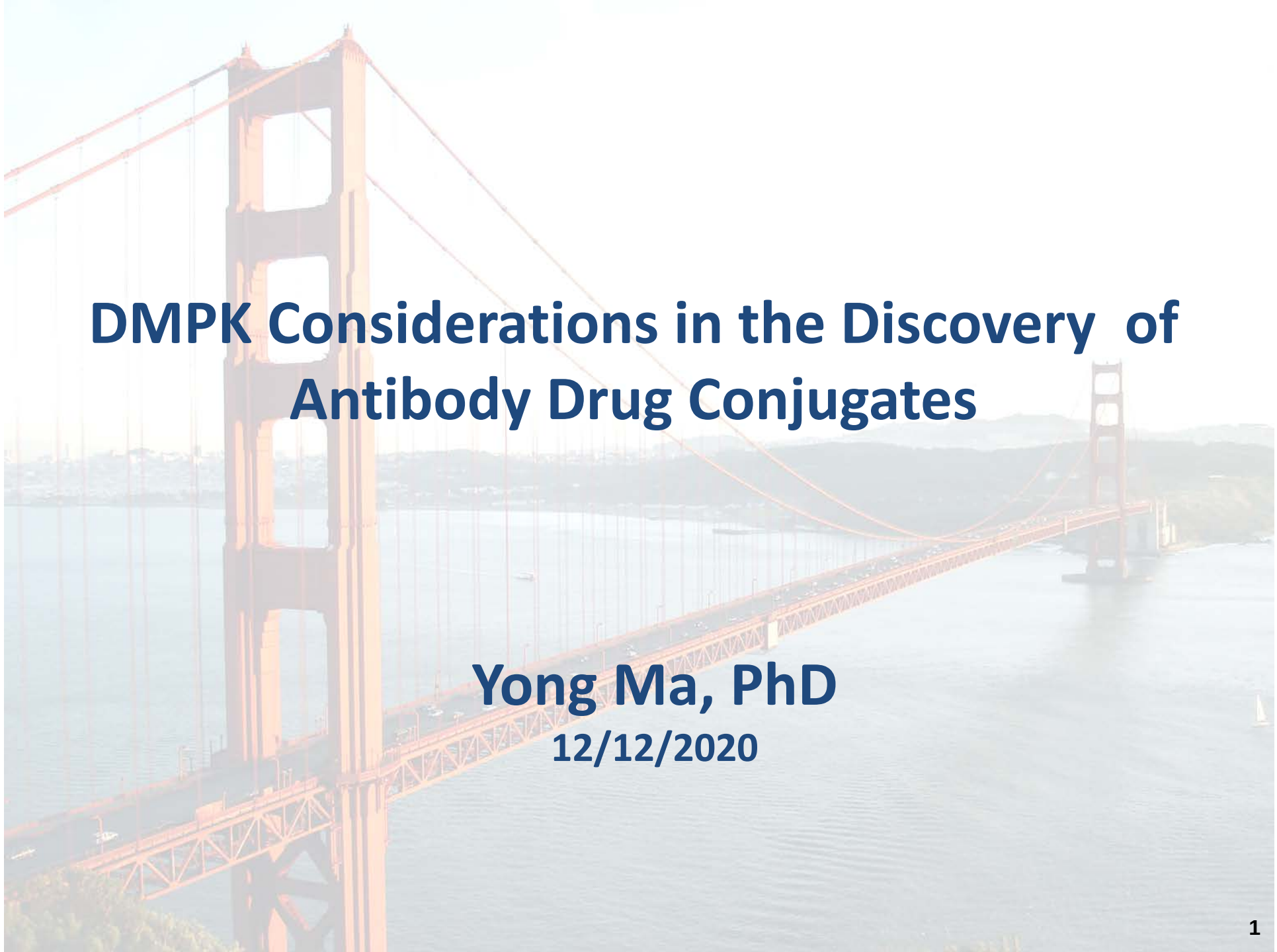

# DMPK Considerations in the Discovery of Antibody Drug Conjugates

Yong Ma, PhD

12/12/2020

# Outline

- Basics of Antibody-drug Conjugate
- Case Study 1: ADCs with a DNA Alkylator Payload
- Case Study 2: ADCs with a Mitotic Inhibitor Payload
- Case Study 3: Carfilzomib to Be an ADC Payload

# Outline

- **Basics of Antibody-drug Conjugate**
- Case Study 1: ADCs with a DNA Alkylator Payload
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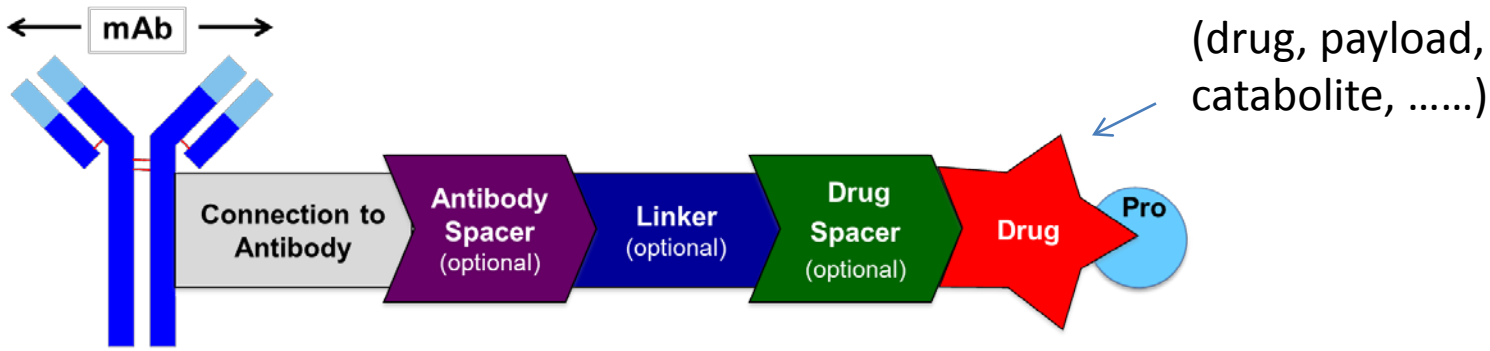
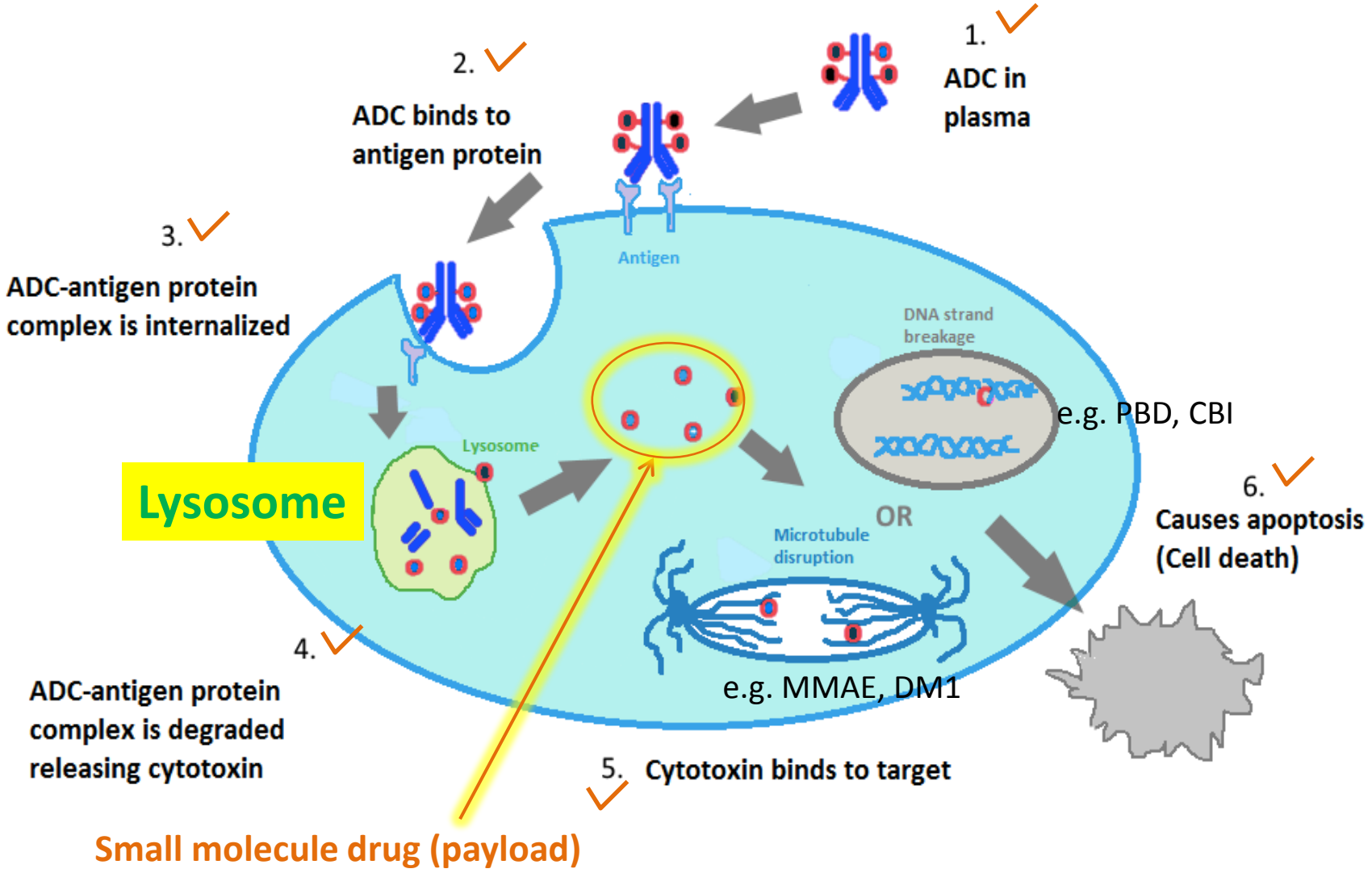
# Basics of Antibody-drug Conjugate (ADC)




- **Antibody**: to target the cancer cells
- **Linker**: to determine when and how the small molecule drug will be released from the antibody
  - **Cleavable linker**: peptide linker (cleavage by proteases),
    disulfide linker (cleavage by GSH),
    hydrazone linker (cleavage by pH change) …
  - **Non-cleavable linker**: a linker which will not leave the payload (linker-drug complex is active)
- **Drug (payload)**: extremely cytotoxic agents to induce cell death
  - **Microtubule binder**: Monomethyl auristatin E (MMAE), emtansine (DM1), …
  - **DNA cutter/alkylator**: Calicheamicin, Pyrrolobenzodiazepine(PBD), cyclopropylbenz[e]indolone(CBI), …
  - **Others**: under investigation.

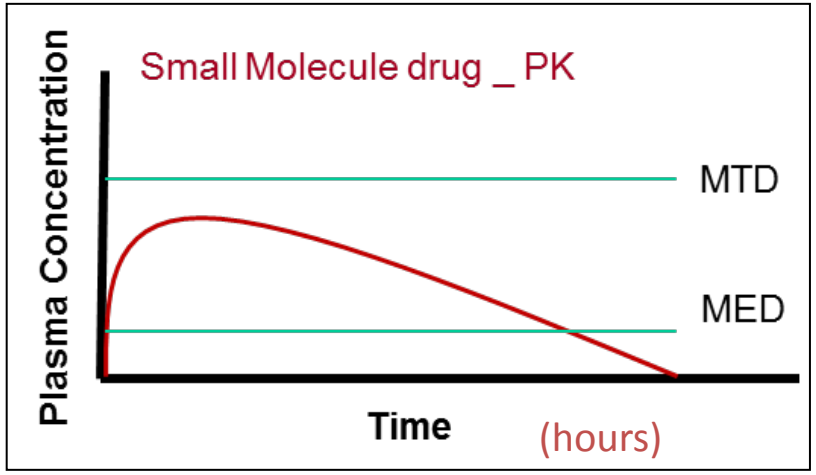
# ADC: Mechanism of Action (MOA)

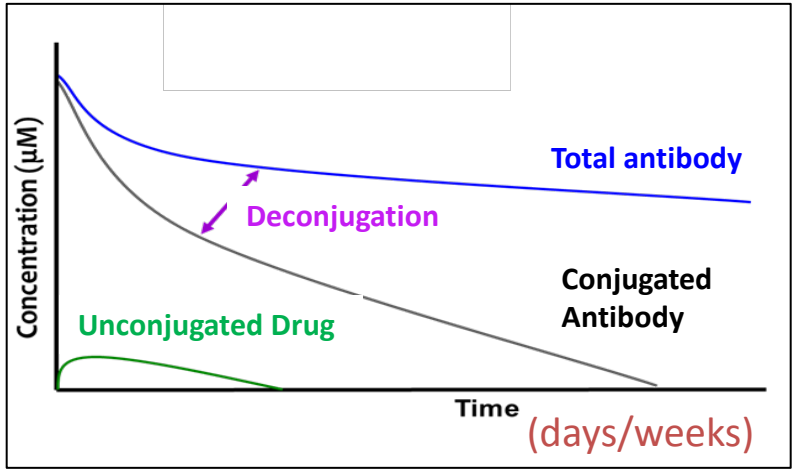
**Use DMPK approaches to understand more about steps 2-5**




