Antibody drug conjugate (ADC)

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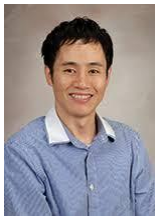
Texas Therapeutics Institute

August 15, 2023
TIPS-CTTP Drug Discovery Course

The Brown Foundation
Institute of Molecular Medicine
for the Prevention of Human Diseases

The University of Texas
Health Science Center at Houston

Next-Generation Antibody-Drug Conjugates by Novel Linker Technologies

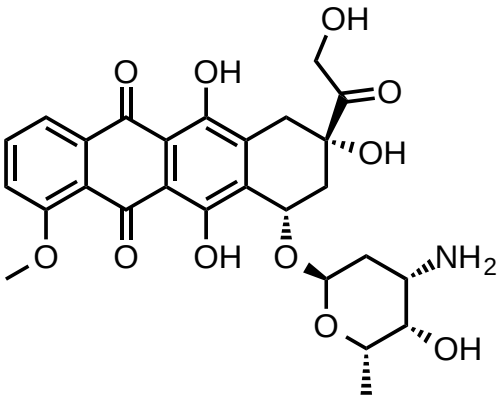


Kyoji Tsuchikama, Ph.D. Medicinal chemist (Associate Professor, UTHealth)

- 8-year experience as PI
- A well-equipped lab for chemistry and pharmacology research
- Successful funding record (NIH R35, DOD BCRP levels 1&2)
- Developed novel ADC linkers (*a key component in this proposal*)
- Published in high-profile journals (*Nature Communications*, *Cell Reports*, *JACS*, etc.)

Why Antibody-Drug Conjugate (ADC)?

Conventional chemotherapeutics



ADC

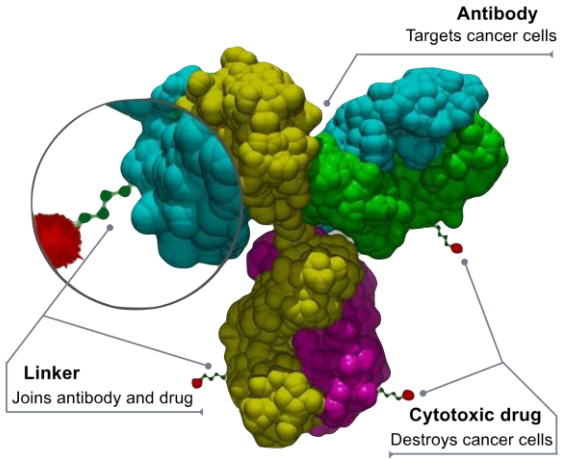


Image from Wikipedia

- Off-target cytotoxicity
- Adverse effects
- Short circulation life ($t_{1/2}$ ~ hours)
  = frequent administrations needed

✓ Powerful cell killing
✓ Broader therapeutic window
✓ Longer circulation life ($t_{1/2}$ ~ 21 days)
  = injection once every 2-3 weeks

An emerging drug class for relapsed and refractory cancers

12 ADCs on the market, ~$20B market share by 2030, >100 ADCs in clinical trials

Success of ADCs

DESTINY Breast04 Trial
(heavily pretreated patients with unresectable or metastatic HER2-low breast cancer)

