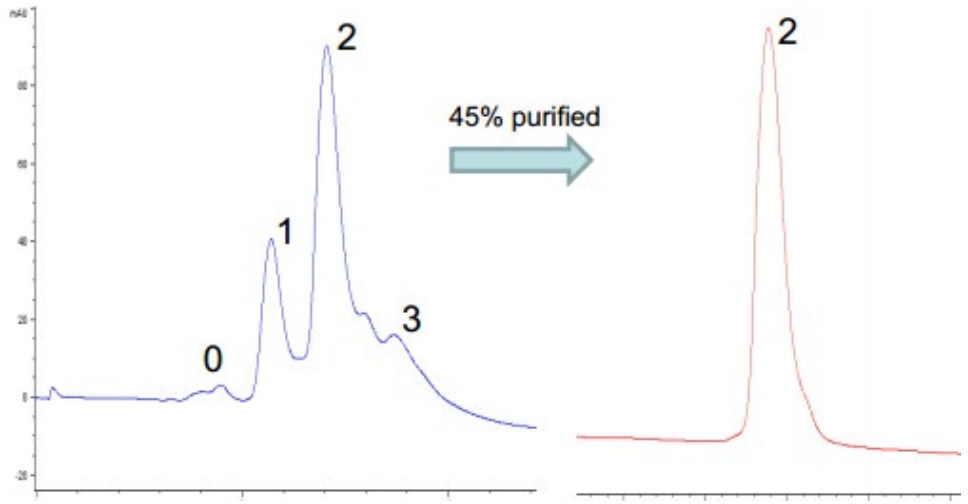
Volker Schellenberger, Amunix. 2013. PEGS Boston

ABZENA

Concortis' K-Lock™ lysine conjugation



- Claims to take advantage of the microenvironmental differences of each lysine side chain (PKa, solvent exposure, hydrophobic pockets)
- No information on efficiency of conjugation, stability, PK, in vivo efficacy

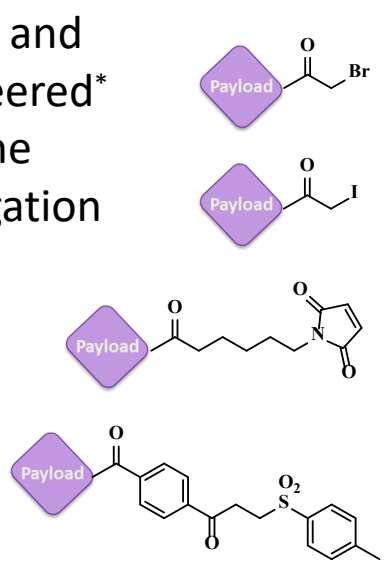


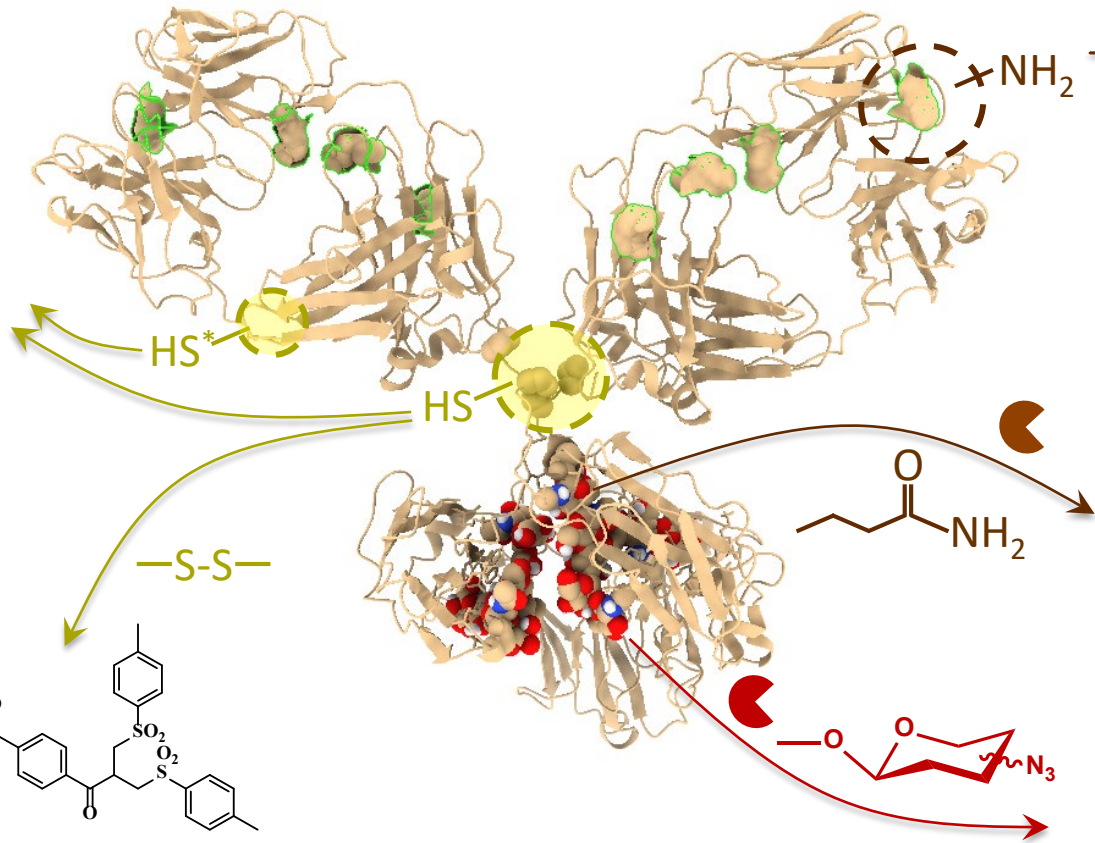
Source: David Miao, Concortis, World ADC Summit San Francisco, October 16, 2013

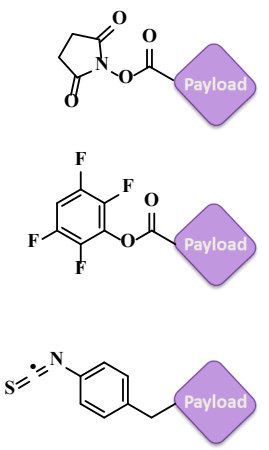
ABZENA

Multiple conjugation options can be evaluated to identify suitable functional attachment as part of the design stage

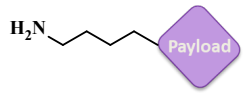
Native and engineered* cysteine conjugation