Pharm Res, 32 (11), 3526-40

# Attributes of Small Molecule Drugs and ADCs

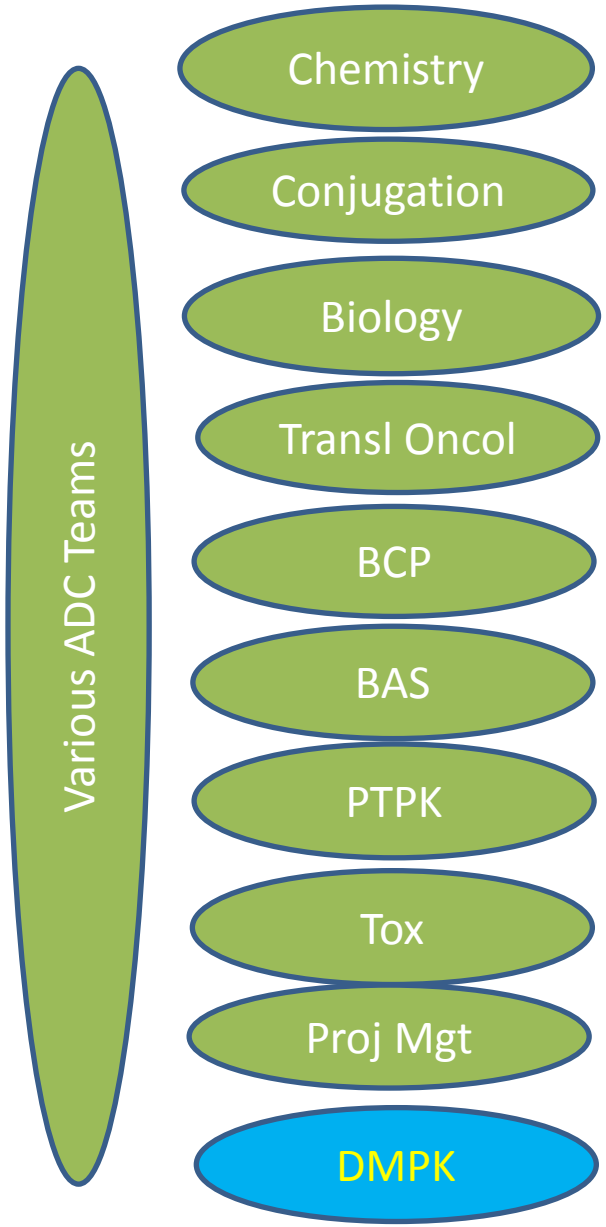


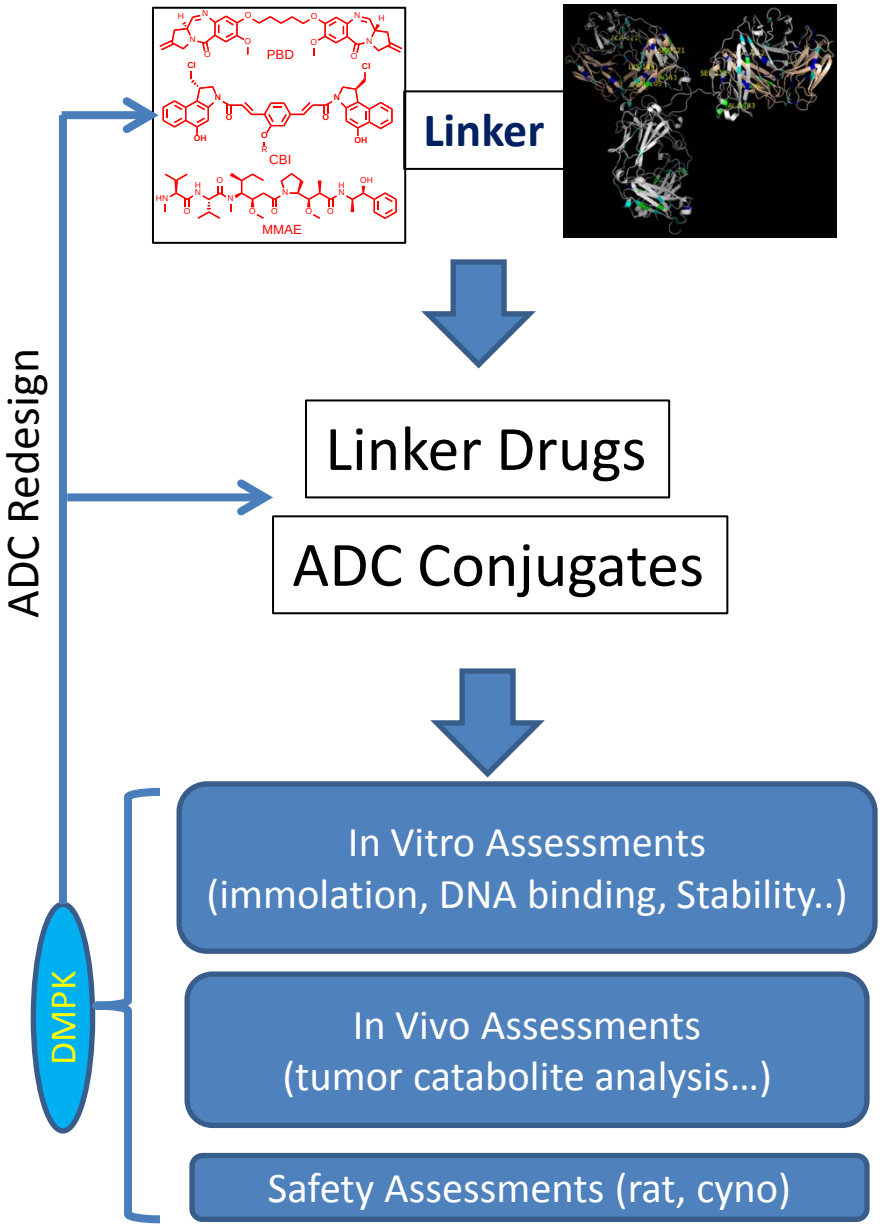




| Parameters | Small Molecules | ADC |
|---|---|---|
| Size (Da) | ~500 | ~150,000 |
| Active species | Parent | Catabolite (payload) |
| **Dose (mg/kg)** | **0.1-30** | **0.3-10 (~ 100 µg payload)** |
| Dosing Route | PO/IV | IV |
| $T_{1/2}$ | hours | Days-weeks |
| Metabolism | CYP and others | Catabolism |
| $V_d$ (parent) | Small to large | Small |

Drug Metab Dispos, 42 (11), 1914-20; Bioanalysis, 5 (2), 201-26; Pharm Res, 32 (11), 3470-9; Anal Biochem, 412 (1), 56-66

# DMPK Functions in the ADC Team



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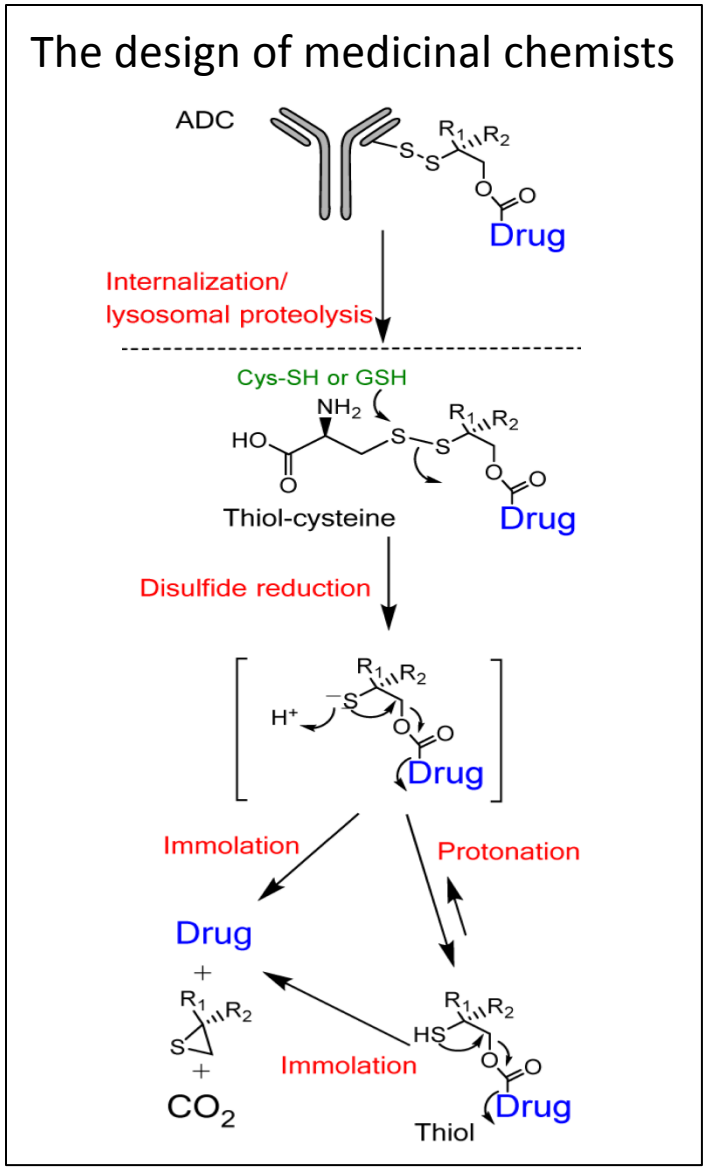
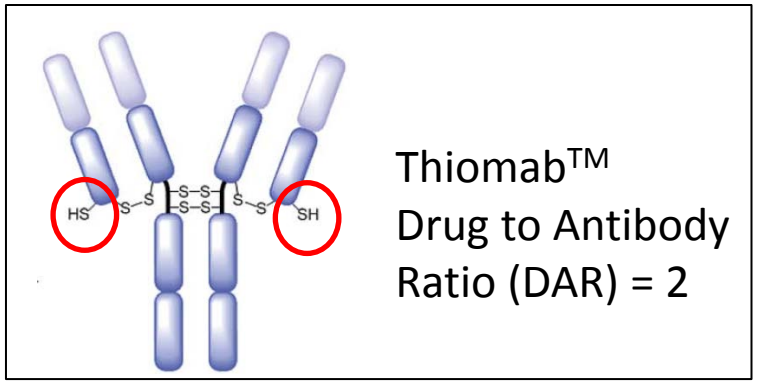
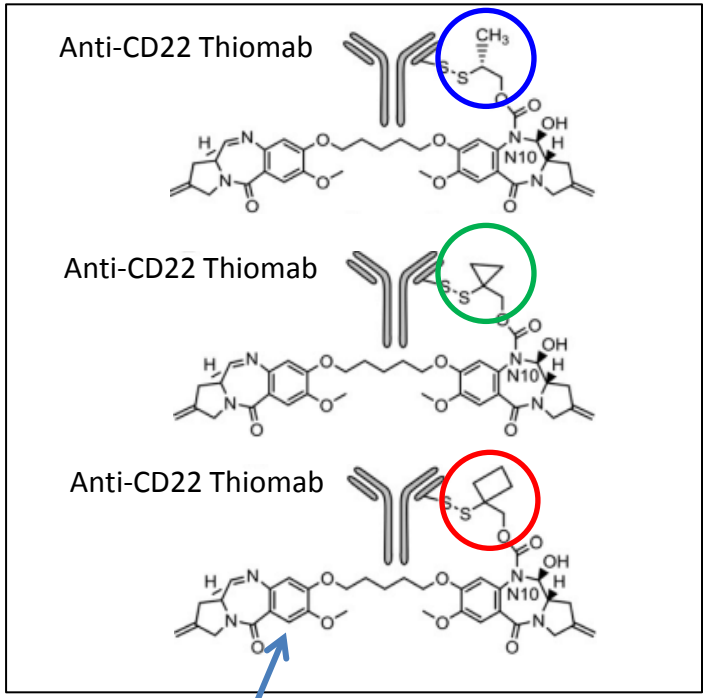
# Case Study 1: ADCs with an DNA Alkylator Payload