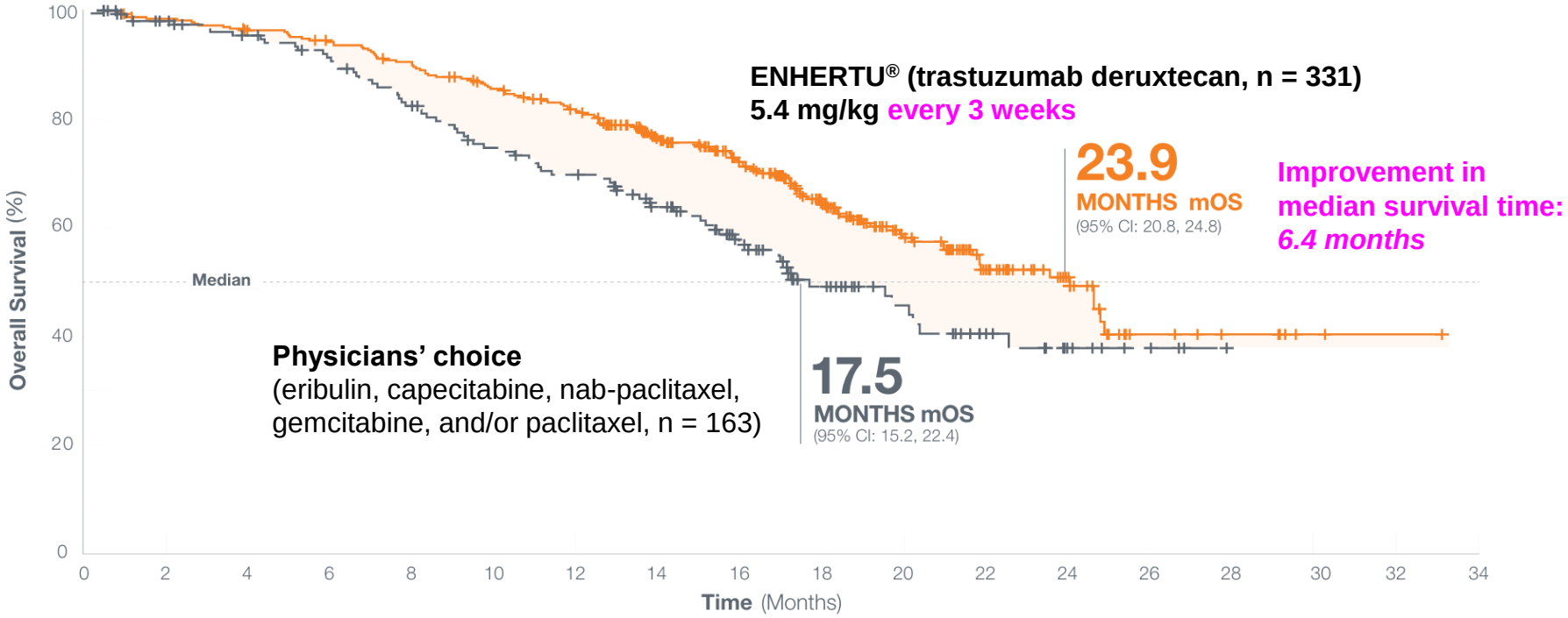
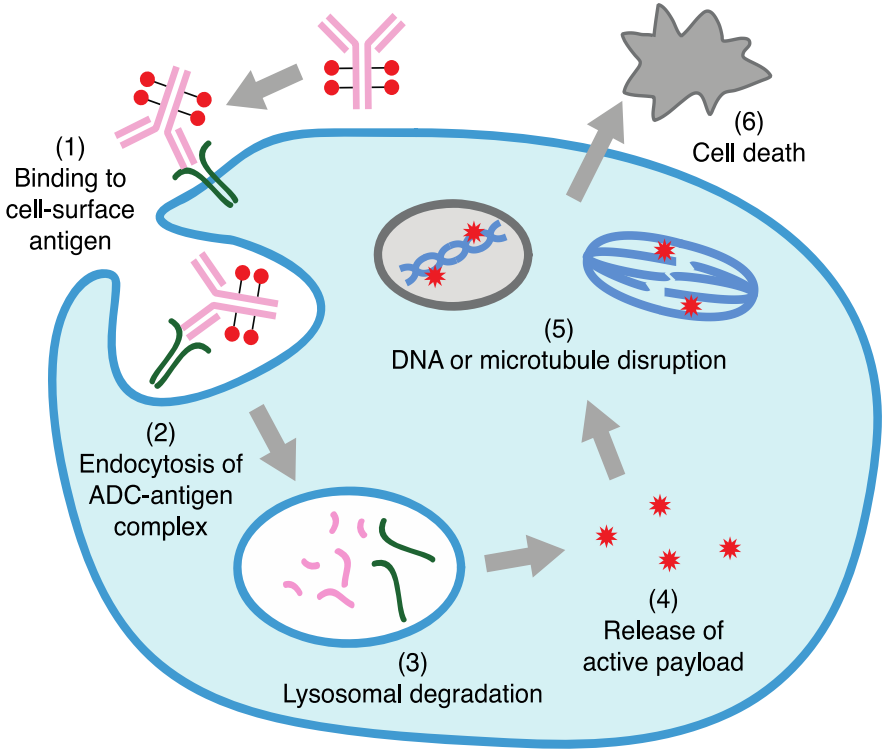


Figure from https://www.enhertuhcp.com/en/her2-low-breast with modifications

Mechanism of Action



Key factors

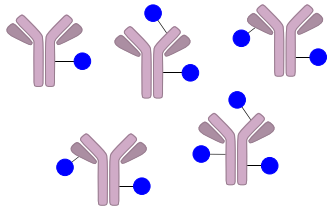
- Tumor-specific antigen
- Internalization rate
- Turnover rate
- Tissue penetration
  (mAb & released payloads)
- Potency of the payload
- (nM–pM IC_{50}, not too low, not too high)
- PK/PD
- ADC Hydrophobicity
- Immunogenicity
- Tolerability

Linker & Conjugation chemistries impact many of these parameters

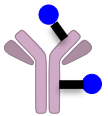
Tsuchikama, K.; An, Z. *Protein Cell* **2018**, *9*, 33–46.

Linker Chemistry Is a Key for Successful ADC Generation

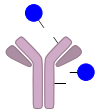
1. ADC homogeneity
   - Most FDA-approved ADCs are heterogeneous; suboptimal for maximal efficacy and safety



2. Linker stability in circulation
   - Premature release of toxic payloads before reaching tumors can lead to systemic toxicity



3. Linker cleavability for rapid release of active payloads
   - To maximize potency, ADC linkers should be degraded immediately after getting into target tumor cells

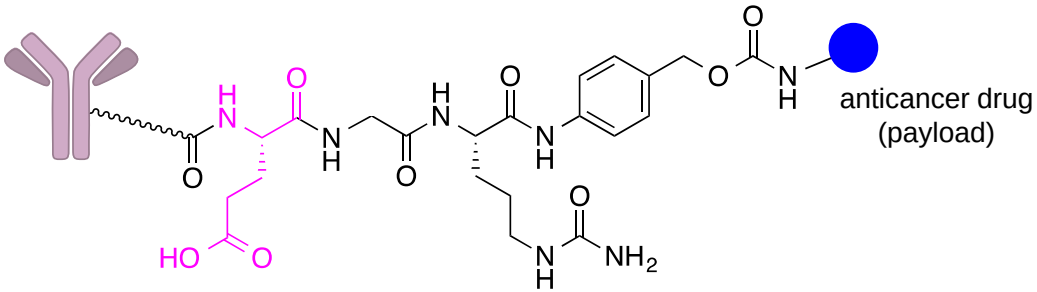


If <u>ALL</u> these issues are optimized *at the same time*…

More efficacious and safer ADCs can be generated!