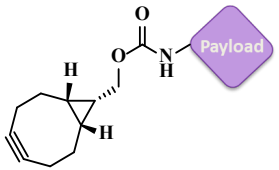


Native lysine conjugation



Transglutaminase conjugation

Native disulfide conjugation
ThioBridge®

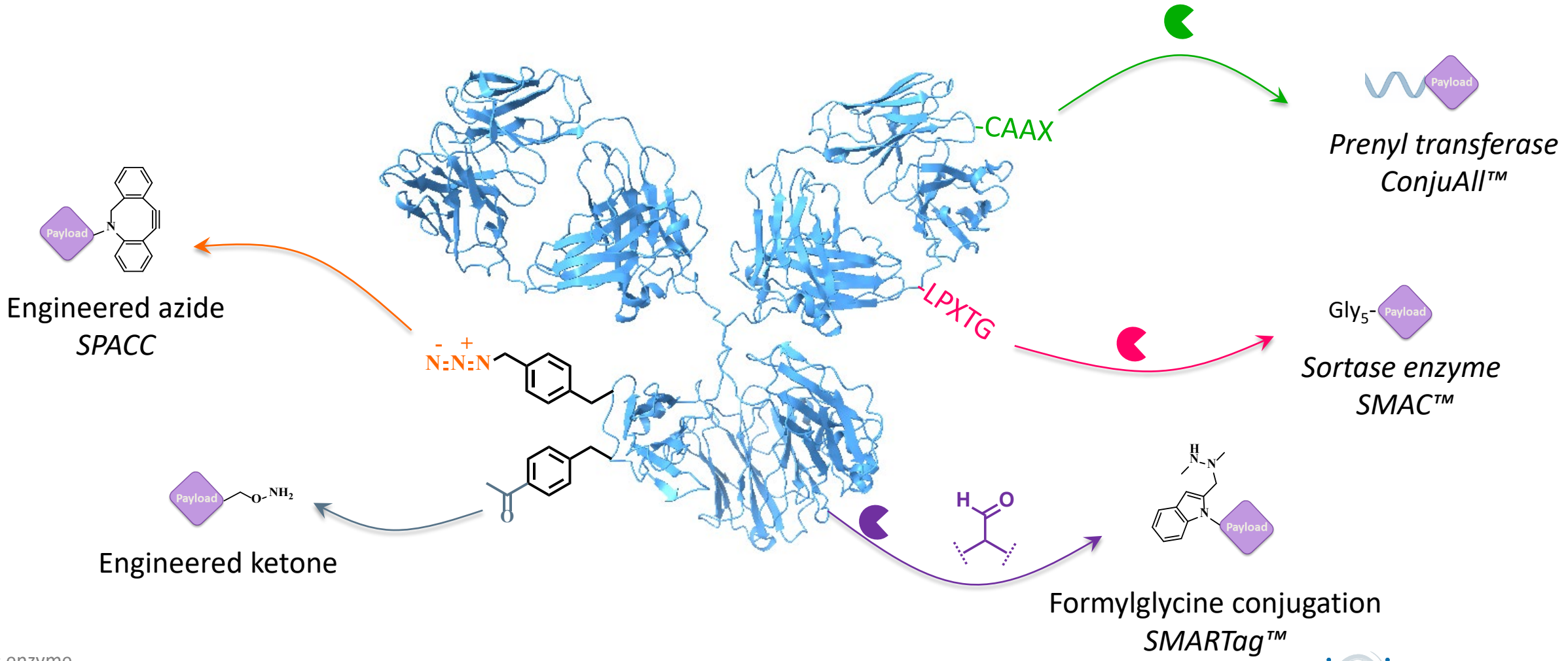


Glycan conjugation
GlycoConnect™

= enzyme

ABZENA

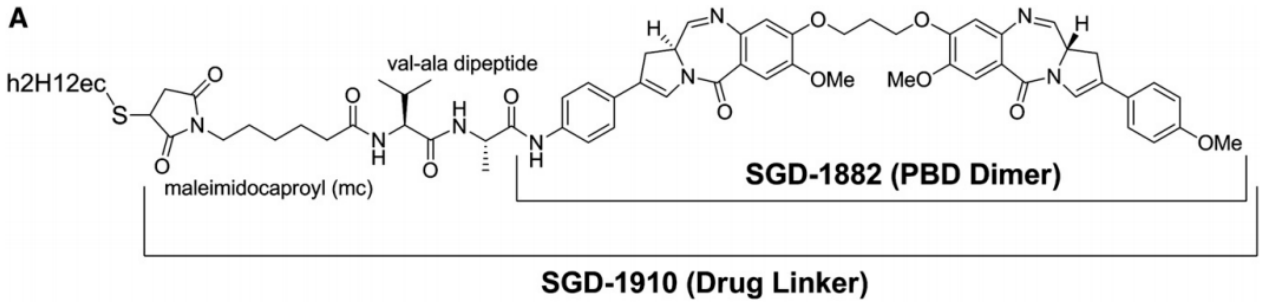
Multiple conjugation options can be evaluated to identify suitable functional attachment as part of the design stage

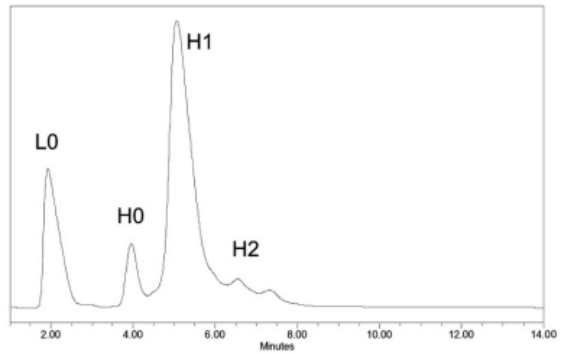


= enzyme

ABZENA

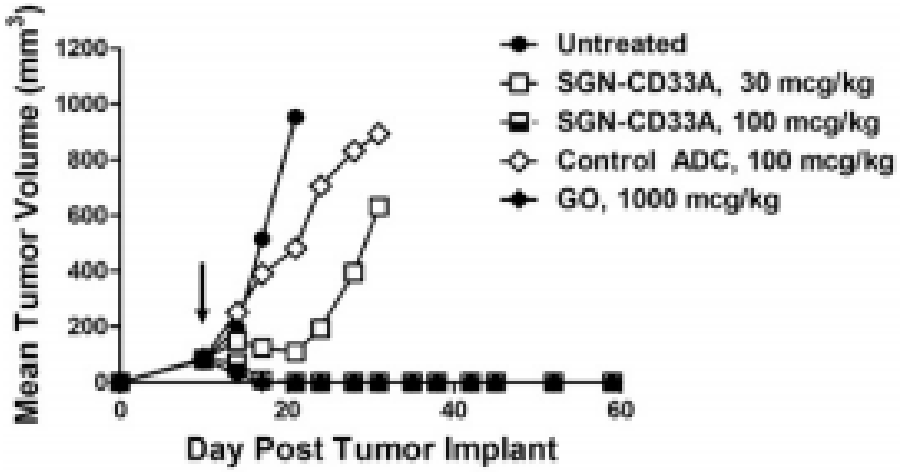
SGN-CD33A: Engineered cysteine with Spirogen's PBD payload