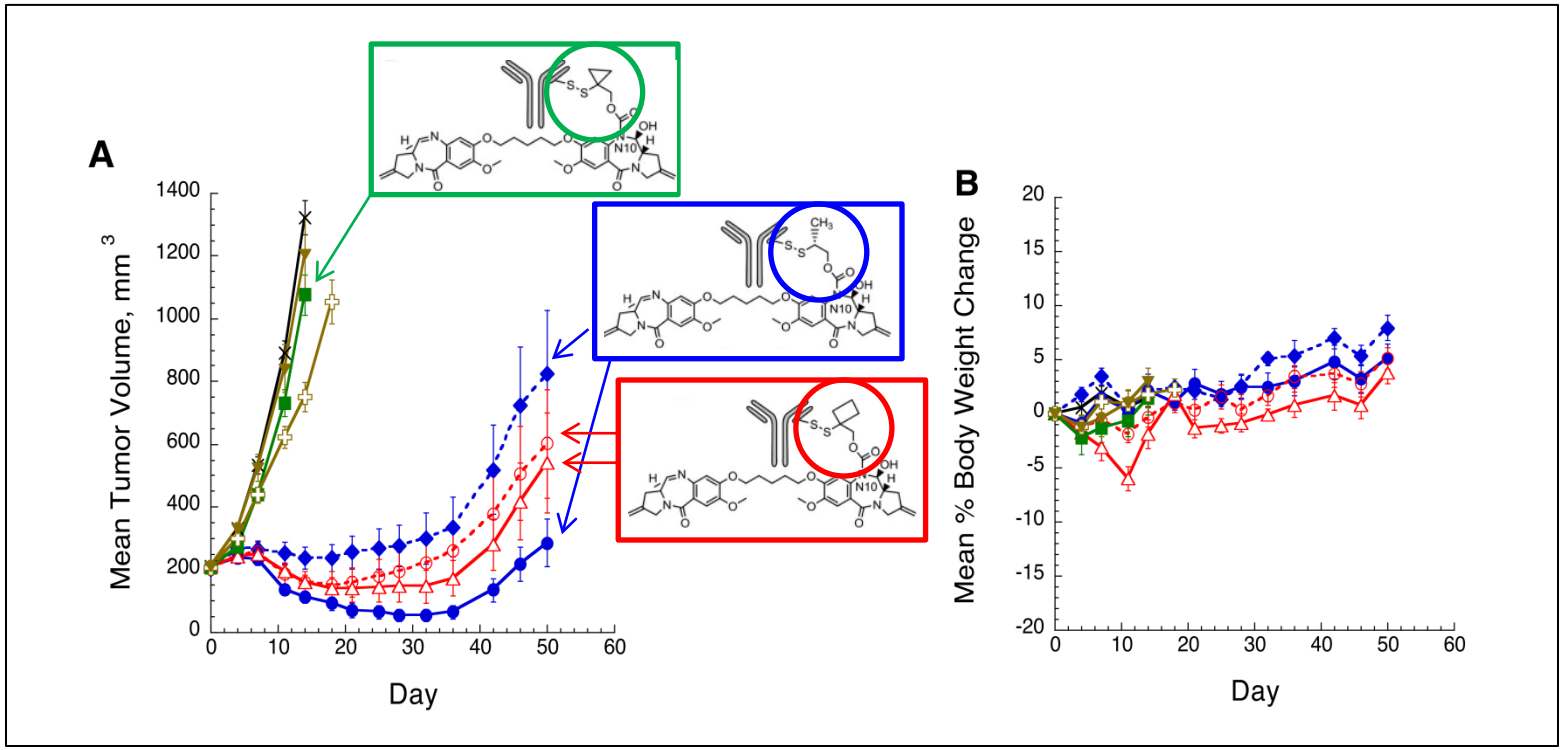




ACS Med Chem Lett, 7 (11), 988-993

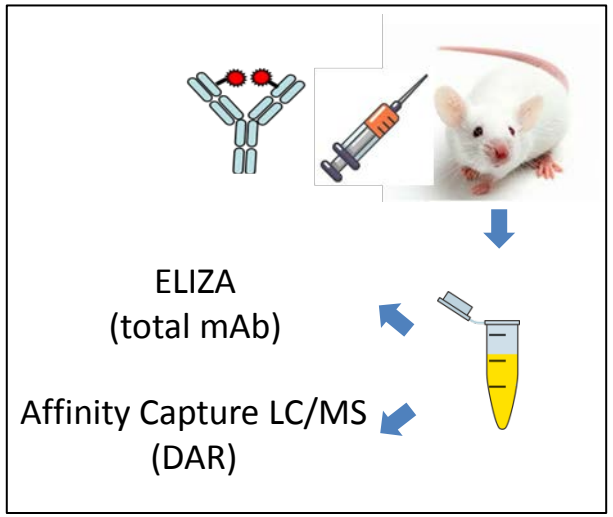
# Efficacy of 3 ADCs in a Xenograft Mouse Model

**Model**: SCID mice inoculated with CD22-expressing human diffuse large B-cell lymphoma WSU-DLCL2 cells

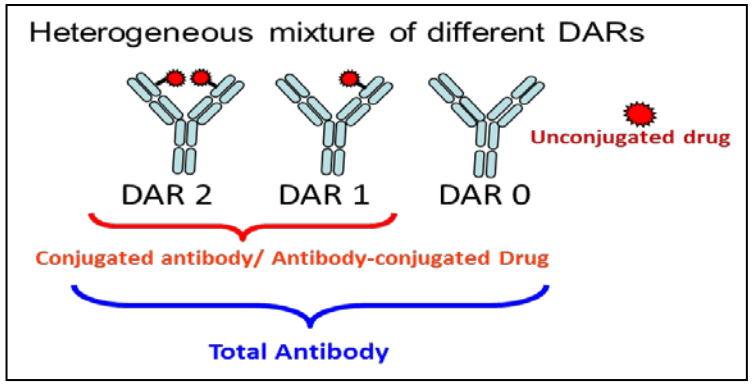



Drug Metab Dispos, 44 (9), 1517-23

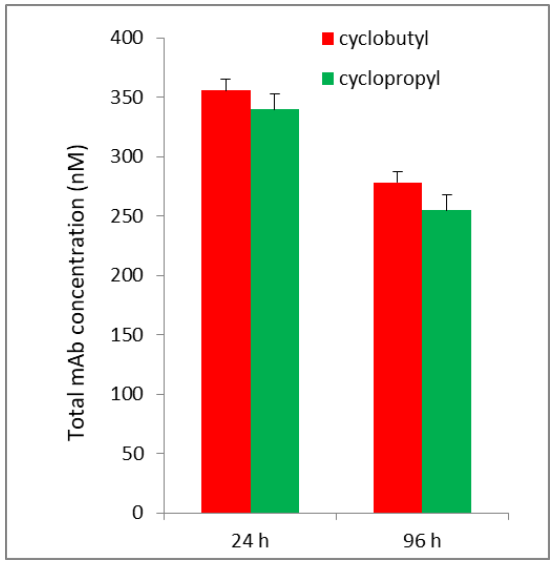
# Characterization of ADC in the Circulation

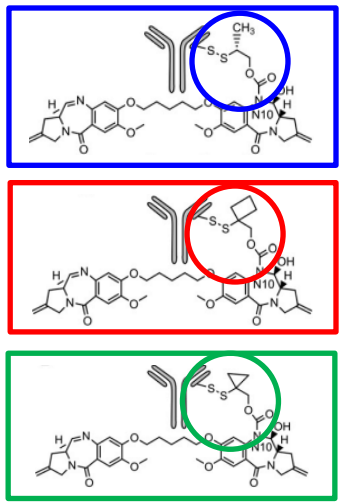



## DAR: Drug to Antibody Ratio

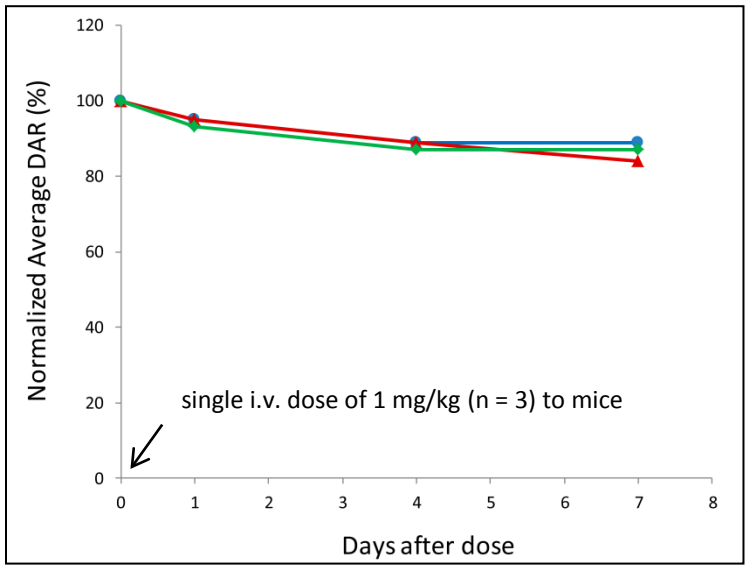



## Total mAb in circulation



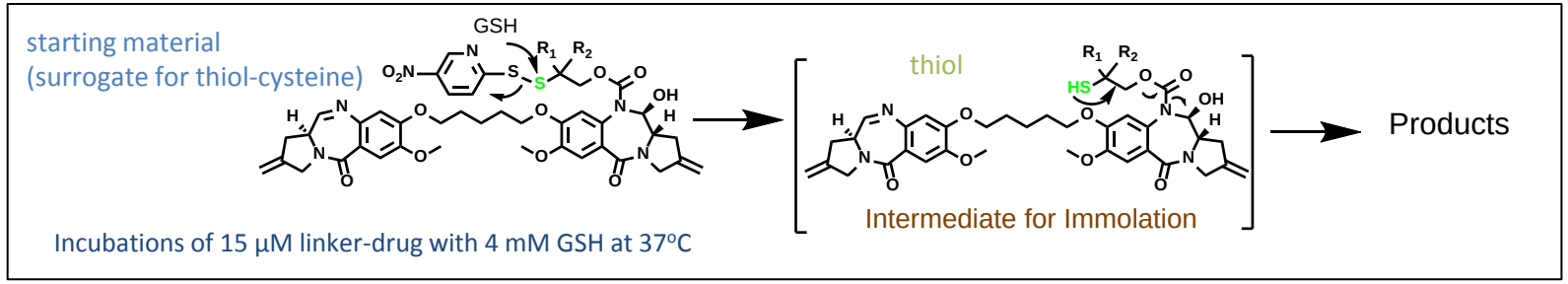



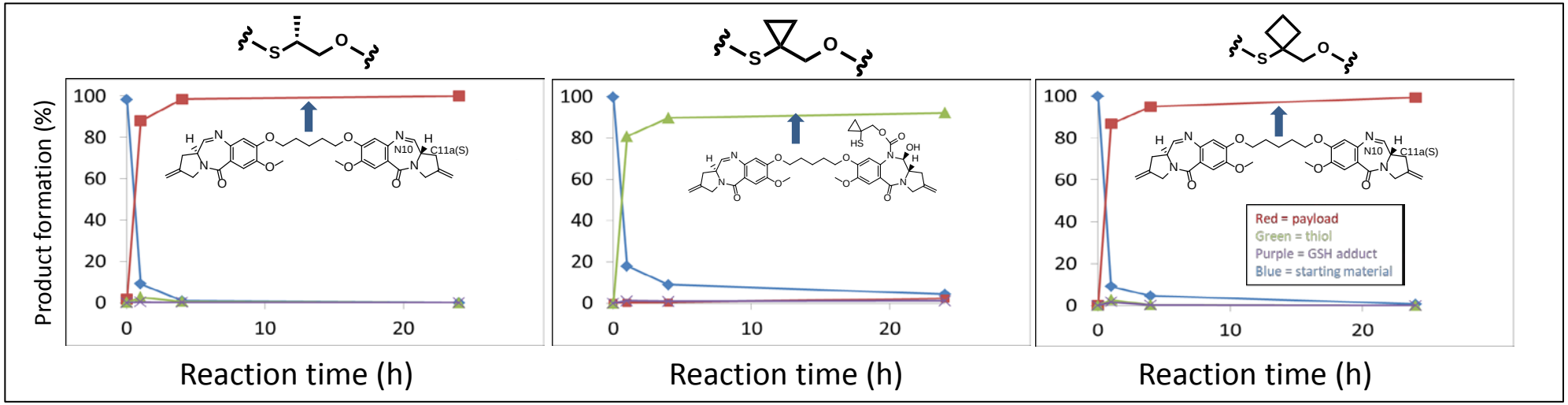
## DAR of ADC in circulation

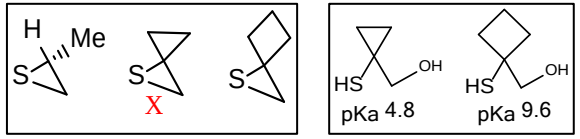



Drug Metab Dispos, 44 (9), 1517-23

# *In Vitro* PBD Release from Linker-Drugs



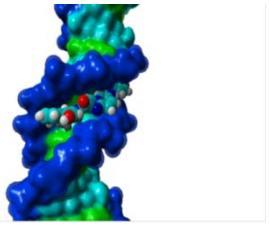



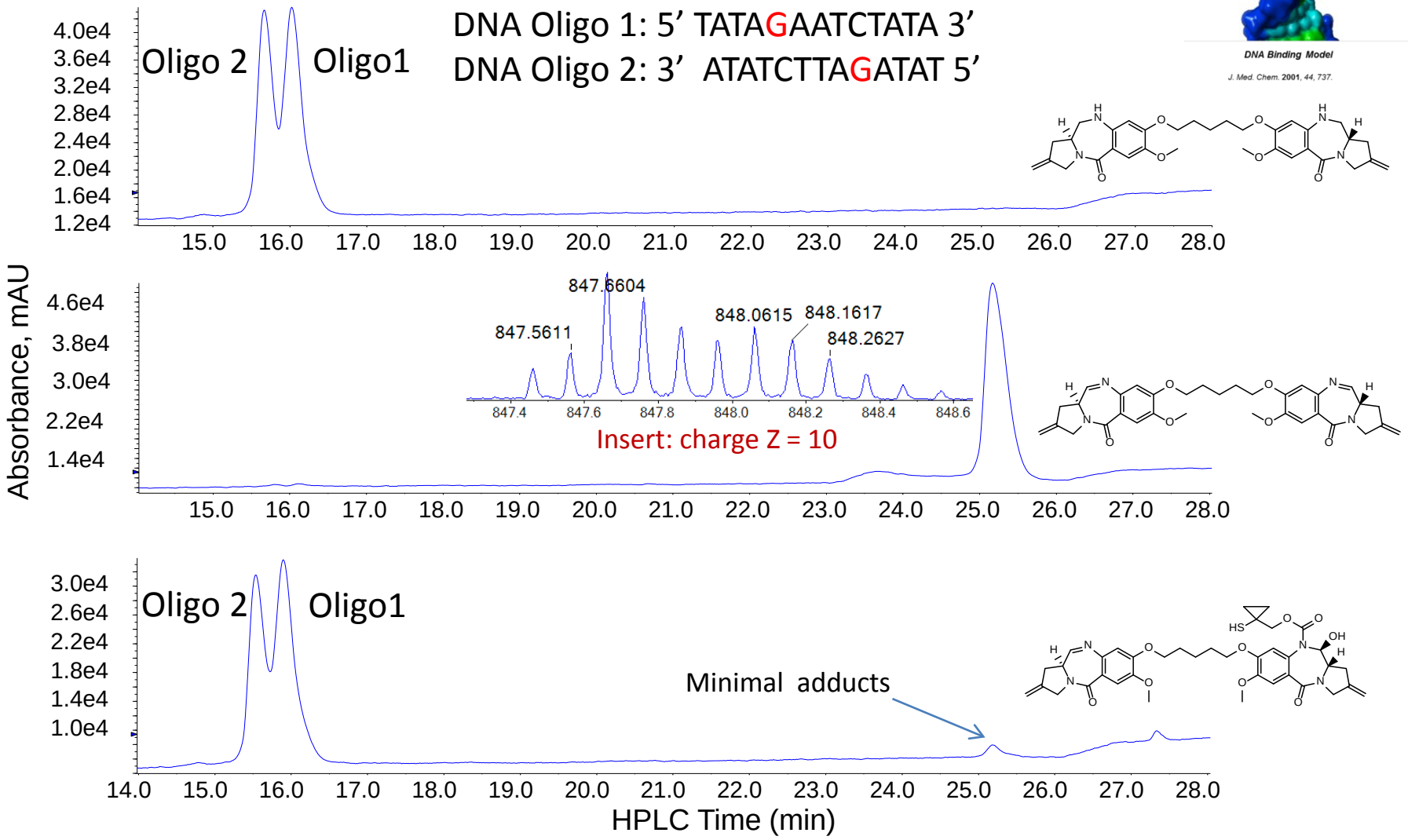

- **Different immolation was observed.**
- **The linker-drugs are useful in testing drug release, because the same intermediates for immolation are generated from ADCs.**