Our ADC Linker Platform

1) E**G**Cit (Glu**Gly**Cit) enzymatically cleavable linker (a provisional patent filed)

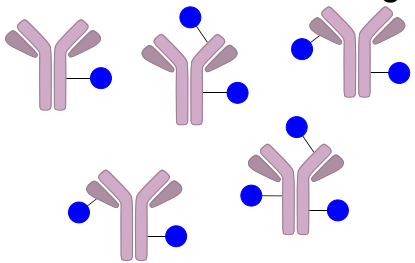


Glu – glutamic acid
Gly – glycine
Cit - citrulline

✓ **Excellent stability, tolerability, and hydrophilicity**
✓ **Rapid intracellular release of active payloads**

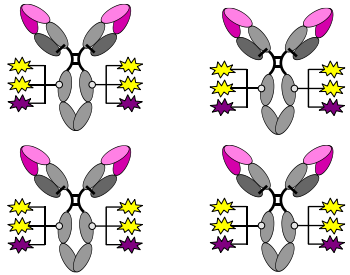
Broader therapeutic window compared to existing ADCs!

2) Enzymatic conjugation using branched linkers (WO2018218004A1, US11629122B2 granted)



Conventional ADC

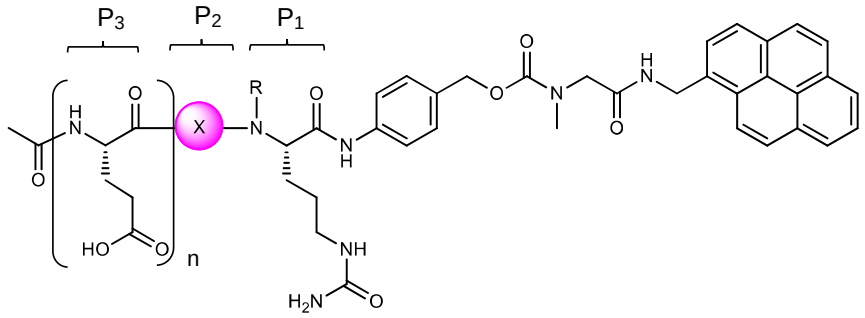
- heterogeneous mixture
- 1 payload/linker
- Strict quality control required

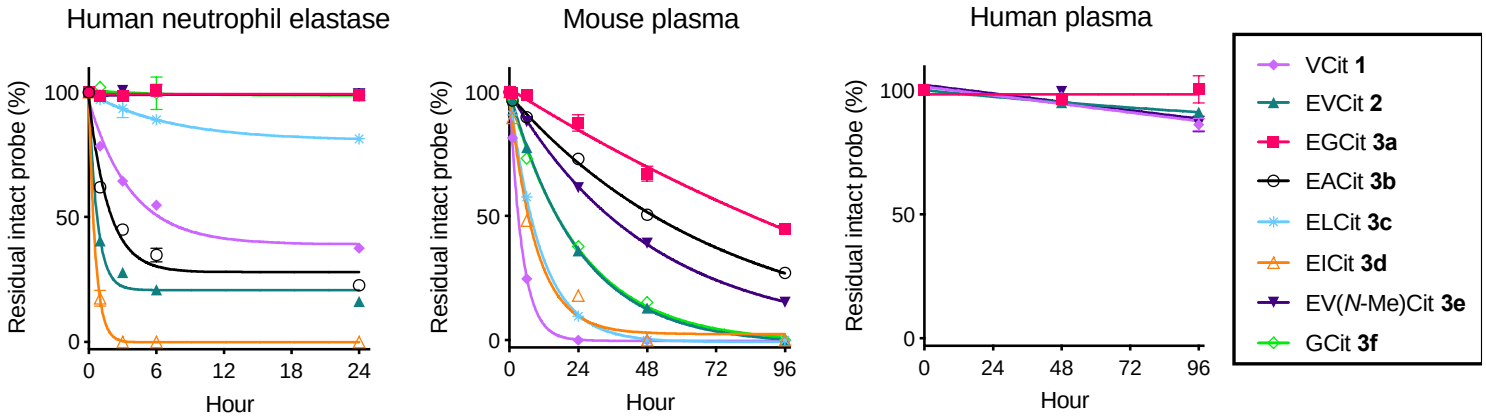


Our ADC

✓ **Homogeneous product**
✓ >2 payload/linker, **dual-drug mode available**
✓ Facile, versatile, simple production, “plug-and-play”

Stability Test



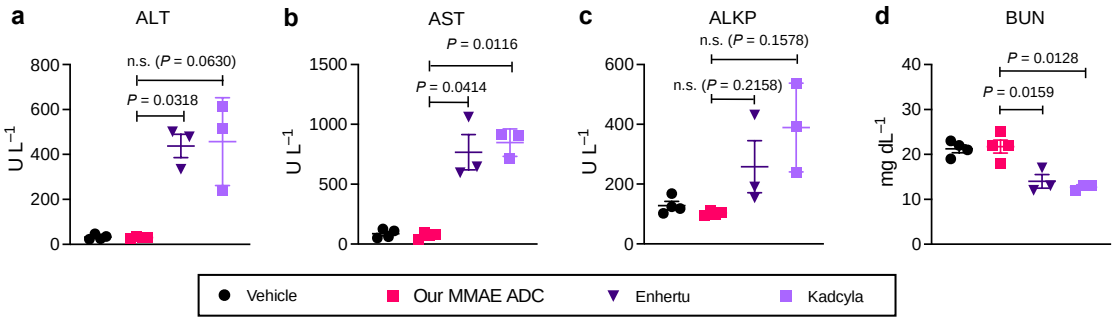


Promising linkers with high stability: EGCit and EV(*N*-Me)Cit

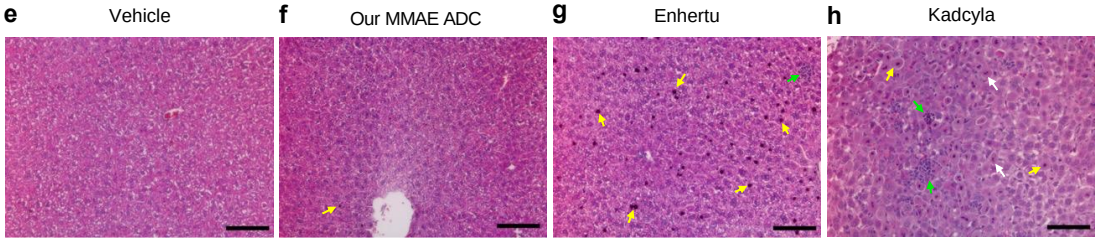
Ha, S.Y.Y., Anami, Y., …, Tsuchikama, K. *Mol. Cancer Ther.* **2022**, *21*, 1449.