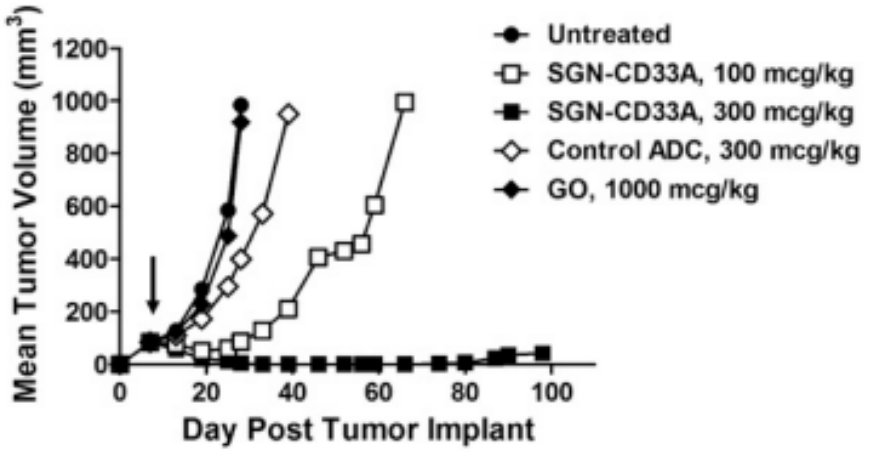


SGN-CD33A – average of 1.9 drugs / antibody

MDR^{-} HL-60



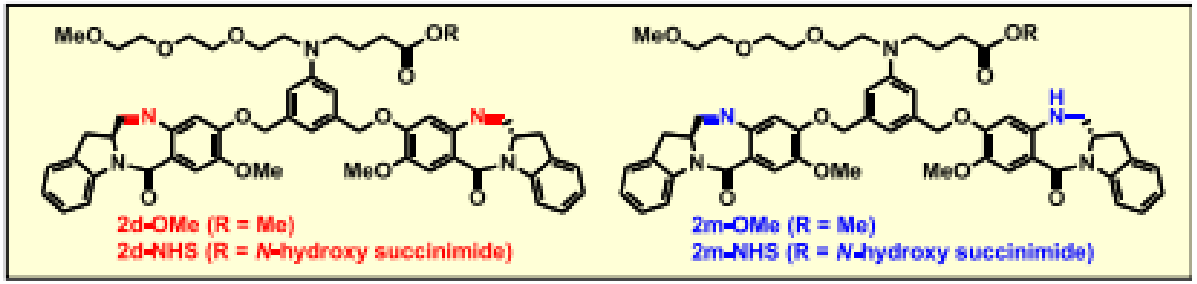
MDR^{+} TF1-alpha



Sutherland et al., 2013. Blood. 122(8):1455-1463

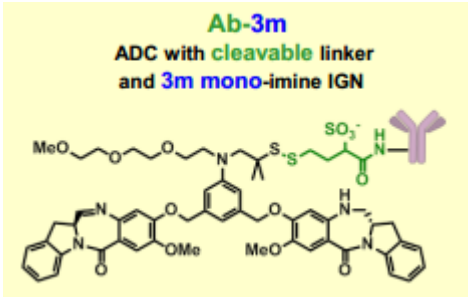
ABZENA

Immunogen: Indolinobenzodiazepine dimers

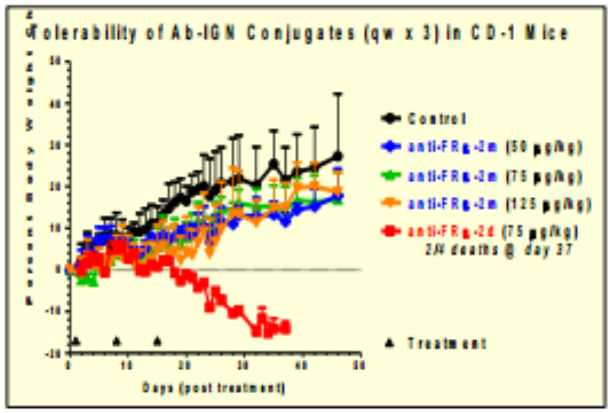


2d (di-imine) Both allkylates and cross-links DNA

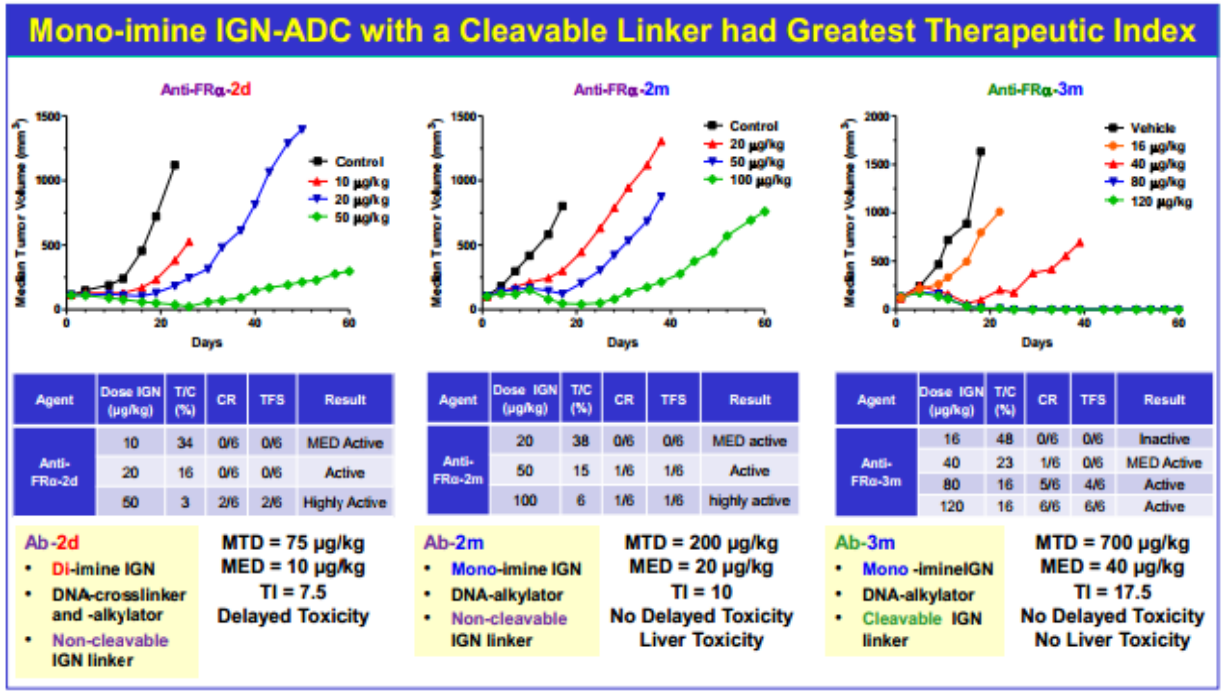
2m (mono-imine) Only alkylates DNA Non-cleavable



3m (mono-imine) Only alkylates DNA Cleavable



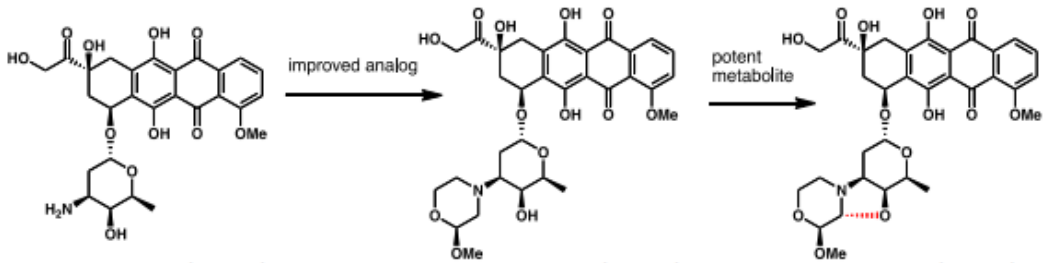
- Delayed toxicity observed for 2d but not for 2m
- Cleavable linker had greatest TI



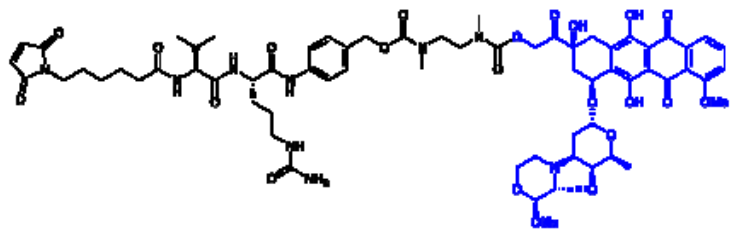
Miller et al., 2013. #C160. AACR-NCI-EORTC
Whiteman et al., 2013. #C161. AACR-NCI-EORTC

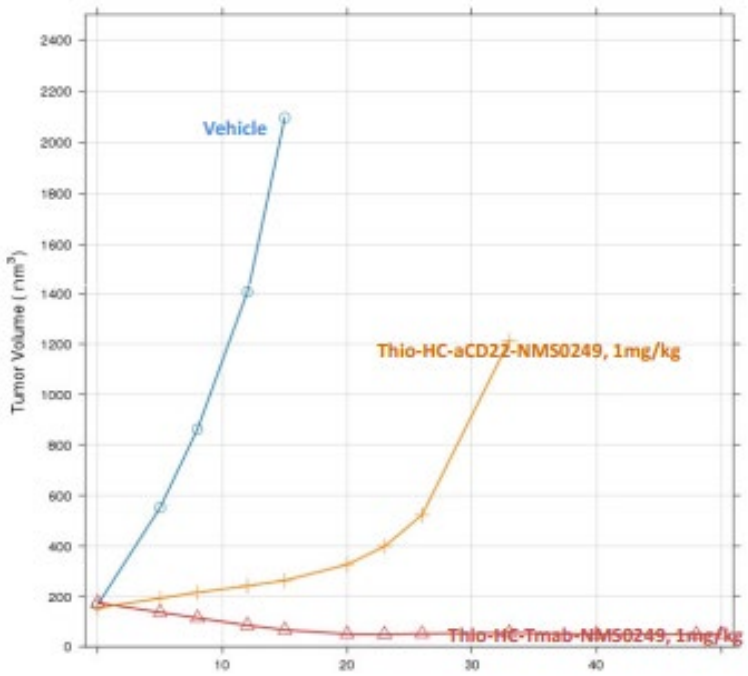
ABZENA

Nerviano Medical Sciences / Genentech: PNU-159682



NMS249
(MC-VC-PAB-EDA-PNU159682)