ACS Med Chem Lett, 7 (11), 988-993

# Did Cyclopropyl Thiol Bind to Oligo Model?



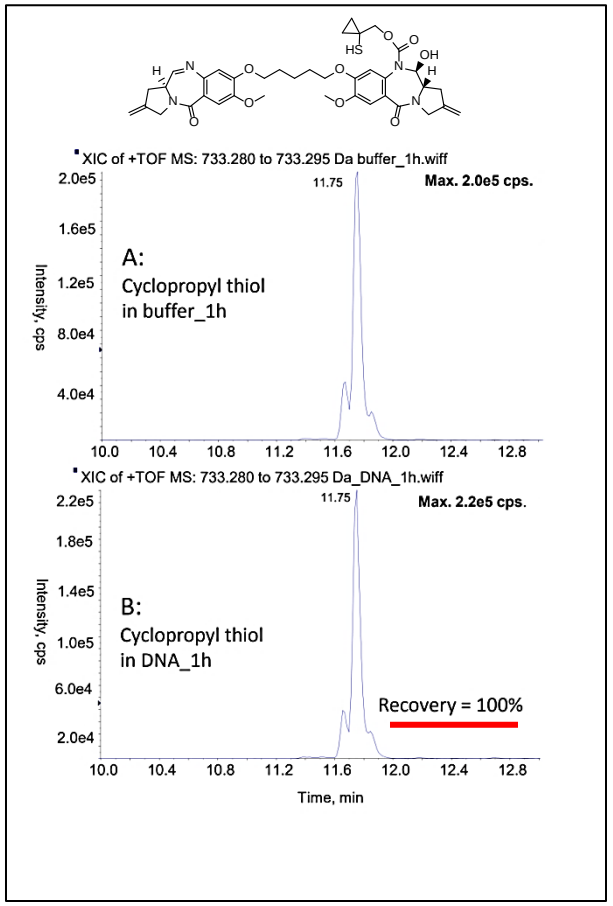

DNA Oligo 1: 5’ TATAGAATCTATA 3’
DNA Oligo 2: 3’ ATATCTTAGATAT 5’



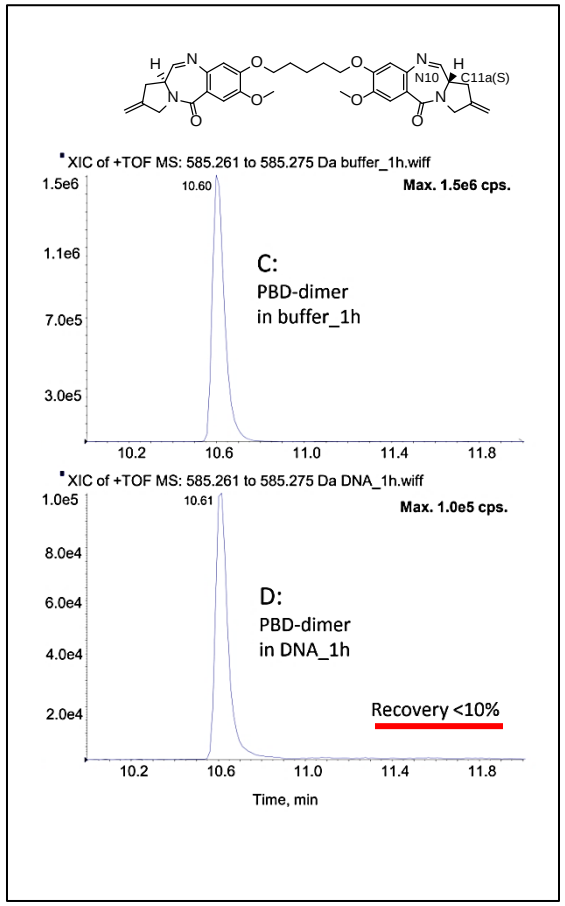
# DNA Binding Potential of PBD-dimer and Cyclopropyl Thiol

In vitro incubation: 1 µM PBD-dimer or cyclopropyl thiol + 1 mg/mL calf thymus DNA for 1 hour in 0.5 mL of 10 mM Bis-Tris, pH 7.1 at 37°C.

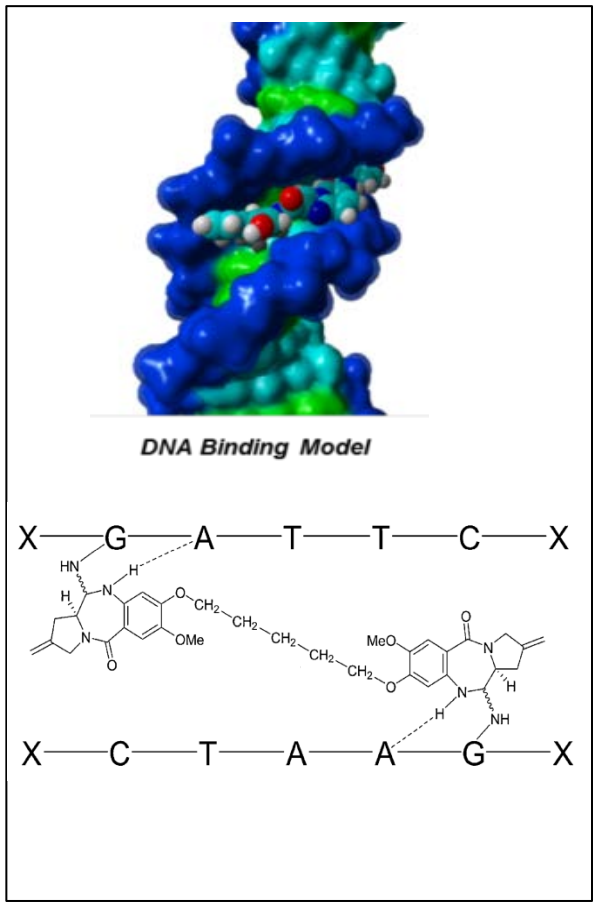
**cyclopropyl thiol + DNA**




**PBD-dimer + DNA**



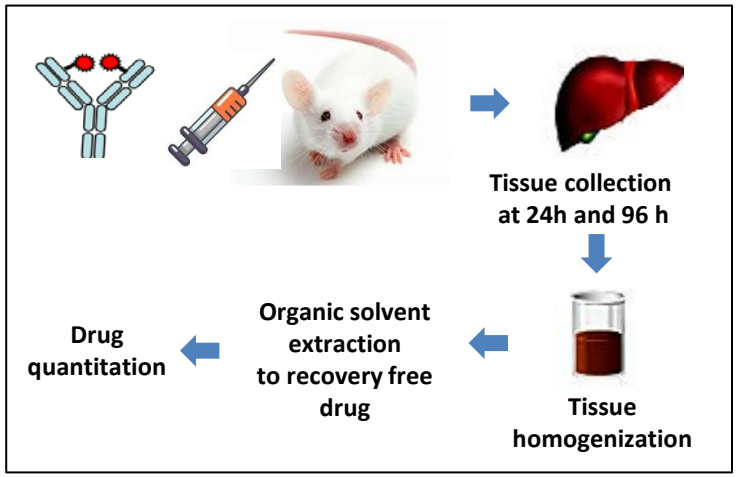

**PBD-DNA adduct**

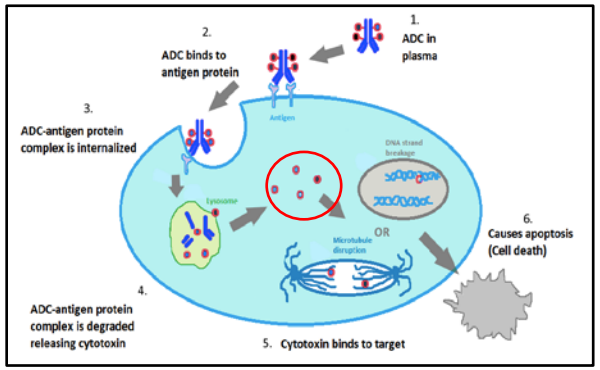


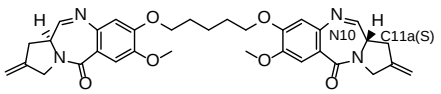

Drug Metab Dispos, 44 (9), 1517-23

Invest New Drugs, 30 (3), 950-8

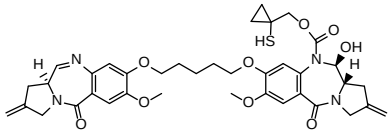
# Free PBD-dimer and Cyclopropyl Thiol in Tumor and Other Tissues



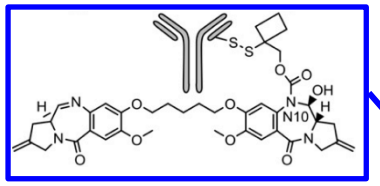



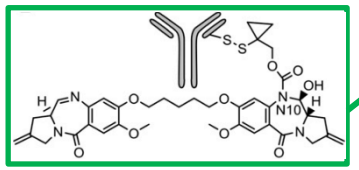




**PBD-dimer**



**Cyclopropyl-thiol**



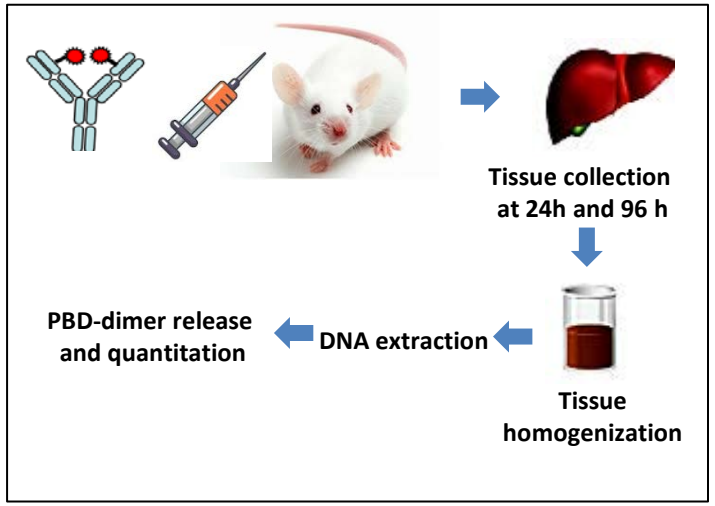



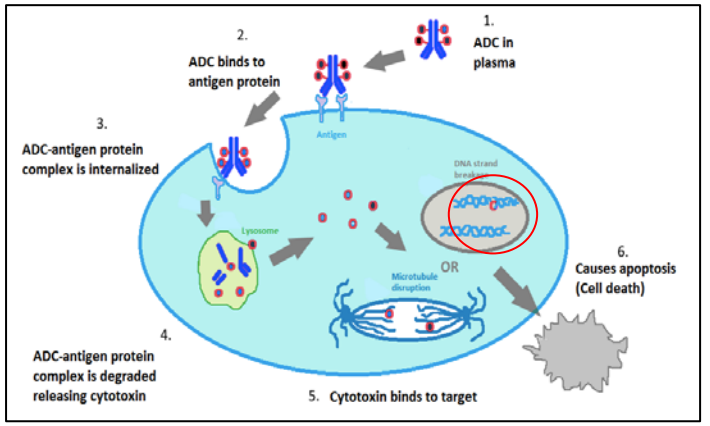

| ADC | Time | PBD-dimer Plasma | PBD-dimer Liver | PBD-dimer Tumor | Cyclopropyl-thiol Plasma | Cyclopropyl-thiol Liver | Cyclopropyl-thiol Tumor | mAb Plasma | mAb Liver | mAb Tumor |
|---|---|---|---|---|---|---|---|---|---|---|
| | *h* | *nM* | *nM* | *nM* | *nM* | *nM* | *nM* | *nM* | *nM* | *nM* |
| Cyclobutyl-containing | 24 | 0.43 | 0.26 | 1.03 | NA | NA | NA | 370 | 24.8 | 43.5 |
| | | 0.55 | 0.55 | 2.09 | NA | NA | NA | 342 | 19.5 | 25.0 |
| | 96 | <LLOQ | <LLOQ | 1.93 | NA | NA | NA | 285 | 20.9 | 25.3 |
| | | <LLOQ | <LLOQ | 2.05 | NA | NA | NA | 272 | 21.1 | 56.0 |
| Cyclopropyl-containing | 24 | ND | ND | ND | 0.74 | 1.42 | 7.58 | 294 | 28.4 | 59.4 |
| | | ND | ND | ND | 0.57 | 0.63 | 6.76 | 216 | 18.6 | 27.4 |
| | 96 | ND | ND | ND | <LLOQ | <LLOQ | 4.33 | 349 | 23.4 | 23.5 |
| | | ND | <LLOQ | ND | 0.30 | 0.34 | 4.69 | 331 | 16.6 | 16.6 |

NA, not applicable; LLOQ, lower limit of quantitation, 0.24 nM for both analytes in homogenates (nanomolar concentration in tumor and liver was estimated on the basis of an assumption of tissue density of 1 g/ml).