EGCit ADCs Show Promising Tox Profile in Mice

Serum Chemistry (80 mg/kg IV single dose, 5 days)



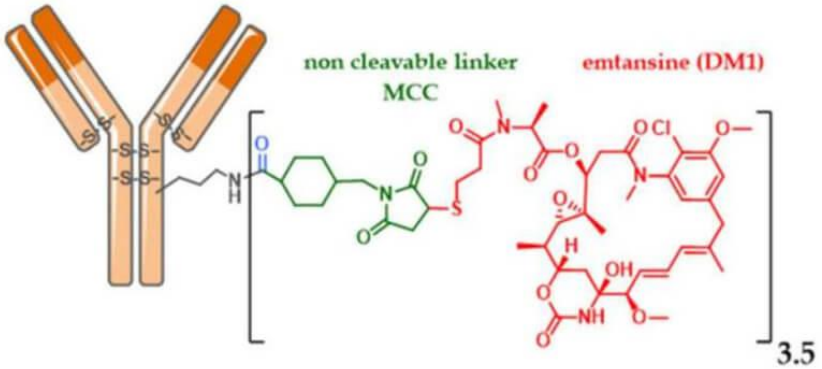
Liver histology (80 mg/kg IV single dose, 5 days)



- Maximum tolerated dose in healthy mice: >> 80 mg/kg
- No sign of liver or blood toxicity, *including neutropenia* at 80 mg/kg
- Rat & monkey tox studies to be performed

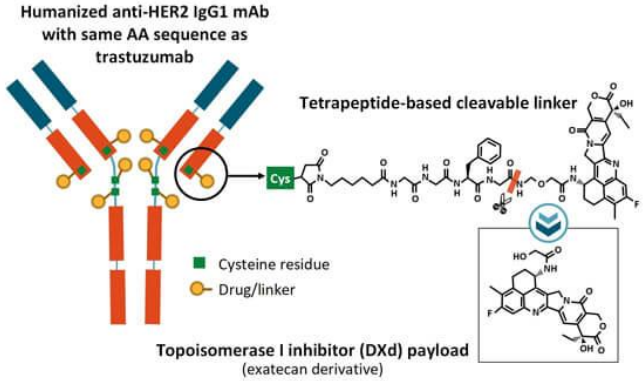
ALT - alanine transaminase
AST - aspartate aminotransferase
ALKP - alkaline phosphatase
BUN - blood urea nitrogen

Ha, S.Y.Y., Anami, Y., …, Tsuchikama, K. *Mol. Cancer Ther.* **2022**, *21*, 1449.



anti-HER2 Kadcyla® (ado-trastuzumab emtansine)

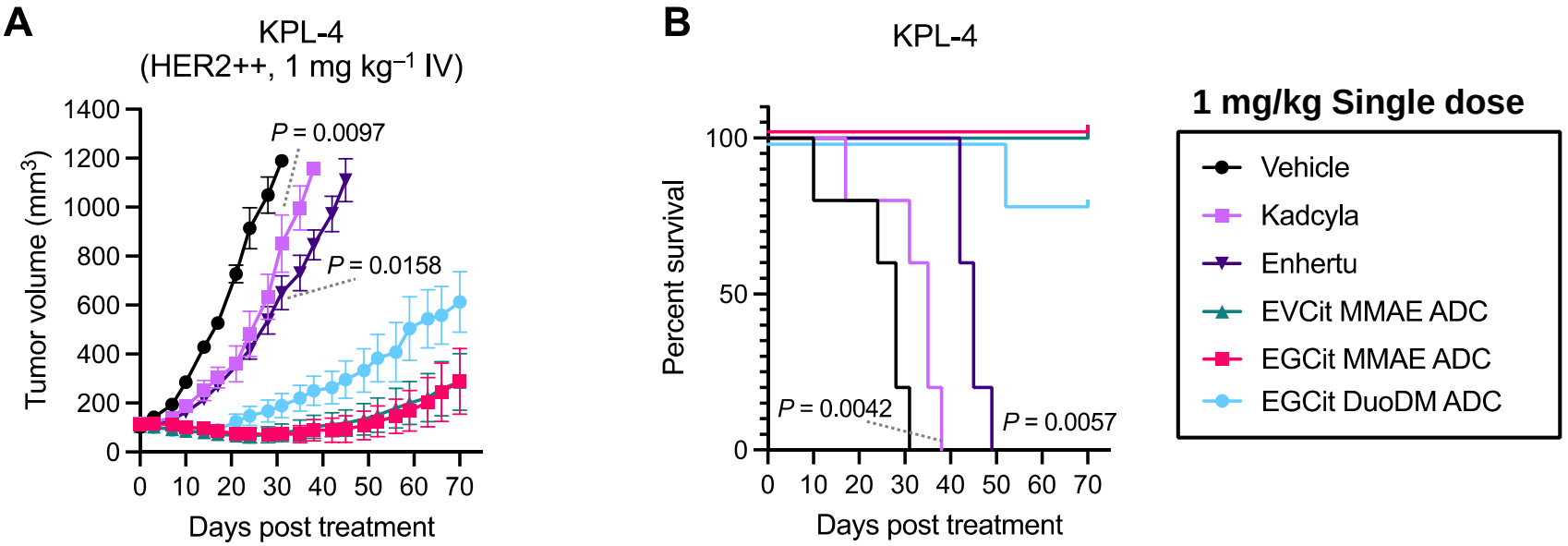
HER2-Targeted ADC: Trastuzumab Deruxtecan