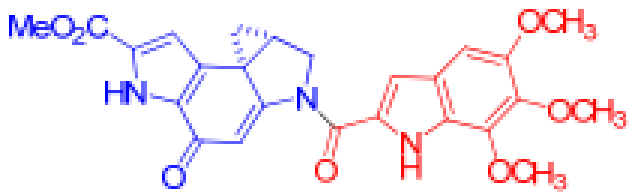


- Metabolite of nemorubicin
- Picomolar potency
- Thio-HC-Tmab-NMS249 efficacious at 1 mg/kg in Fo5 mammary tumour transplant model

Thomas Pillow, Genentech, 2014. PEGS Boston

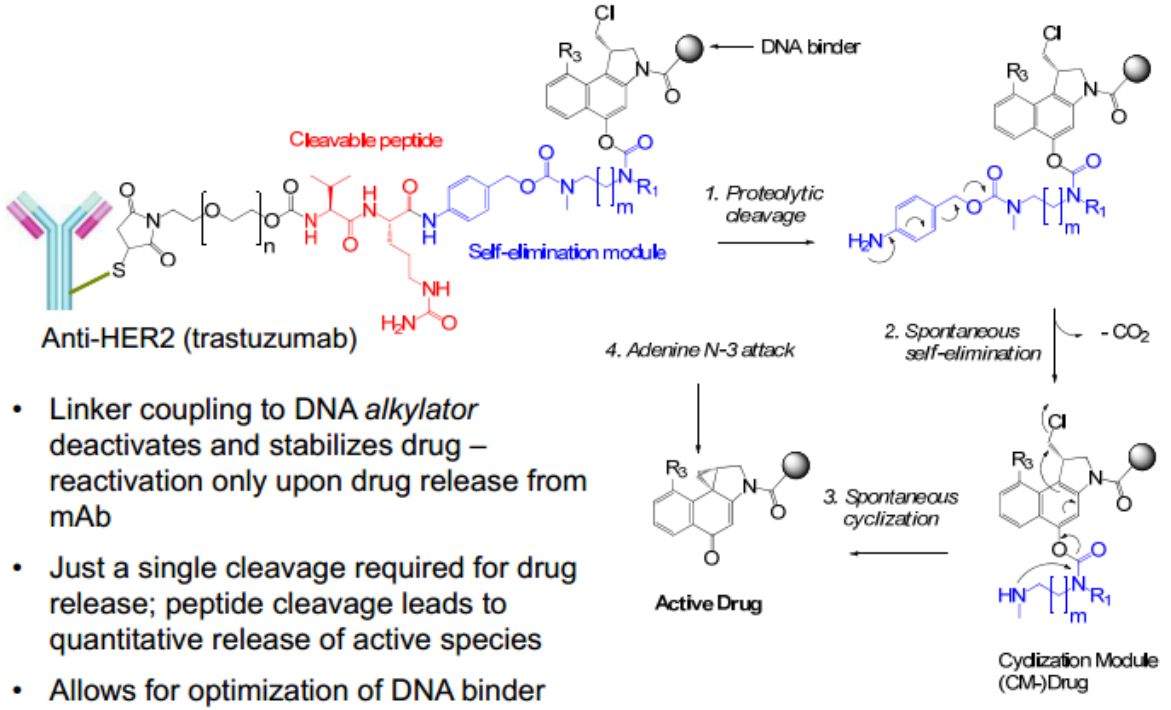
ABZENA

Synthon: *SpaceLink* - Duocarmycin



DNA-alkylating unit in blue

DNA-binding unit in red



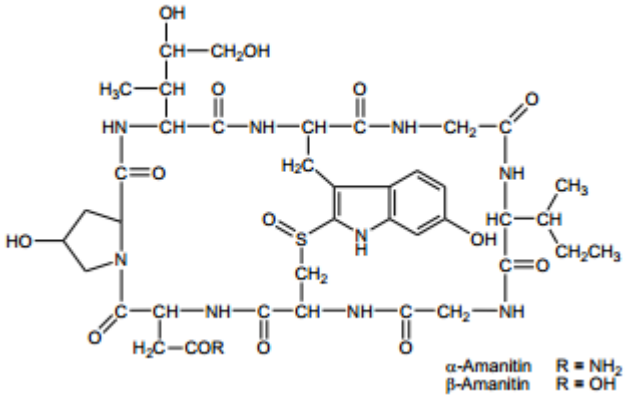
- Linker coupling to DNA *alkylator* deactivates and stabilizes drug – reactivation only upon drug release from mAb
- Just a single cleavage required for drug release; peptide cleavage leads to quantitative release of active species
- Allows for optimization of DNA binder

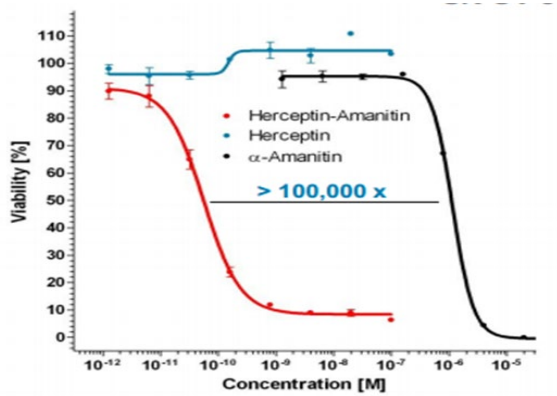
- DNA-alkylating agents; bind to DNA minor groove
- Fully synthetic, picomolar potency
- Proprietary cleavable linker – cleaved by esterases in mice – knockout mutants needed for pre-clinical models
- SYD983 (anti-HER2) selected for development

Gijs Verheijden, Synthon, 2013. PEGS Boston

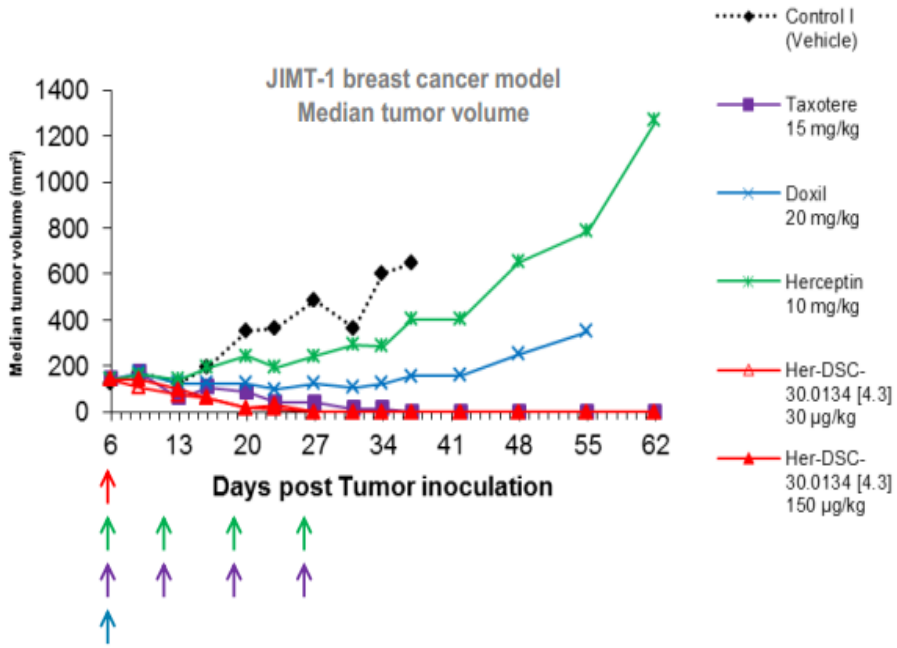
ABZENA

Heidelberg Pharma: Alpha-amanitin





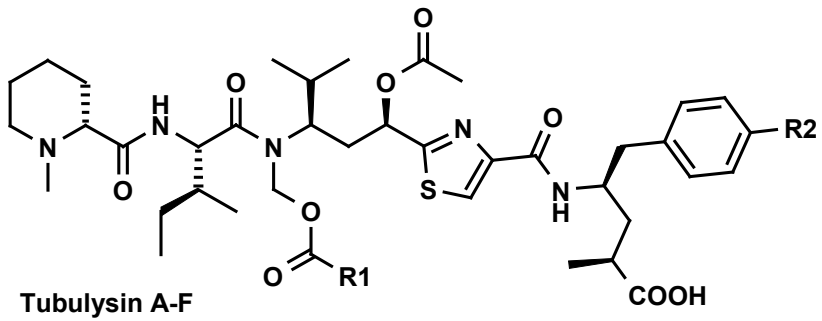
- From green death-cap mushroom (A. phalloides)
- Hydrophillic bicyclic octapeptide
- RNA polymerase II inhibitor
- Hepatotoxicity mediated by OATP1B3
- Active at very low doses, cell-cycle independent, active in MDR-positive cells
- Process of amanitin production by fermentation establish