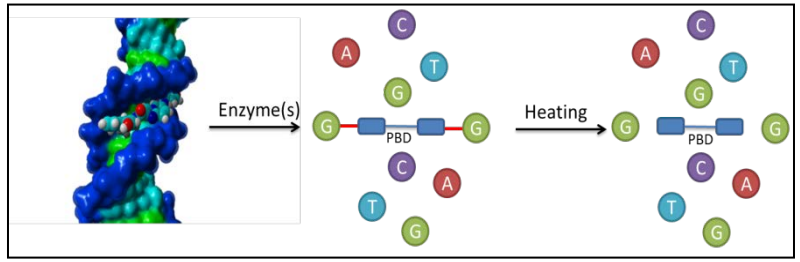
Drug Metab Dispos, 44 (9), 1517-23

# PBD-dimer Recovery from Tissue DNA

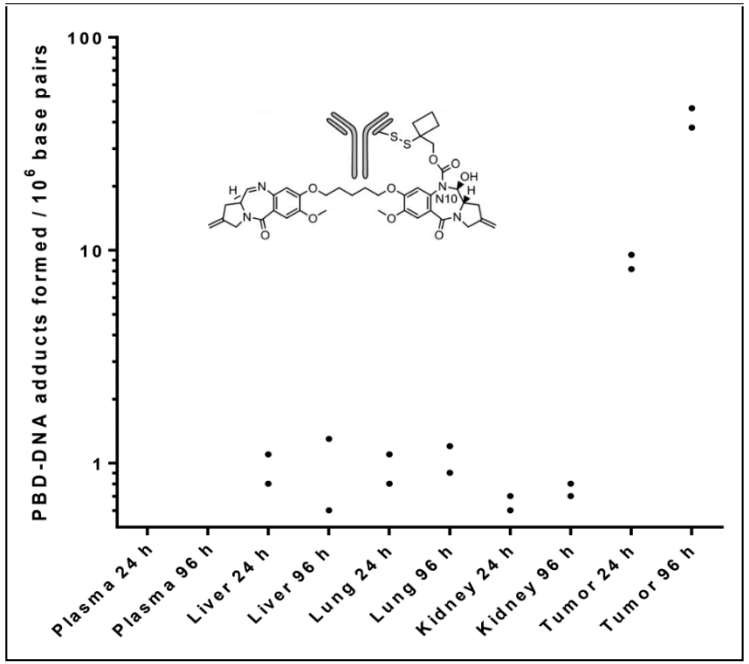



## DNA Digestion and heating to release PBD




## PBD-DNA adducts number in tissues and tumor




**Conclusion: The driving force of ADC efficacy comes from the small molecule payload.**

Drug Metab Dispos, 44 (12), 1958-1962

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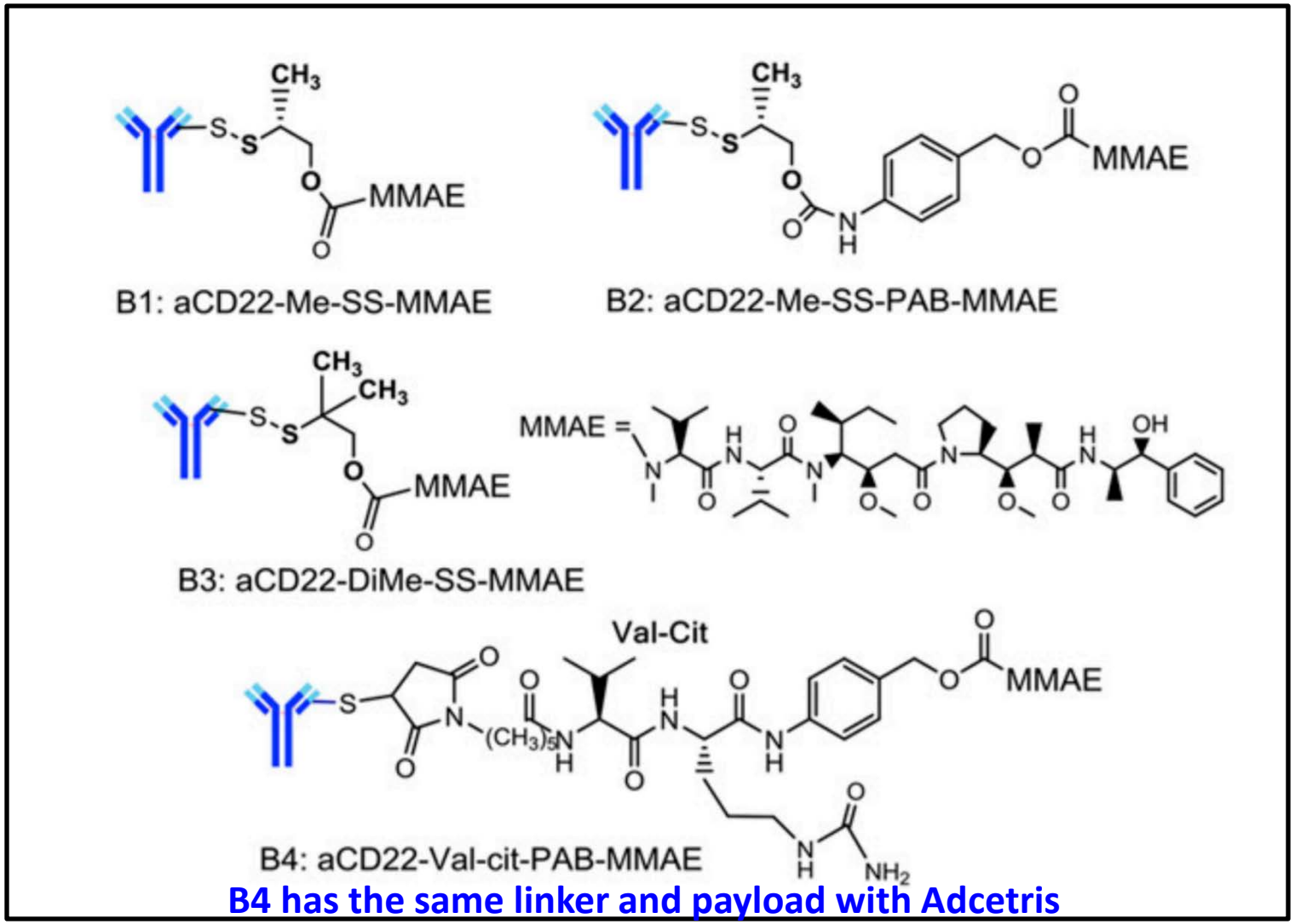
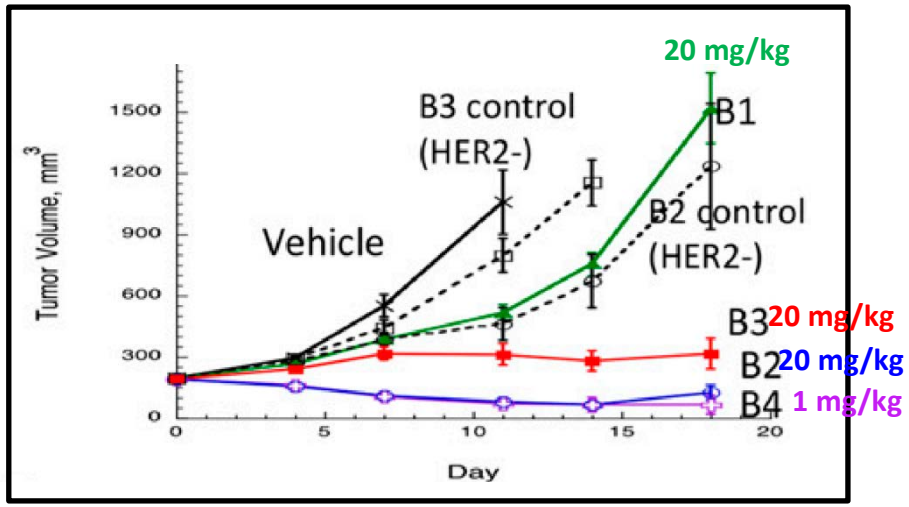
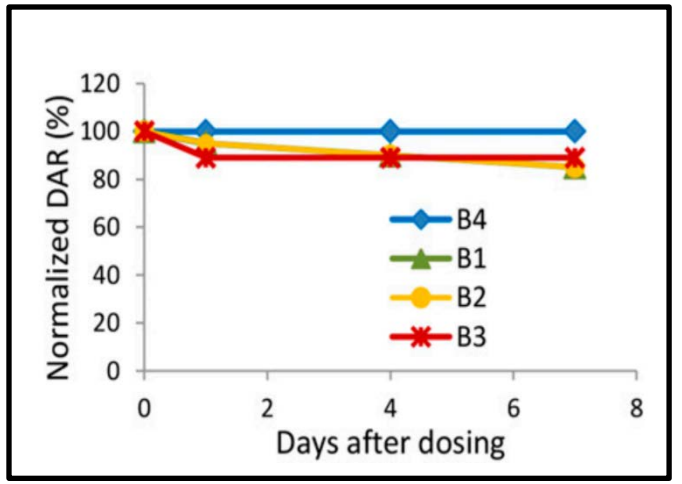
# Case Study 2: ADCs with a Mitotic Inhibitor Payload

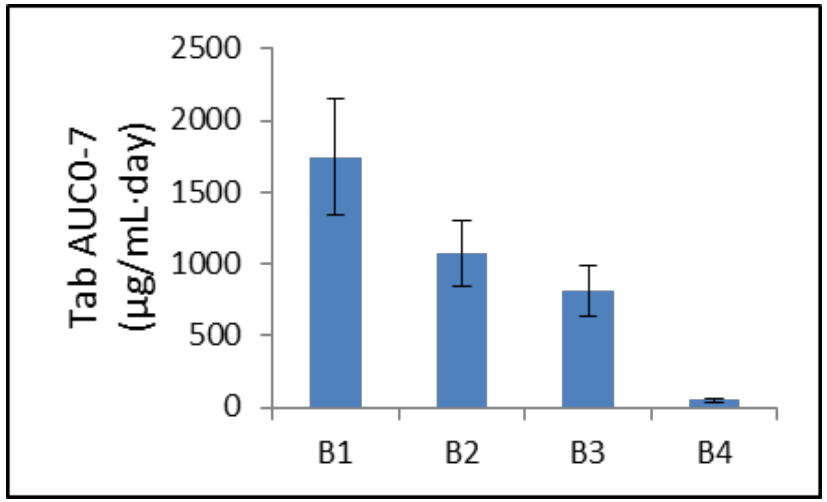


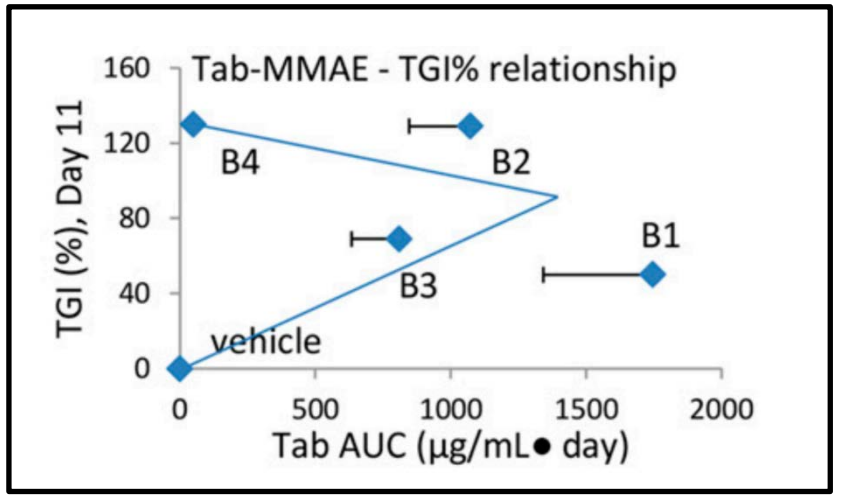

**B4 has the same linker and payload with Adcetris**

# Exposure-Efficacy Analysis: Total antibody AUC in Plasma



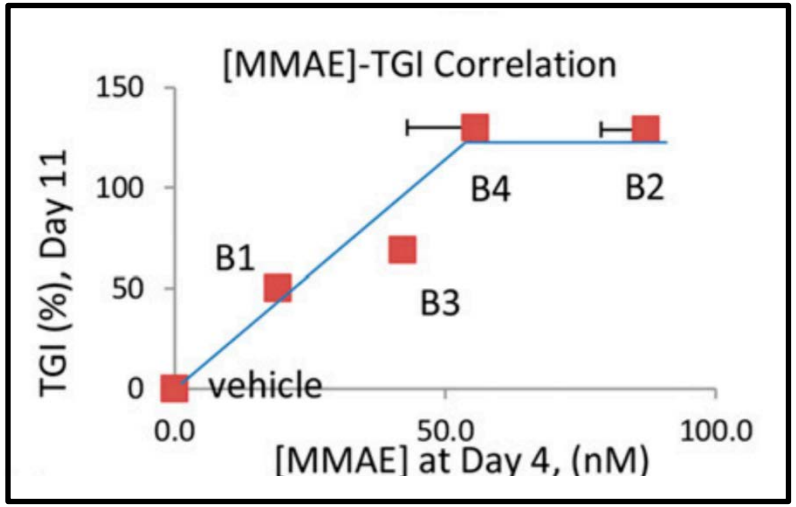



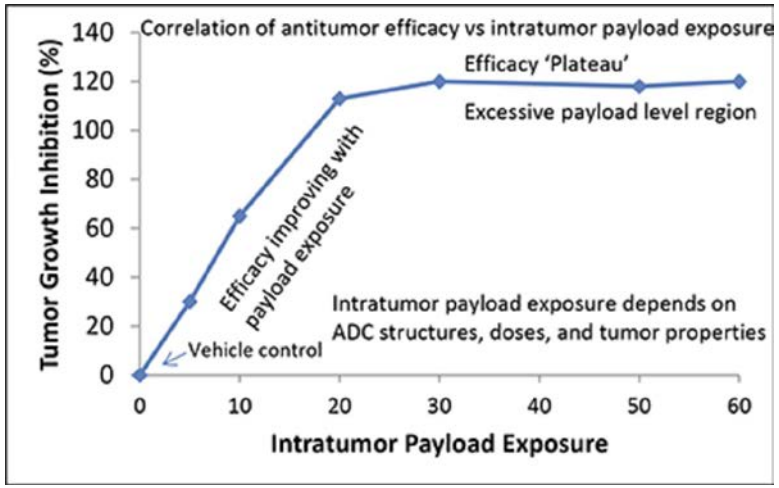

**Total antibody AUC in the circulation can not be correlated with the efficacy.**

Drug Metab Dispos, 47 (10) 1146-1155

# Exposure-Efficacy Analysis: Intratumoral MMAE

| | [MMAE], nM, in tumors Dose (mpk) | Day 4 | Linker |
|---|---|---|---|
| B1 | 20 | 19.1 | Me-S-S- |
| B2 | 20 | 87.1 | Me-S-S-PAB |
| B3 | 20 | 42.1 | DiMe-S-S- |
| B4 | 1 | 55.6 | Val-cit-PAB |