- High drug:antibody ratio: ~8
- Stable linker-payload
- Tumor-selectable cleavable linker
- High potency, membrane-permeable payload with short systemic half-life
- Bystander killing effect

Nakada. Chem Pharm Bull (Tokyo). 2019;67:173. Trail. Pharmacol Ther. 2018;181:126. Ogitani. Cancer Sci. 2016;107:1039.

EGCit linker ensures maximal in vivo efficacy

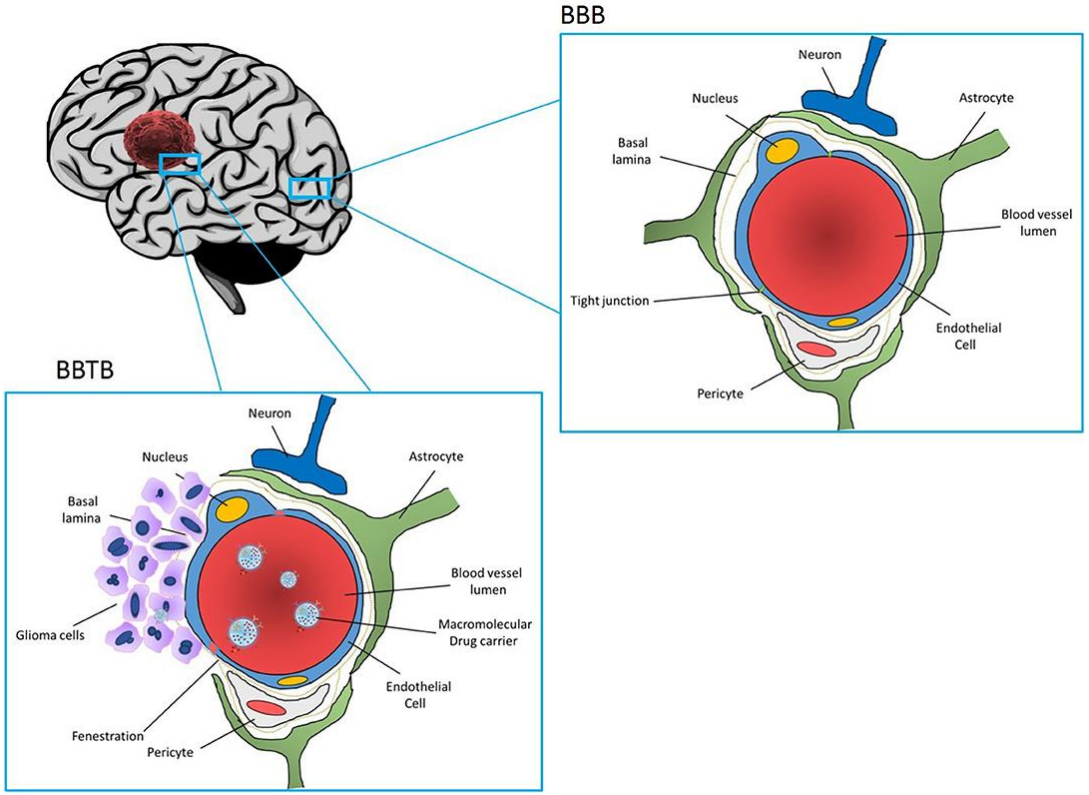


- **EGCit ADC** showed excellent treatment efficacy
- No negative impact on efficacy by replacing conventional Valine (V) with Glycine (G)

KPL-4 – breast cancer cell line

Anami, Y., Yamazaki, C.M., …, Tsuchikama, K. *Nat. Commun.* **2018**, 9:2512.
Ha, S.Y.Y., Anami, Y., …, Tsuchikama, K. *Mol. Cancer Ther.* **2022**, *21*, 1449.

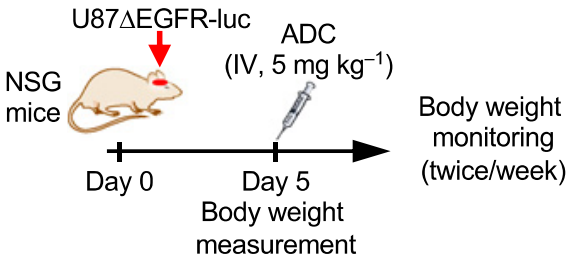
ADC Delivery to Brain Tumors Is a Big Challenge