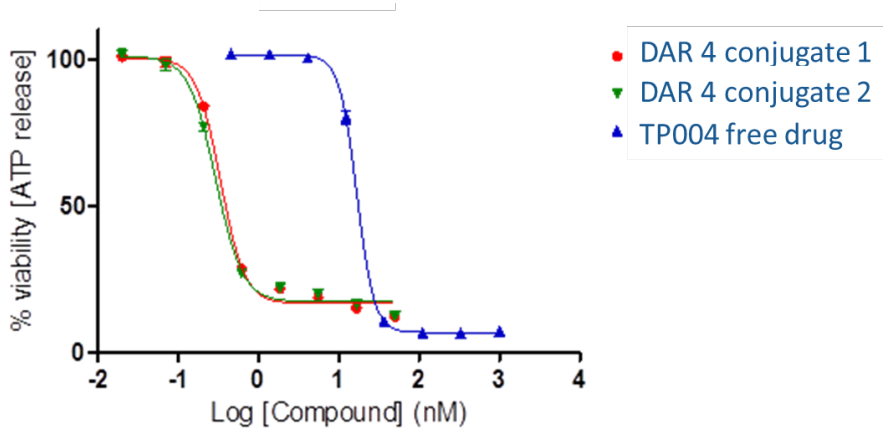
Jan Aderl, 2014. Bioconjugates San Francisco

ABZENA

Tube Pharma: Cytolysins – fully synthetic tubulysin payloads



Tubulysin A-F
R1 = iBu, Et, Pr, tBu R2 = H, OH

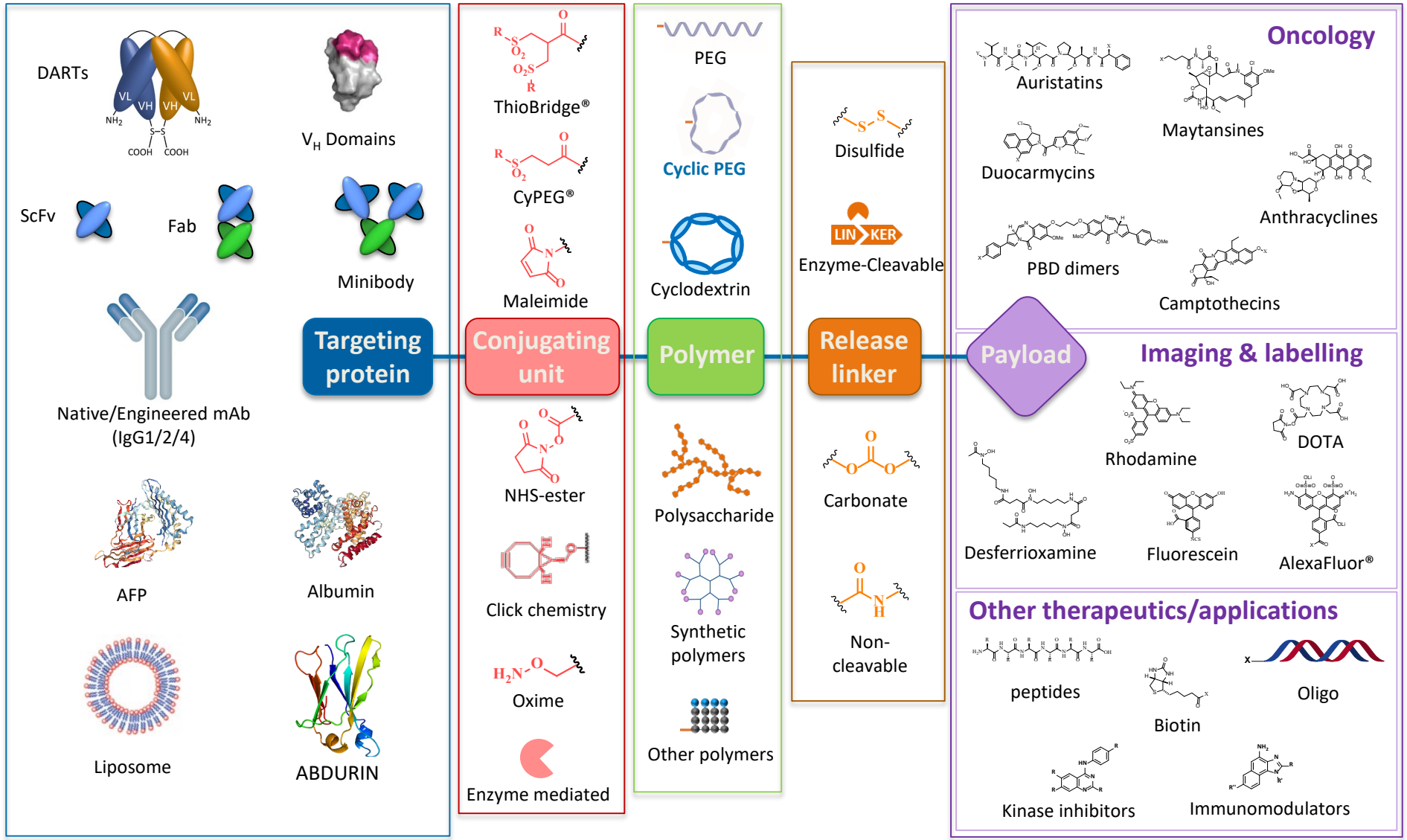


- Tubulysins isolated from Myxobacteria
- Bind at vinblastin binding site of tubulin, destabilize the tubulin skeleton
- Highly cytotoxic, induce apoptosis and have anti-angiogenic activity
- Cytolysins are tetrapeptidic analogues of the natural class Tubulysins
- Many different Tubulysin/Cytolysin derivatives are accessible by total synthesis in sufficient quantities for preclinical and clinical development