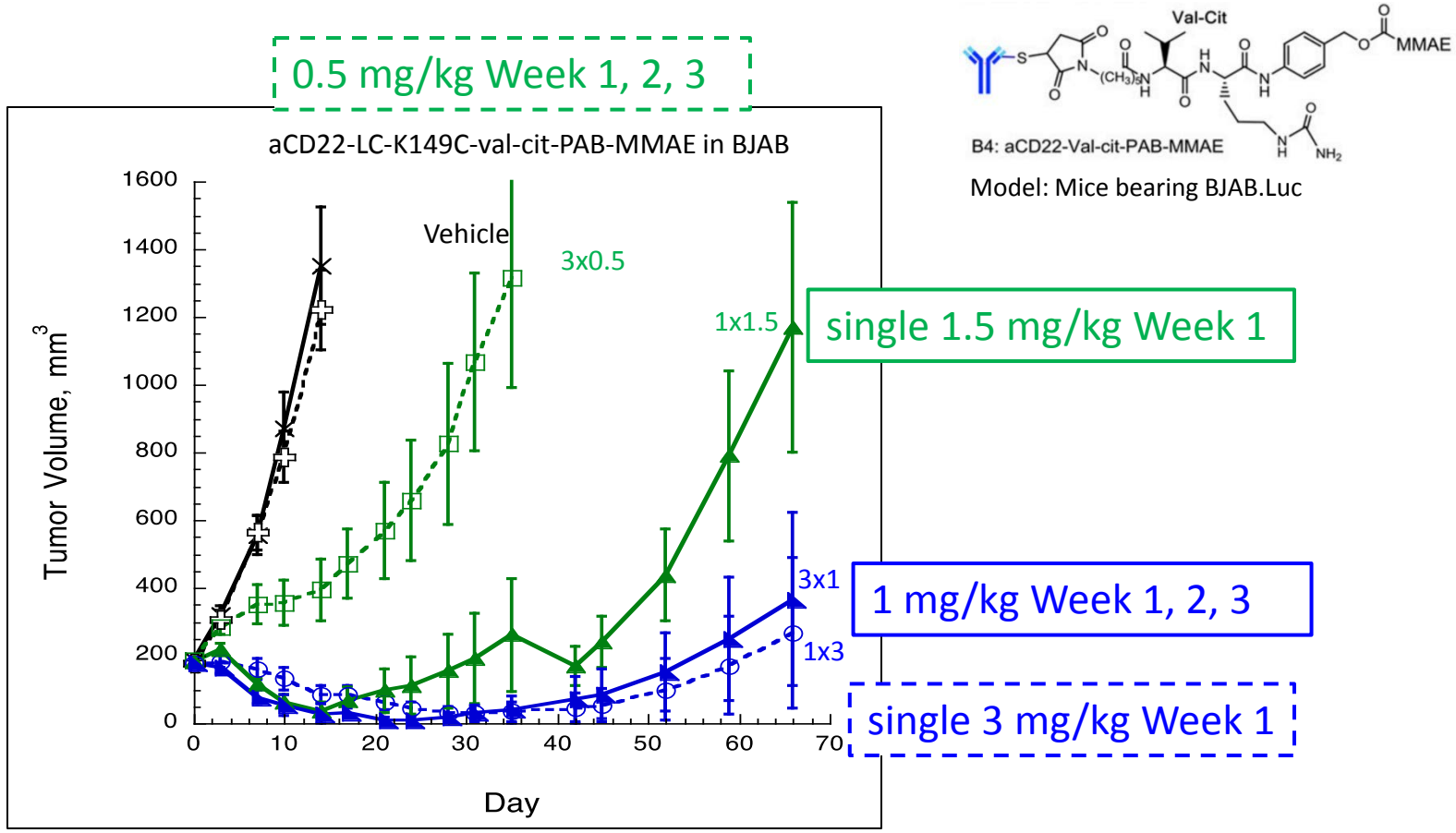



**1) Intratumoral MMAE correlates with ADC efficacy. Intratumoral MMAE concentration on Day 4 is predictive for the TGI on Day 11.**

**2) Excessive drug in tumor did not further improve efficacy.**

# Dose-fractionation Efficacy Studies




**1) The single-dose regimen shows a better efficacy than fractionated dosing, suggesting delivery of payload to a level during the initial period is important.**

**2) When the dose was too high, fractionation did not show difference for efficacy, suggesting delivery of excessive payload to tumor did not further improve the efficacy.**

Drug Metab Dispos, 47 (10) 1146-1155

# Summary on Case 1 and Case 2

- **Intratumoral catabolite correlates with the ADC efficacy.**
- **The systemic exposures of total antibody (Tab) may not rationalize the observed ADC efficacies.**
- **Linker and dose can greatly affect delivery of payload to tumors.**
- **Anti-tumor efficacy can be saturated (‘plateau’). ADC can deliver a threshold level of payload beyond which the efficacy is not further improved but may generate payload in normal tissues for toxicity.**

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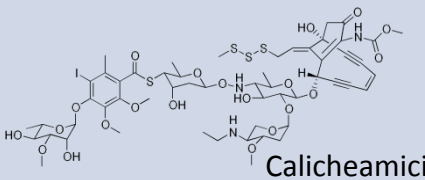
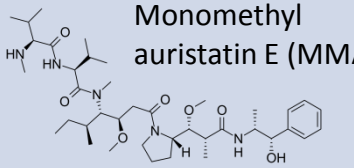
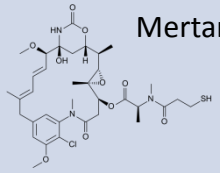
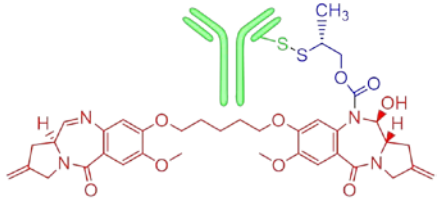
# Case Study 3: Carfilzomib to Be an ADC Payload

***Payloads of ADCs approved before 2019:***

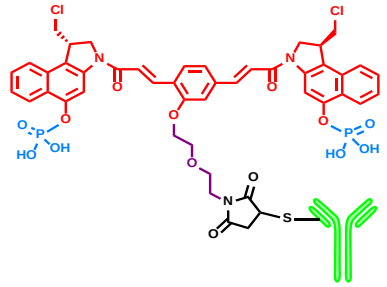
| Drug | Maker | Condition | Trade name | Anti-body | Payload |
|---|---|---|---|---|---|
| Gemtuzumab ozogamicin | Pfizer/Wyeth | relapsed AML | Mylotarg | anti-CD33 |  Calicheamicin |
| Inotuzumab ozogamicin | Pfizer/Wyeth | relapsed or refractory B-cell precursor ALL(CD22+) | Besponsa | anti-CD22 | |
| Brentuximab vedotin | Seattle Genetics/Takeda | relapsed HL and relapsed sALCL | Adcetris | anti-CD30 |  Monomethyl auristatin E (MMAE) |
| Polatuzumab vedotin-piiq | Genentech, Roche | relapsed or refractory DLBCL | Polivy | anti-CD79b | |
| Trastuzumab emtansine | Genentech, Roche | mBC(Her2+) | Kadcyla | anti-Her2 |  Mertansine (DM1) |
| Moxetumomab pasudotox | AstraZeneca | relapsed or refractory HCL | Lumoxiti | anti-CD22 | *Pseudomonas* exotoxin A (PE38) |

# Different kinds of ADC payload

***Payloads of ADCs in R&D:***

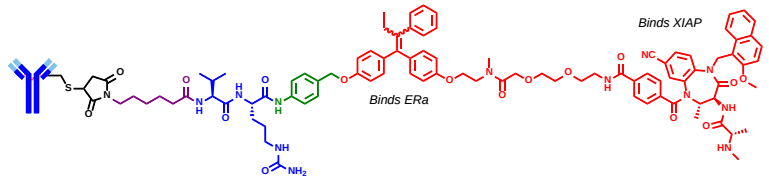


pyrrolobenzodiazepine (PBD)

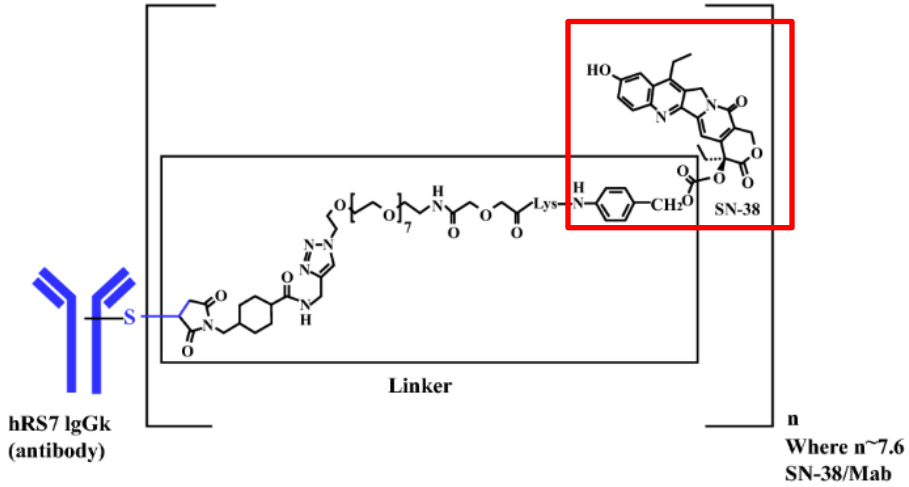


cyclopropabenzindolone (CBI)

proteolysis targeting chimera (PROTAC)

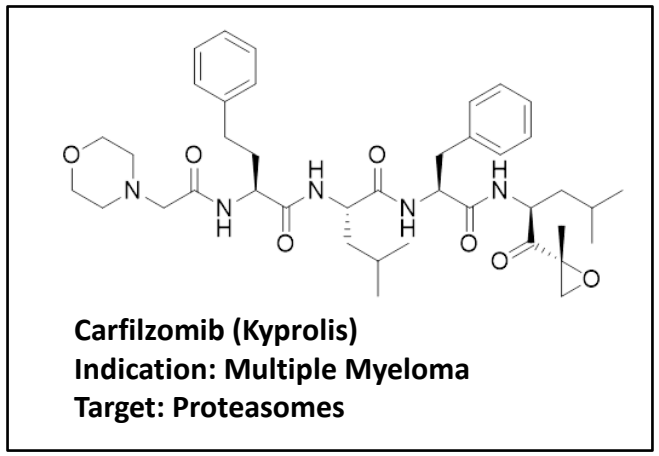



***Sacituzumab govitecan has been approved to treat TNBC in patients in April 2020***

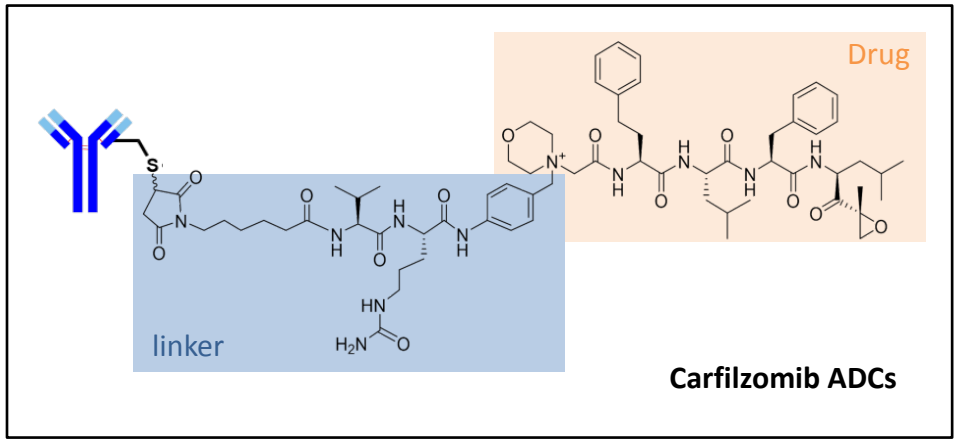



SN-38: topoisomerase I inhibitor; active metabolite of irinotecan, a chemo drug to treat colon cancer and small cell lung cancer