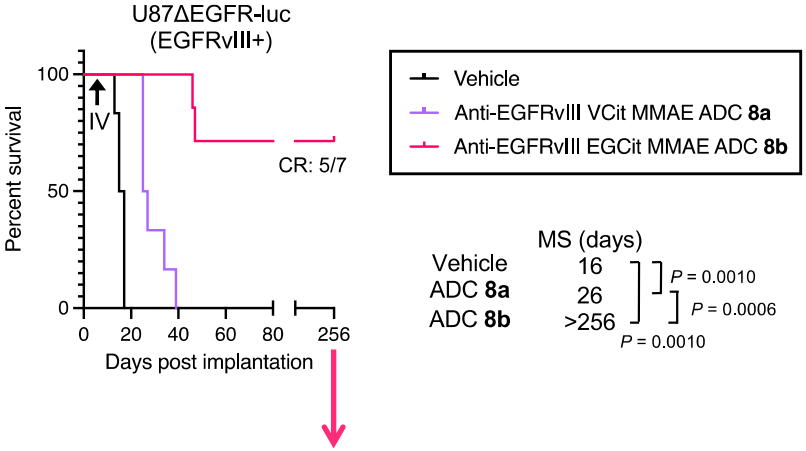


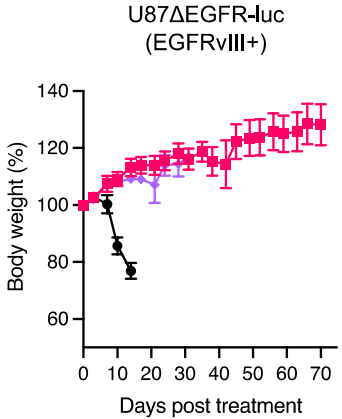
Both blood-brain barrier (BBB) and blood-brain tumor barrier (BBTB) restrict an influx of ADCs.

Figure from Raucher et al. (2018) Front. Oncol. 8:624.

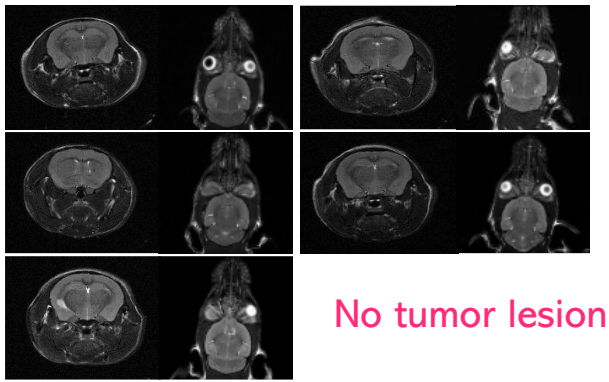
Our EGCit ADC Eradicated GBM Effectively







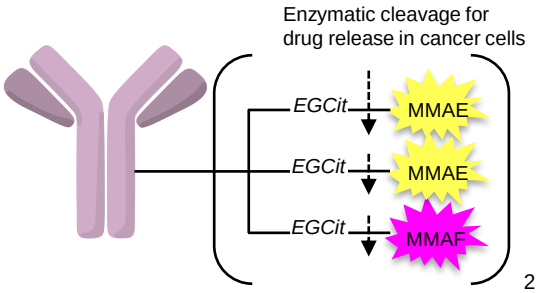
MRI (EGCit MMAE ADC **8b**, Day 256 post implantation)



No tumor lesion detected (CR: 5/7)

Anami et al., Cell Reports, **2022,** *39,* 110839.
Ha, S.Y.Y., Anami, Y., …, Tsuchikama, K. *Mol. Cancer Ther.* **2022**, *21*, 1449.

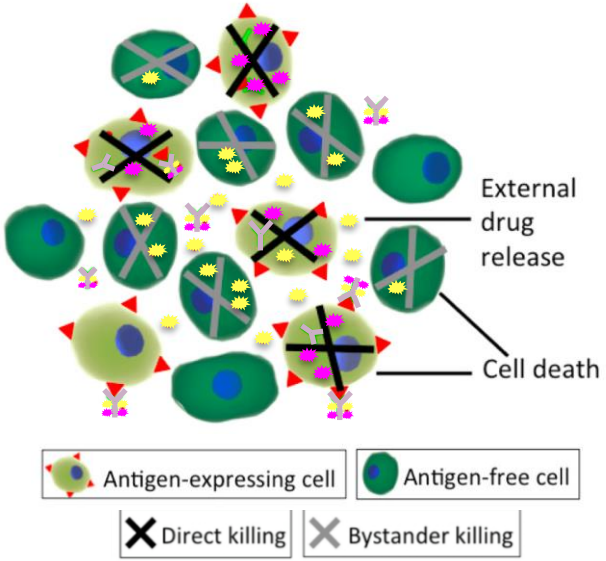
Dual-Drug ADC: a Two-in-One Platform



2

ADC equipped with two different drugs (Dual-drug ADC)

- MMAE kills both antigen +/– cells upon release (bystander effect)
- MMAF quickly kills drug-resistant antigen + cells