Tube Pharma

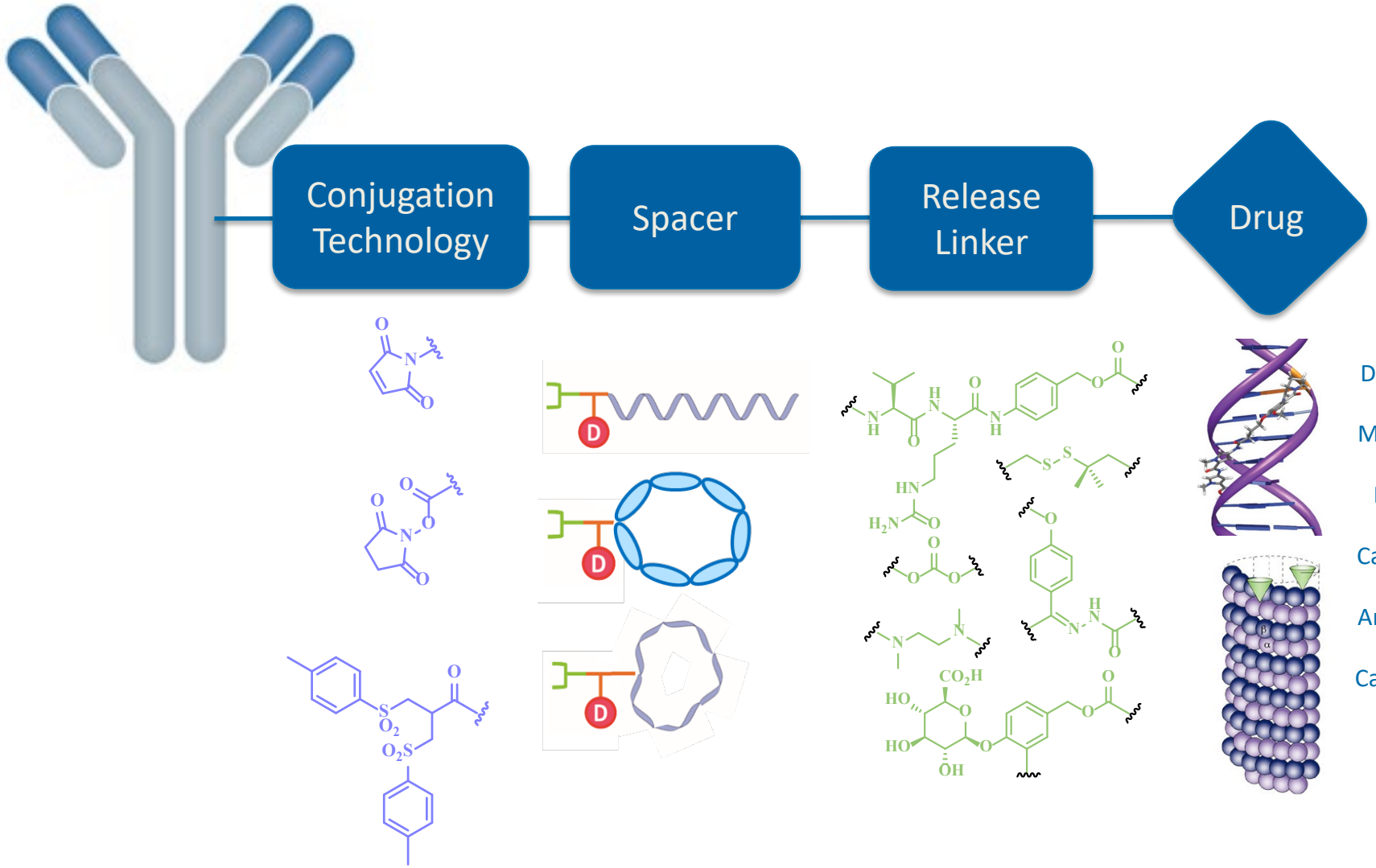
ABZENA

ADC Chemistry Design & Developability Options



ABZENA

Tools For The Design, Optimisation and Manufacture of Antibody Drug Conjugates (ADCs)



ABZENA

The importance of linker selection in relation to why ADCs fail

Invest New Drugs
https://doi.org/10.1007/s10637-017-0520-6

REVIEW

Clinical toxicity of antibody drug conjugates: a meta-analysis of payloads

Joanna C. Masters[1] · Dana J. Nickens[2] · Dawei Xuan[3] · Ronald L. Shazer[4] · Michael Amantea[2]

- **Major clinical toxicities** of ADCs include hematologic, hepatic, neurologic, and ophthalmic events, which are often dose-limiting. These events may be off-target effects **caused by premature release of payload** in circulation

BioDrugs (2017) 31:521–531
DOI 10.1007/s40259-017-0254-1

REVIEW ARTICLE

Recent Developments in ADC Technology: Preclinical Studies Signal Future Clinical Trends

Penelope M. Drake[1] · David Rabuka[1]

- FcγR-mediated internalization …….. a possible explanation for the **dose-limiting toxicity of thrombocytopenia** that is associated with certain ADC treatments, including Kadcyla®

ONCOIMMUNOLOGY
2018, VOL. 7, NO. 3, e1395127 (11 pages)
https://doi.org/10.1080/2162402X.2017.1395127

REVIEW

Antibody structure and engineering considerations for the design and function of Antibody Drug Conjugates (ADCs)

Ricarda M. Hoffmann[a,b,#], Ben G. T. Coumbe[a,c,#], Debra H. Josephs[a,d], Silvia Mele[a], Kristina M. Ilieva[a,e], Anthony Cheung[a,e], Andrew N. Tutt[e], James F. Spicer[d], David E. Thurston[f,g], Silvia Crescioli[a,b], and Sophia N. Karagiannis[a,b,e]

- T-DM1 has been demonstrated to be internalized by megakaryocytes in vivo via **FcγRIIa binding**. This has been proposed to be involved in the **development of thrombocytopenia** induced by T-DM1.

antibodies

Review

Factors Affecting the Pharmacology of Antibody–Drug Conjugates

Andrew T. Lucas [1,2,3], Lauren S. L. Price [1], Allison N. Schorzman [1], Mallory Storrie [2], Joseph A. Piscitelli [2], Juan Razo [2] and William C. Zamboni [1,2,3,*]

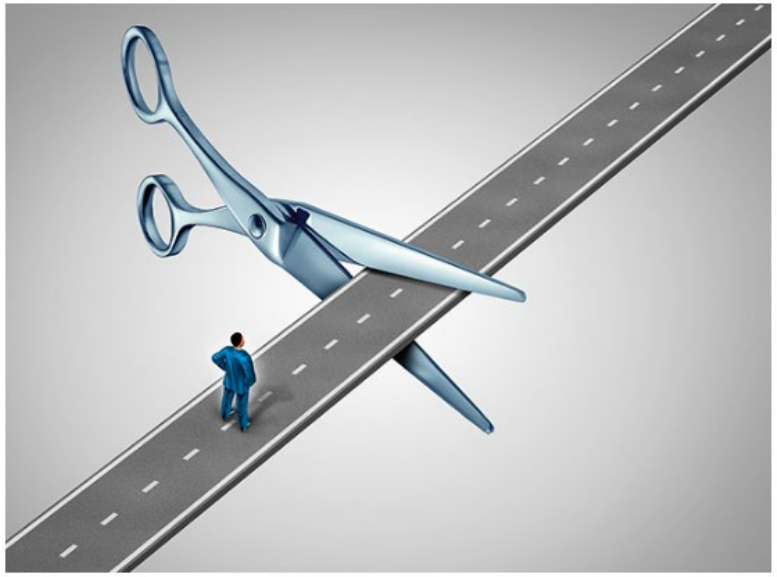
- …..certain formulation characteristics of Adcetris® may make it recognisable to the host's immune system and **mononuclear phagocyte system** (MPS) resulting in hepatic accumulation due to MPS-based clearance.

ABZENA

The importance of linker selection in relation to why ADCs fail

AbbVie discontinues Rova-T programme

30th August 2019



by
Anna Smith

AbbVie has announced the discontinuation of its Rova-T (rovalpituzumab tesirine) research and development programme, following a failure to demonstrate survival benefit at a pre-planned interim analysis.

AbbVie Out $5.8 Billion After Scrapping Rova-T Program

ImmunoGen Announces Top-Line Results from Phase 3 FORWARD I Study of Mirvetuximab Soravtansine in Ovarian Cancer

March 1, 2019 at 6:30 AM EST

Trial Did Not Meet Primary Endpoint of Progression-Free Survival

Efficacy Signal Seen in High Folate Receptor Alpha Patients; Additional Analyses to be Conducted

Favorable Tolerability Profile Confirmed

Combination Regimens to be Evaluated as an Independent Path Forward to Support Registration in Ovarian Cancer

ImmunoGen Announces Completion of Operational Review

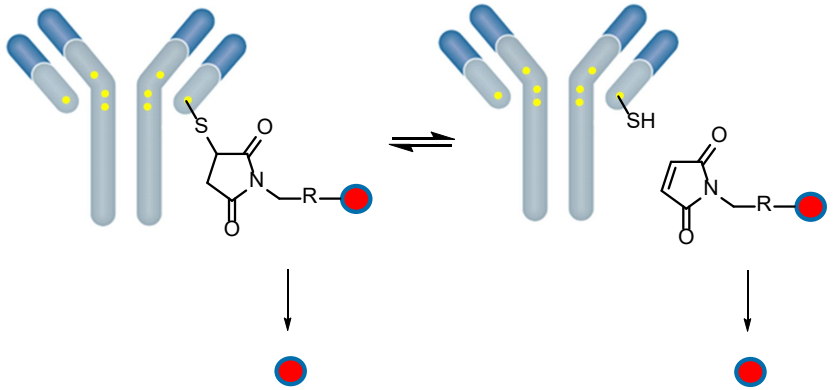
June 27, 2019 at 6:30 AM EDT

Company Will Prioritize Continued Development of Mirvetuximab Soravtansine and a Select Portfolio of Earlier-Stage Candidates