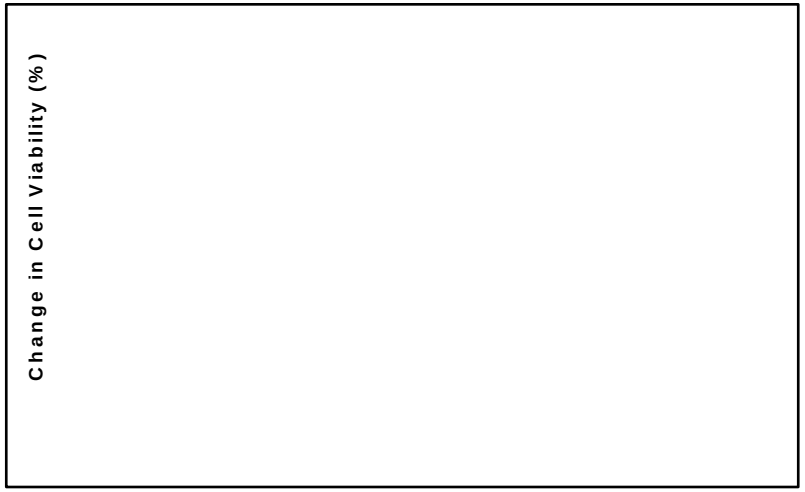
# Carfilzomib: Conjugation to Antibodies



**Carfilzomib (Kyprolis)**
**Indication: Multiple Myeloma**
**Target: Proteasomes**



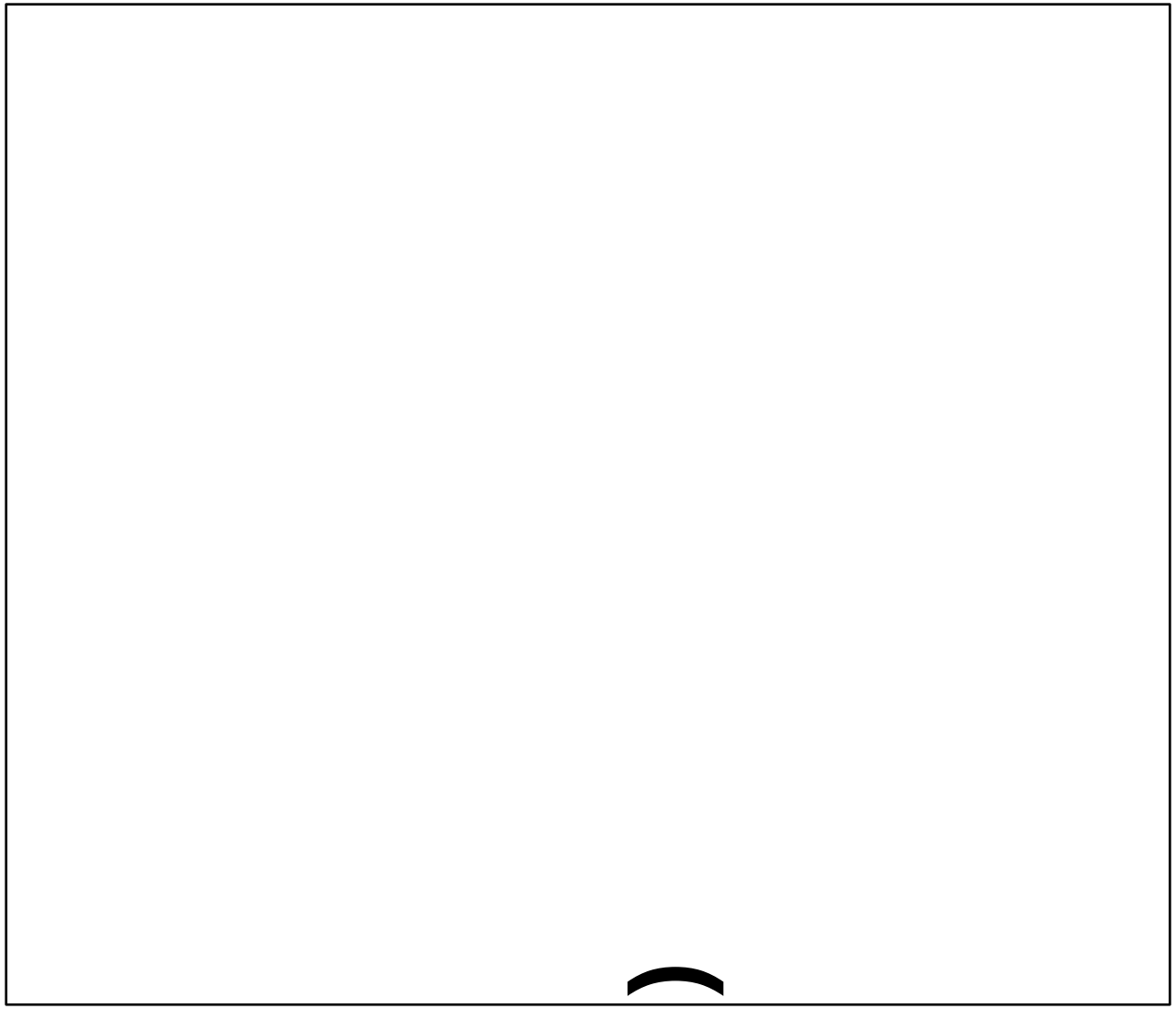

## Free Carfilzomib in cancer cell lines




Drug Metab Dispos, 47 (8), 884-889

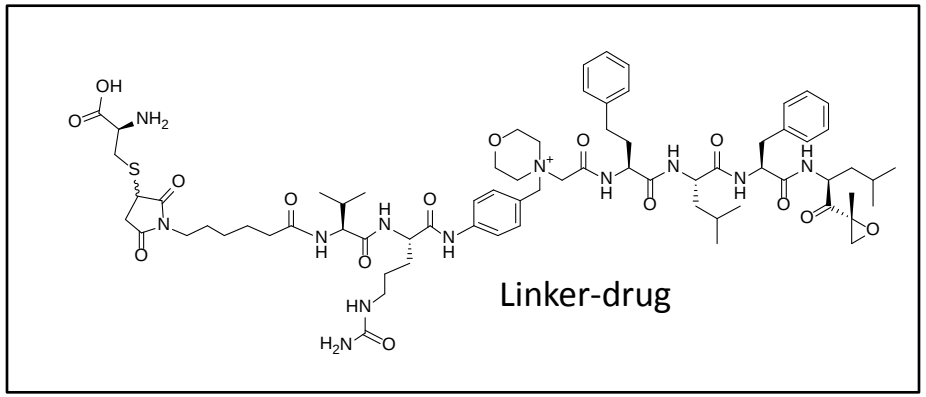
# Carfilzomib: Potent as Free Drug, but NOT as ADC Payload

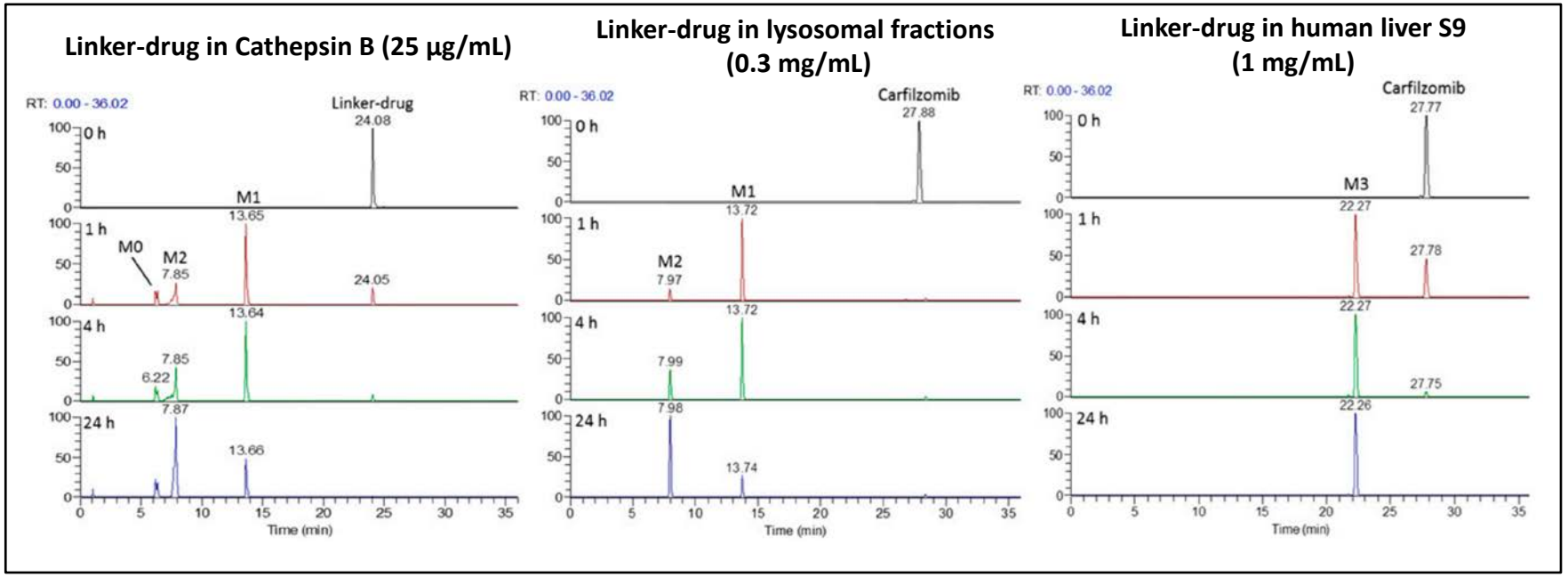
**Carfilzomib ADCs in cancer cell lines**