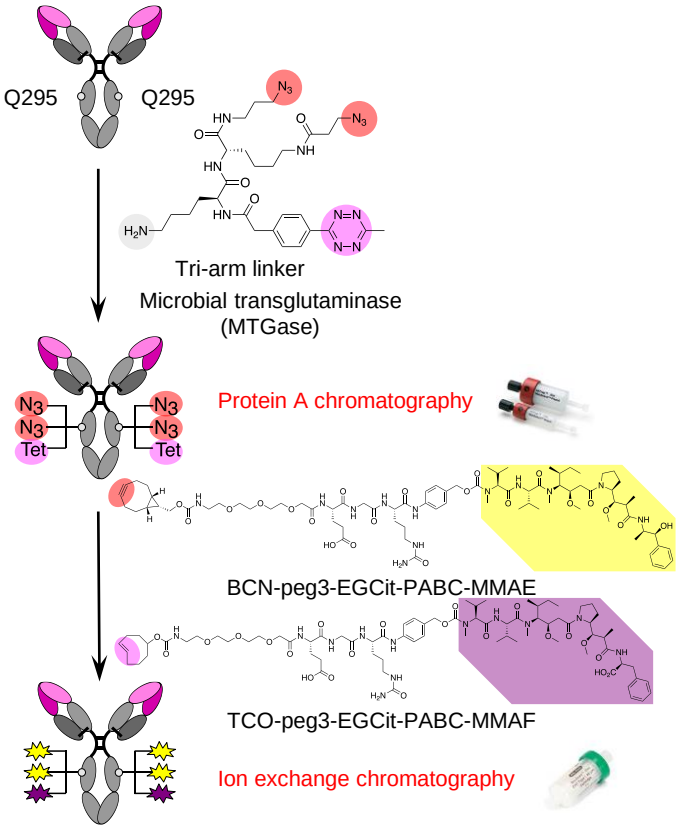


<u>Advantages</u>

✓Dual modes of action

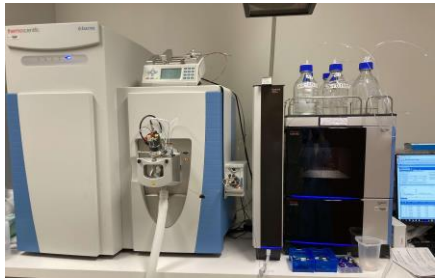
✓May overcome **drug resistance** and **cancer heterogeneity**

Dual-Drug ADC Production (~200 mg, 2 weeks)



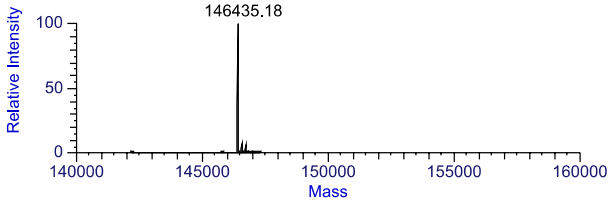
Overall yield: 70-75%

Thermo Q Exactive Orbitrap Mass Spectrometer

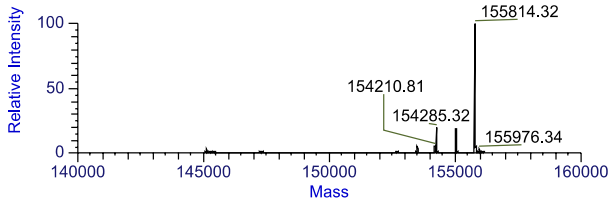


Bio-Rad NGC Liquid Chromatography System

mAb-linker conjugate

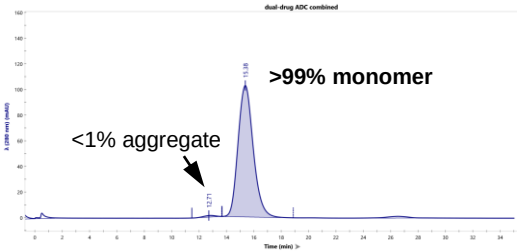


Dual-drug ADC (DAR 4+2)



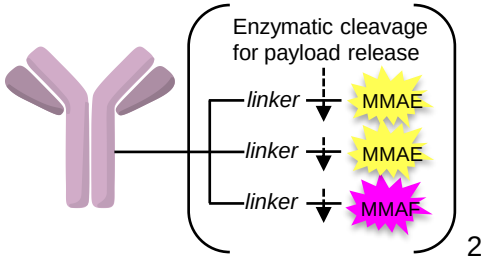
Size-exclusion chromatography

- Final characterization



Aggregate-, bioburden-, and endotoxin-free

Dual-Drug ADC is Effective for Heterogeneous Breast Cancer

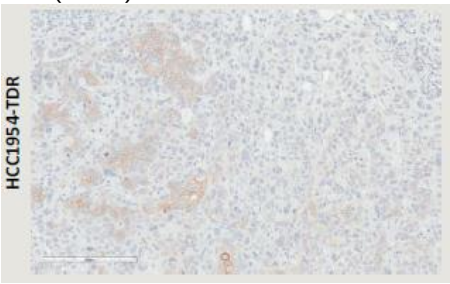


2

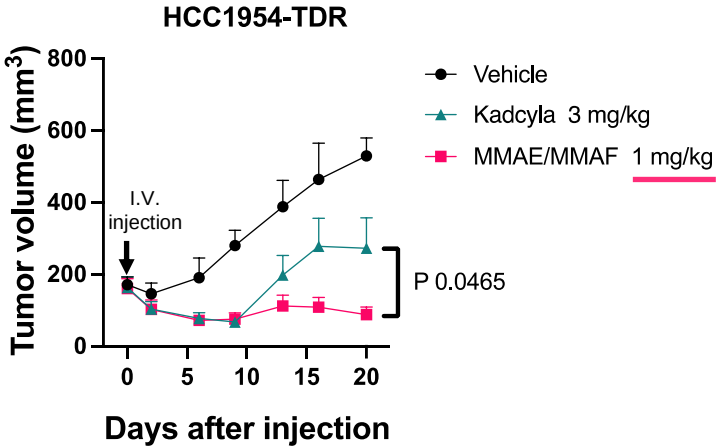
Anti-HER2 ADC equipped with two different drugs

- MMAE: bystander killing of antigen +/- cells
- MMAF: great potency to antigen + cells

IHC (HER2)

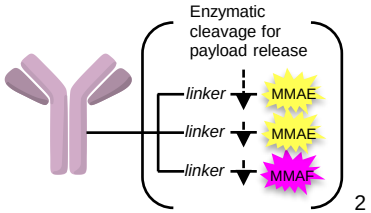


HER2-low heterogeneous breast cancer:
no effective cure is currently available
an unmet need



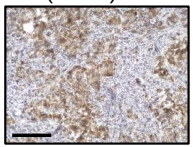
Our dual-drug ADC was more effective (TGI%: 120%) than Kadcyla® (TGI% 69%) *even at a lower dose*!