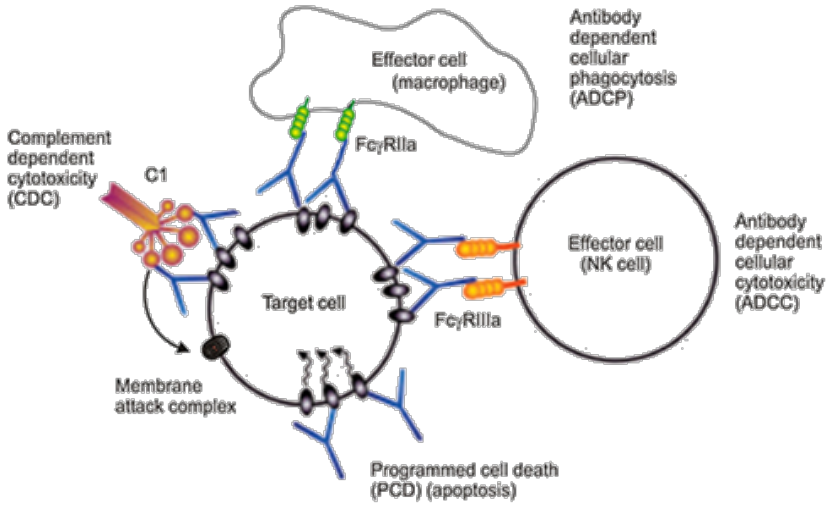
Cash Runway Extended Through Readout of Mirvetuximab Soravtansine Pivotal Trial in Ovarian Cancer

ABZENA

Addressing the failures of ADCs in the clinic: toxicity of ADC is a design issue



1. Instability of linker-payload leads to early payload release

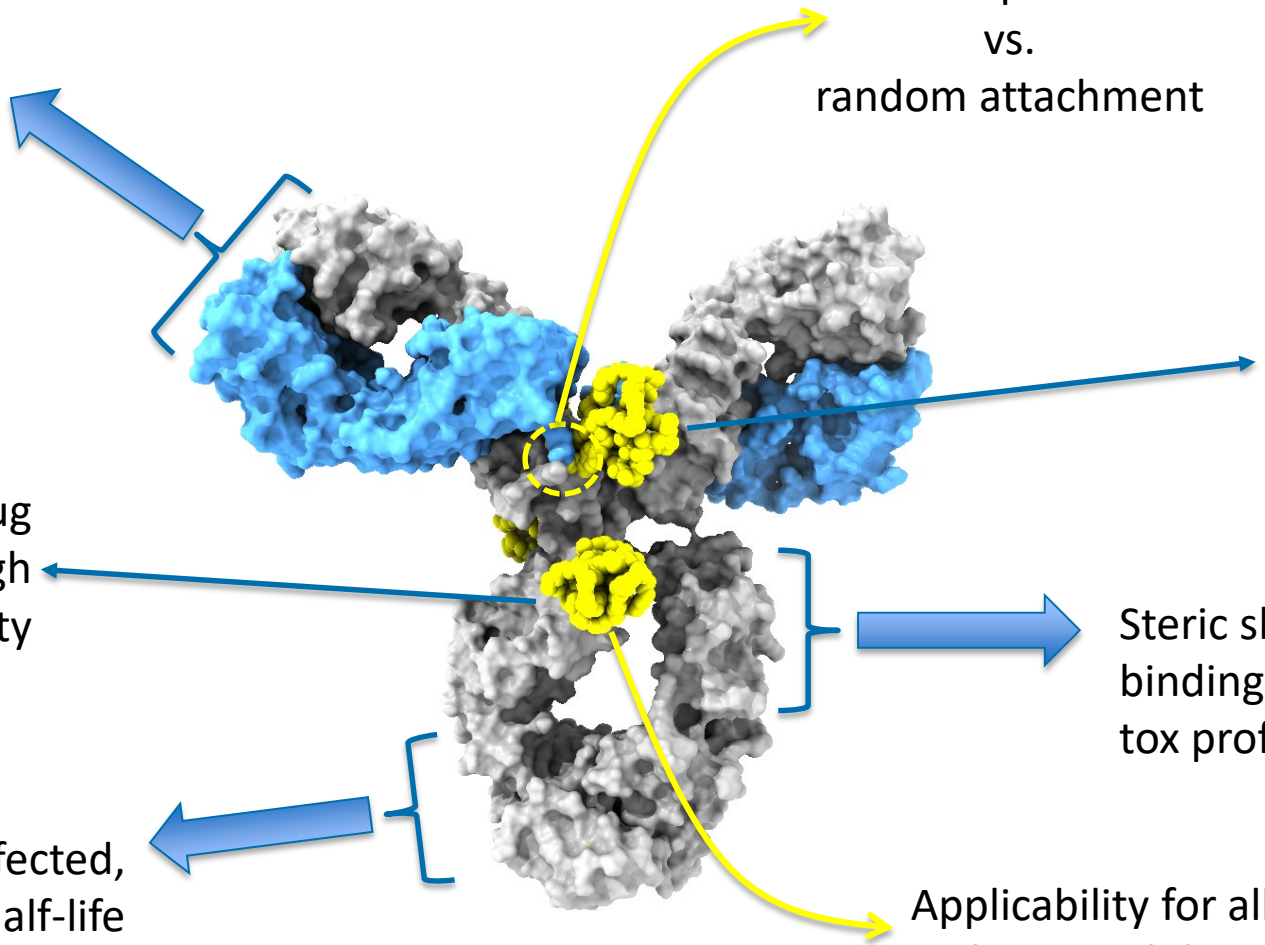


2. Non-specific targeting of via Fcγ-receptor binding

- Hepatic
  - Recognition by host phagocytes causes accumulation
- Dose limiting thrombocytopenia
  - FcγR-mediated internalization
- Dose limiting Neurologic, ophthalmic, vascular
  - Presumed early payload release

ABZENA

Developability properties for de-risking lead candidate selection and widening the therapeutic window



Site-specific
vs.
random attachment

Does not block
Ag+ binding

Stable linker – does not de-conjugate or cross conjugate

Homogeneous drug attachment with high reproducibility

Steric shielding of FcγR binding – improvement in tox profile

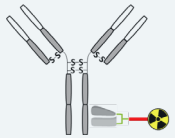
FcRn binding not affected, retaining long-circulatory half-life

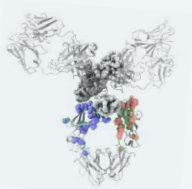
Applicability for all payload classes, and architectural design for DAR optimisation

ABZENA

ADC Design and Developability process

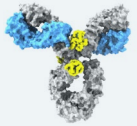
Stage 0: mAb selection



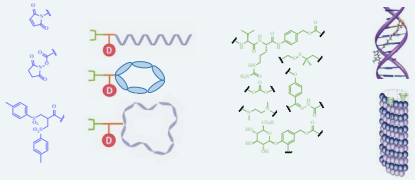


Stage 1: up to 12 Candidates, about 2 months

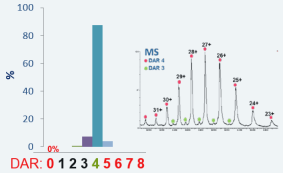
Product Concept & Design



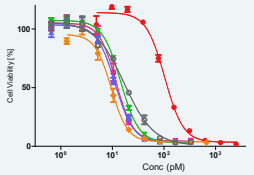
Reagent choice: conjugation, spacer, linker & payload