Drug Metab Dispos, 47 (8), 884-889

# High-resolution MS analysis to identify metabolites

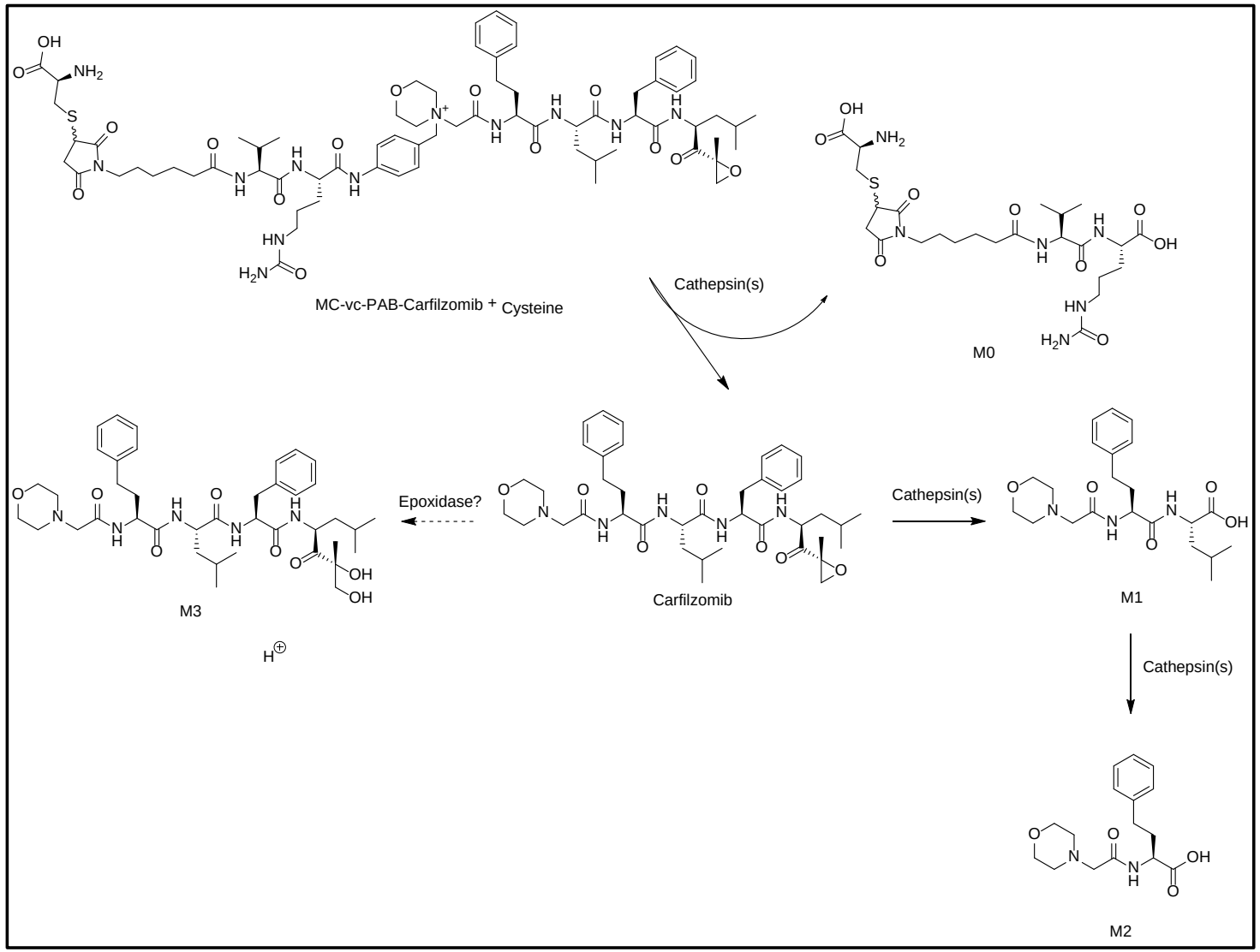



Drug Metab Dispos, 47 (8), 884-889

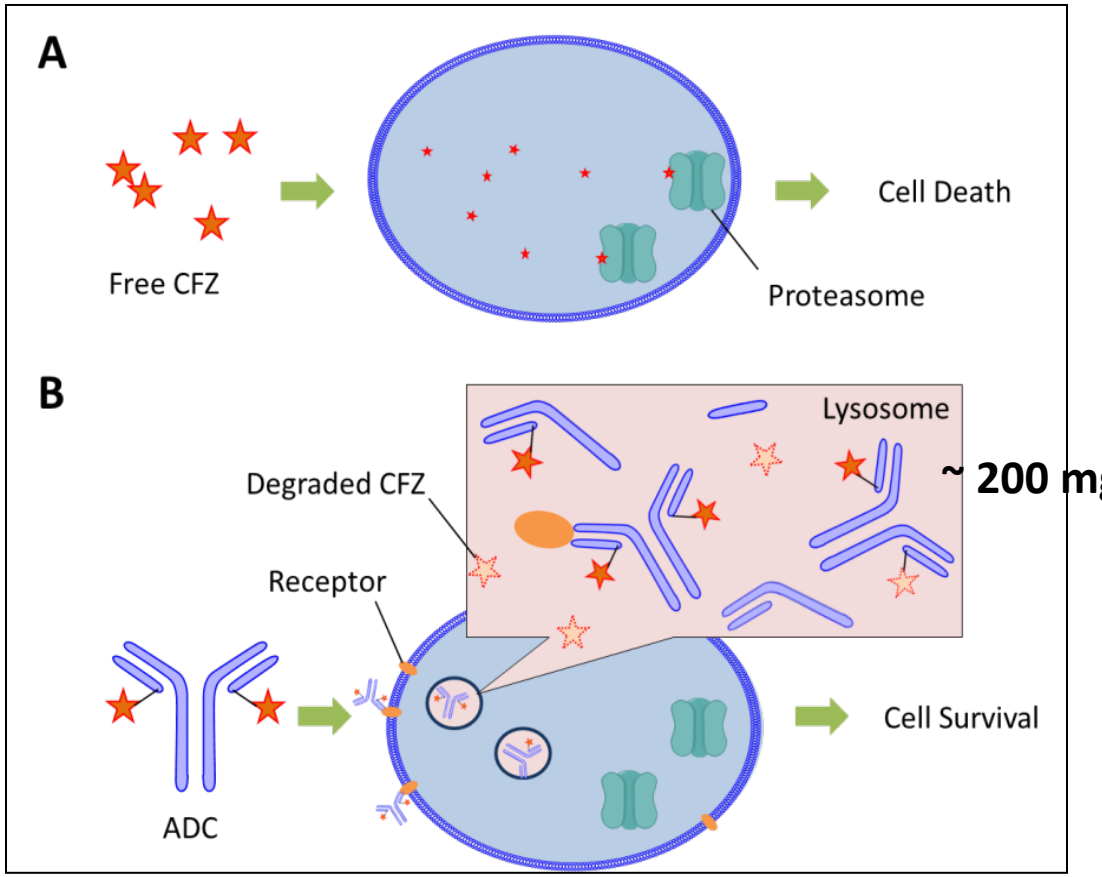
# What Occurs to Carfilzomib in Lysosomes?

## The cleavage of linker-drug in lysosome fractions




Drug Metab Dispos, 47 (8), 884-889

# Carfilzomib: Free Drug or Payload?




**~ 200 mg/mL**

**Conclusion: The payload of ADCs should not be deactivated in the lysosomes.**

Drug Metab Dispos, 47 (8), 884-889

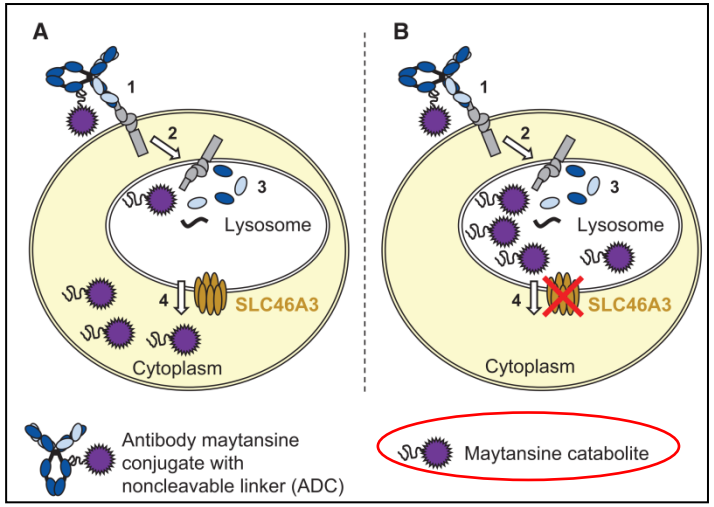
# An Interesting Report from Amgen

**Conclusion: The active species may need a transporter to get out of the lysosomes.**

Therapeutics, Targets, and Chemical Biology

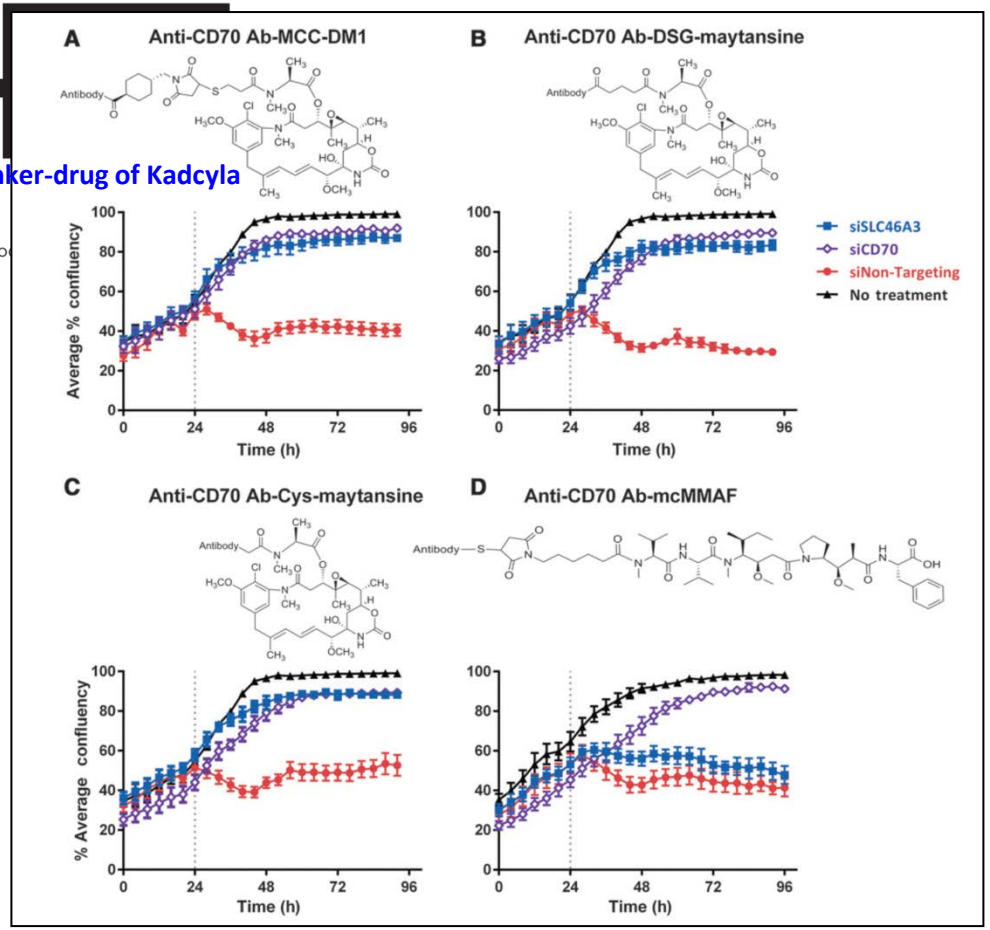
## SLC46A3 Is Required to Transport Catabolites of Noncleavable Antibody Maytansine Conjugates from the Lysosome to the Cytoplasm

Kevin J. Hamblett[1], Allison P. Jacob[1], Jesse L. Gurgel[1], Mark E. Tometsko[1], Brooke M. Ro
Sonal K. Patel[2], Robert R. Milburn[3], Sophia Siu[4], Seamus P. Ragan[5], Dan A. Rock[2],
Christopher J. Borths[3], Jason W. O'Neill[4], Wesley S. Chang[6], Margaret F. Weidner[1],
Matthew M. Bio[3], Kim C. Quon[1], and William C. Fanslow[1]

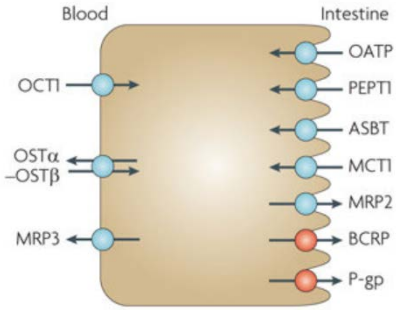



Active species: Linker-drug complex

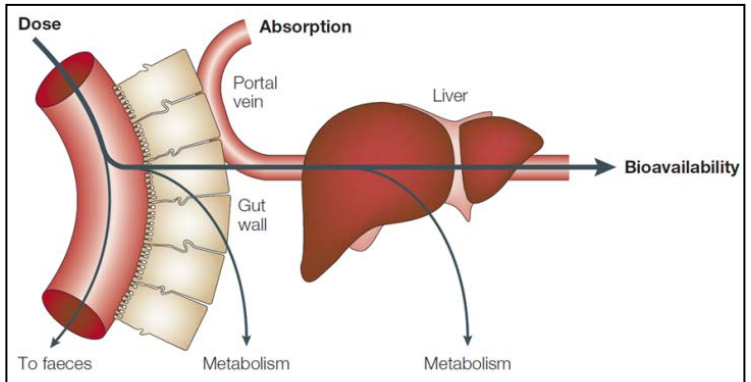
Linker-drug of Kadcyla



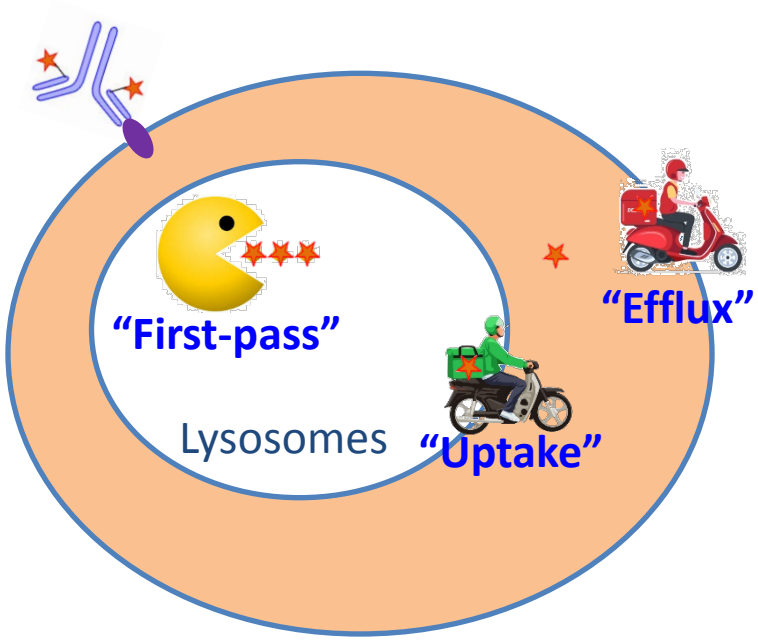
# Similarities: Free Drugs and ADC Payloads




Nat Rev Drug Discov. 2010 Mar;9(3):215-36.




Nat Rev Drug Discov. 2003 Mar;2(3):192-204



# Summary on Case 3

- **A criteria to select a payload: potent, easy to conjugate, and also stable enough in the lysosomes.**

- **Enzymes and tranporters have different effects on the free ADC payload.**

# Acknowledgements


**DMPK**
Donglu Zhang
Hoa Le
Chenghong Zhang
Buyun Chen
Pingping Lu
Liling Liu
Peter Fan
Emile Plise
Jonathan Cheong
Jonathan Wang
Dewakar Sangaraju
Alan Deng
Brain Dean
Marcel Hop

**Chemistry**
Pete Dragovich
Tom Pillow
Leanna Staben
Stefan Koenig
Zhonghua Pei
Bin-Qing Wei
Oleg Mayba

**Biology**
Carter Fields
Jun Guo
Ginny Li
Gail Phillips

**BCP**
Kathy Kozak
Josefa Chuh
Aimee Fourie
Philip Chu
Yichin Liu

**Conjugation**
Hans Erickson
Jack Sadowsky
Breanna Vollmar
Martine Darwish
Pragya Adhikai
Neelie Zacharias
Rachana Ohri
James Ernst
Dick Vandlen

**BAS**
Dian Su
Luna Liu
Carl Ng
Jintang He
Surider Kaur
Keyang Xu

**Transl Oncology**
Shang-Fan Yu
Geoff Del Rosario
Jeff Lau
Rebecca Rowntree
Susan Spencer
Paul Polaski
Andy Polson

**PTPK**
Isabel Figueroa
Amrita Kamath
Doug Leipold
Ben Shen
Saileta Prabhu

**Tox**
Melissa Schutten
Chris Frantz
Hong Wang

# *Thank you!*