Dual-Drug ADC is Effective for Heterogeneous Breast Cancer

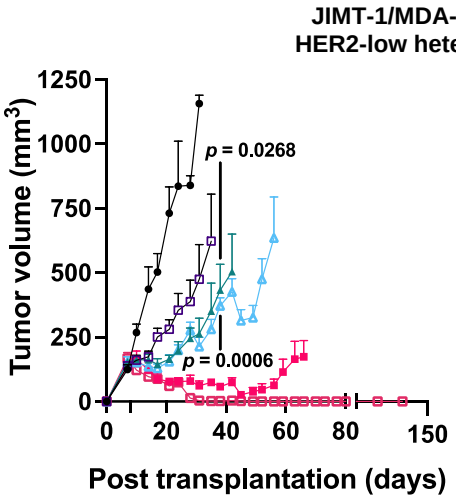


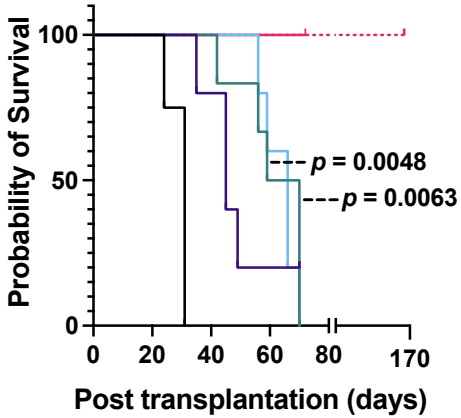
MMAE/MMAF Dual-drug ADC
DAR 4+2

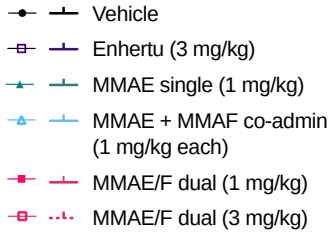
IHC (HER2)



4:1 mix of JIMT-1 (HER2+)
and MDA-MB-231 (HER2–)



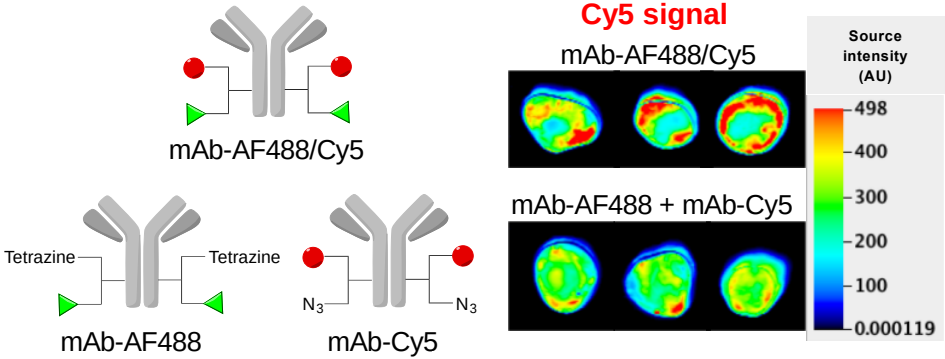


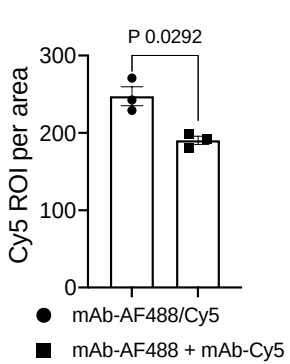


- **MMAE/F dual-drug ADC (drug-to-antibody ratio or DAR 4+2)** showed significantly improved efficacy
- **Dual-drug ADC** was **more effective than a 1:1 cocktail of MMAE and MMAF single-drug ADCs (DAR 4 each)**, demonstrating the advantage of the "two-in-one" ADC design
- Tox studies are underway (preliminary Rat MTD: 25 mg/kg or greater, no neutropenia at MTD)

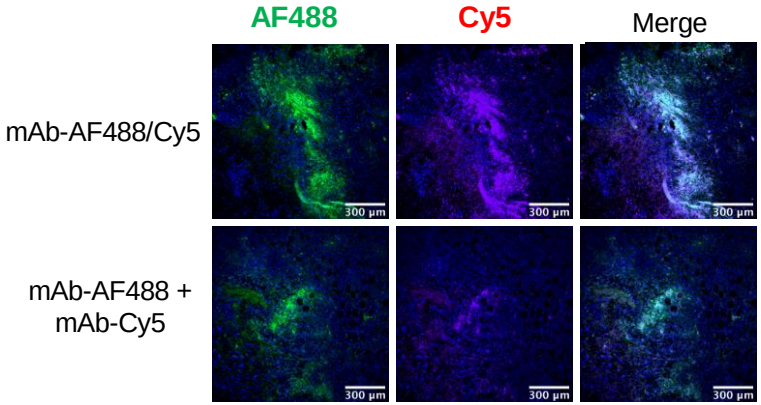
Yamazaki et al., Nature Communications 12:3528 (2021)
Best Preclinical publication in 2021 Award, ADC World San Diego

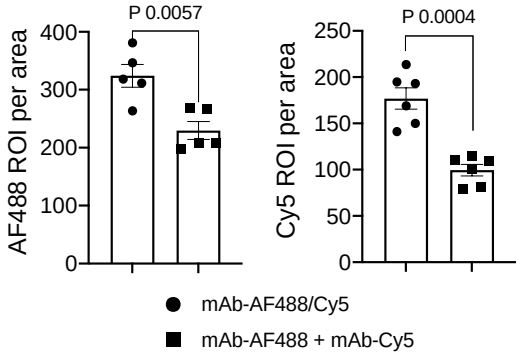
Co-admin of two single ADCs attenuates payload delivery efficiency in HER2-low tumors





JIMT-1/MDA-MB-231 admixed tumors





Yamazaki and Yamaguchi, Nature Communications 12:3528 (2021)

Thank you for your attention!