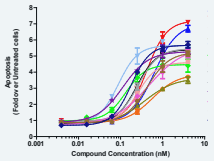
Synthesis of Reagents & ADC candidates



In vitro potency assays



Evaluate binding to cells

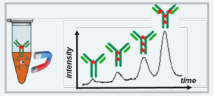


Stage 2: 6 Candidates about 1 month

Payload release Studies

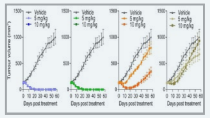
Physchem stability studies

Ex vivo stability evaluation

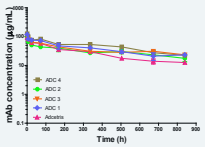


Stage 3: 2 Candidates, 5-6 months

In vivo xenograft studies (mouse)



In vivo PK studies (mouse)



In vivo toxicity (rat)

In vivo tolerability (NVP)

Lead Candidate

The process flow demonstrates requirements for each step of ADC development

ABZENA

ADC Discovery, PD, Scale up and Manufacture Workflow

Discovery Optimization

1-50 mL falcon tube scale

Sartobind nano

ÄKTA purifier ≤1 g scale

25-100 mL PendoTECH

Process Development

1-20 L Sartorius BIOTAT® RM

Sartobind

ÄKTA pilot 1-50 g scale

0.1-1L SARTOFLOW Smart

2-50 L SARTOFLOW Advanced

Manufacture

50-100 L Sartorius Palletank with LevMixer

Sartobind Jumbo

ÄKTA ready gradient 1-250 g scale

50-2000 L Sartorius FlexAct system

ABZENA

Analytical equipment typical for ADC characterisation

- Mass Spectrometry
  - Orbitrap-MS: QE plus with DIONEX nLC (Thermo)
  - Q-TOF-MS: Xevo G2-S with Nano-Acquity UPLC (Waters)
  - TOF-MS: Xevo G2-XS with H Class UPLC (Waters)
- HPLC-UV/FLD systems
  - Three DIONEX HPLCs, Ultimate 3000
  - Two Agilent HPLCs, Bio-Inert 1260
- Other
  - CE system (Perkin Elmer LabChip HT Touch GXII)
  - Qiagen tissue homogeniser
  - Perkin Elmer Lambda 25 UV/VIS spectrophotometer
  - Nanodrop 2000 spectrophotometer
- Additional biophysical capability
  - Unchained Labs UNcle biostability platform
  - Subvisible particle analyser - Malvern Panalytical Zetasizer Ultra
  - Vapro Vapour Pressure Osmometer

QE plus, Thermo

Xevo G2-S, Waters

Uncle biostability platform

Dionex

Qiagen Tissuelyser II

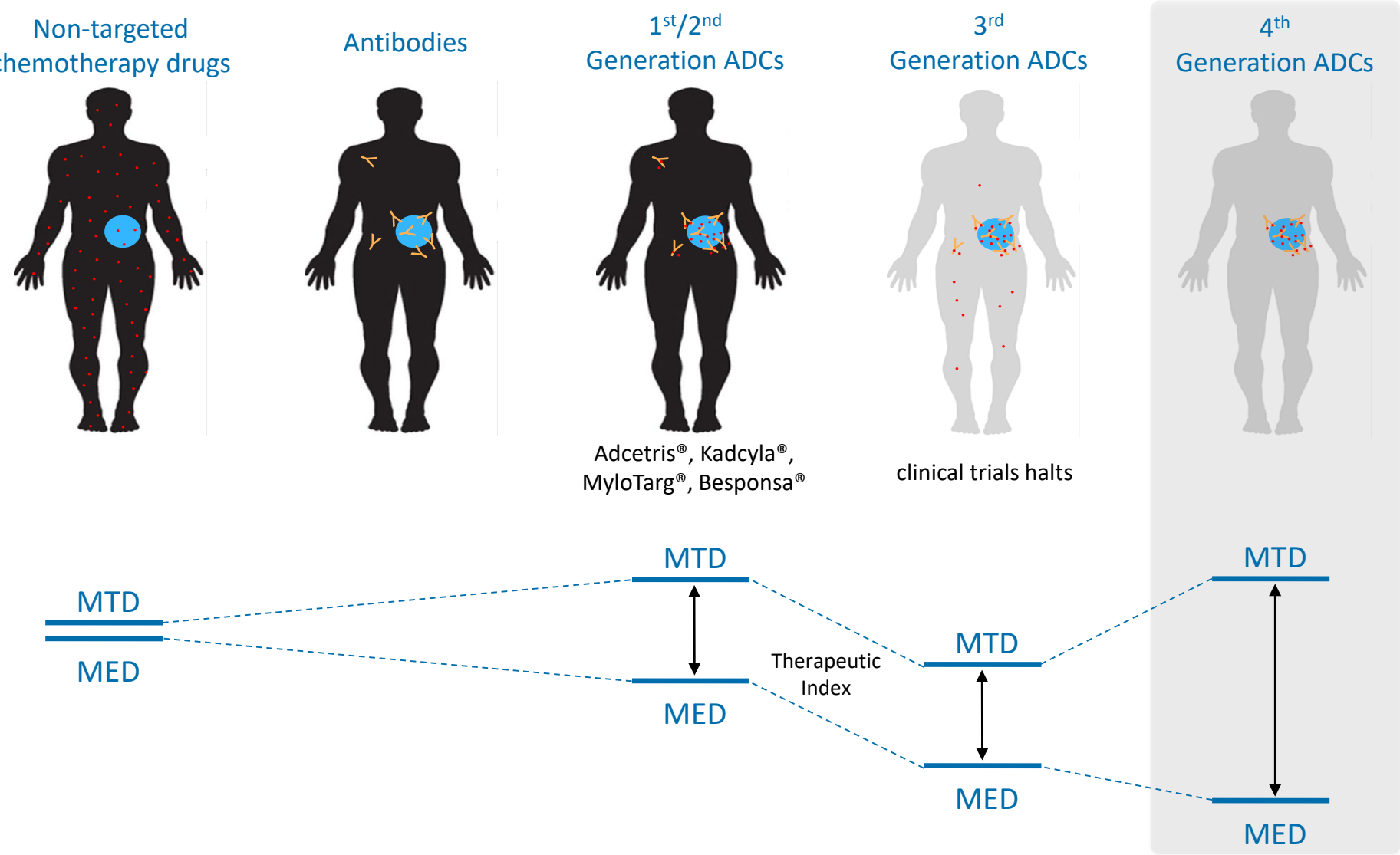
ABZENA

Summary of common ADC analytics

Analysis	Product Quality Attribute	Method
Intact Mass Analysis	Intact and subunit (Heavy and light chain) mass	RP-MS
Peptide Mapping	Peptide fingerprint Sequence coverage Site specific modifications like oxidation, deamidation, lysine clipping, site- specific N-glycosylation	RP-MSMS
Quantitative N-glycan profiling	Major N-glycan distribution on released glycans % Neu5Gc/Neu5Ac	HPLC-FLD
Charge variants	Charge molecular isoforms	CEX-UV Microcapillary CZE
Protein integrity	% intact antibody	Electrophoresis – SDS-PAGE or CE-SDS RP-MS
DAR profile	ADC drug load	HIC-UV
Aggregation profile	% monomeric	SEC-UV DLS, SLS, Fluorescence
Amino acid composition	Sequence	Amino acid analyser with ninhydrin detection RP-FLD
Protein folding	*Higher order structure, protein folding*	*^{1}H NMR fingerprint* DLS/SLS/Fluorescence
Binding	*Antigen binding curve* *Competition assay (e.g. blocking receptor/ligand)* *Affinity*	*ELISA/FACS* *ELISA/FACS* *SPR*
Effector functions	*ADCC* *CDC*	*Cellular assay/SPR* *Cellular assay/SPR*

ABZENA

Targeted delivery of drugs using ADCs can fulfil the full potential of widening the therapeutic index with 4th generation ADC linkers



Summary and future development

- The field of Antibody Drug Conjugation is an exciting area of drug design and development
- ADCs have demonstrated clinical benefits, but toxicity is leading to clinical failures
- Improvements in ADC design and developability including 4th generation linker chemistries are addressing this toxicity issue
- New payloads with novel mechanisms of action are leading ADC design
- Alternative formats to mAbs are leading novel bioconjugate drug development
- New ways of exploiting ADCs are in wider applications outside of oncology

ABZENA

Thank you for listening –

Any questions!