Antibody-drug conjugates

Literature Talk

Oct 8, 2024

Esther Kang

MacMillan Group

ASCO® AMERICAN SOCIETY OF CLINICAL ONCOLOGY

Annual Conference in 2022

ASCO® AMERICAN SOCIETY OF CLINICAL ONCOLOGY

Annual Conference in 2022

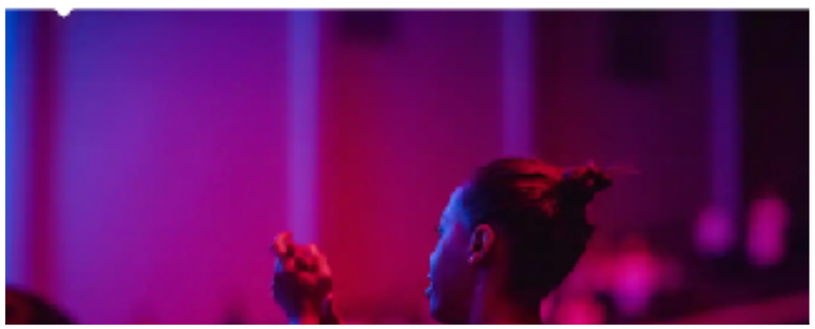
The plenary session at the ASCO 2022 annual meeting saw that rarest of things at a scientific conference: a standing ovation.

The New York Times

Breast Cancer Drug Trial Results in 'Unheard-Of' Survival

For some patients with metastatic tumors not significantly affected by other forms of chemotherapy, the treatment halted their cancer's growth.

A Standing Ovation! Results From DESTINY-Breast04 trial in Breast Cancer



ASCO AMERICAN SOCIETY OF CLINICAL ONCOLOGY

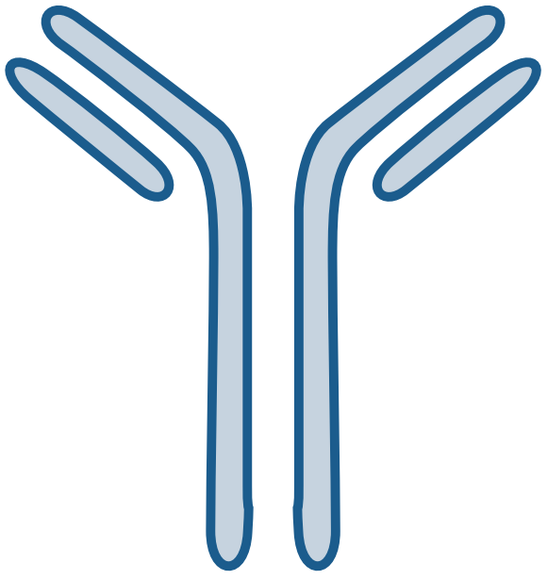
Annual Conference in 2022

Breast cancer trial plenary session

Antibody-drug conjugate (ADC)

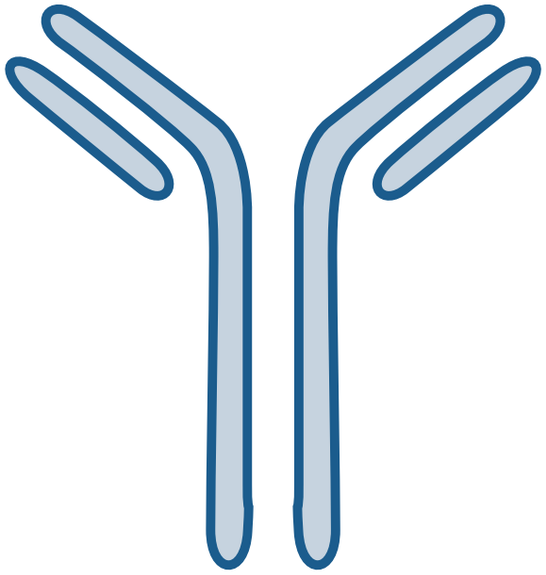
What is antibody-drug conjugate?

What is antibody-drug conjugate?



Monoclonal antibody (mAb)

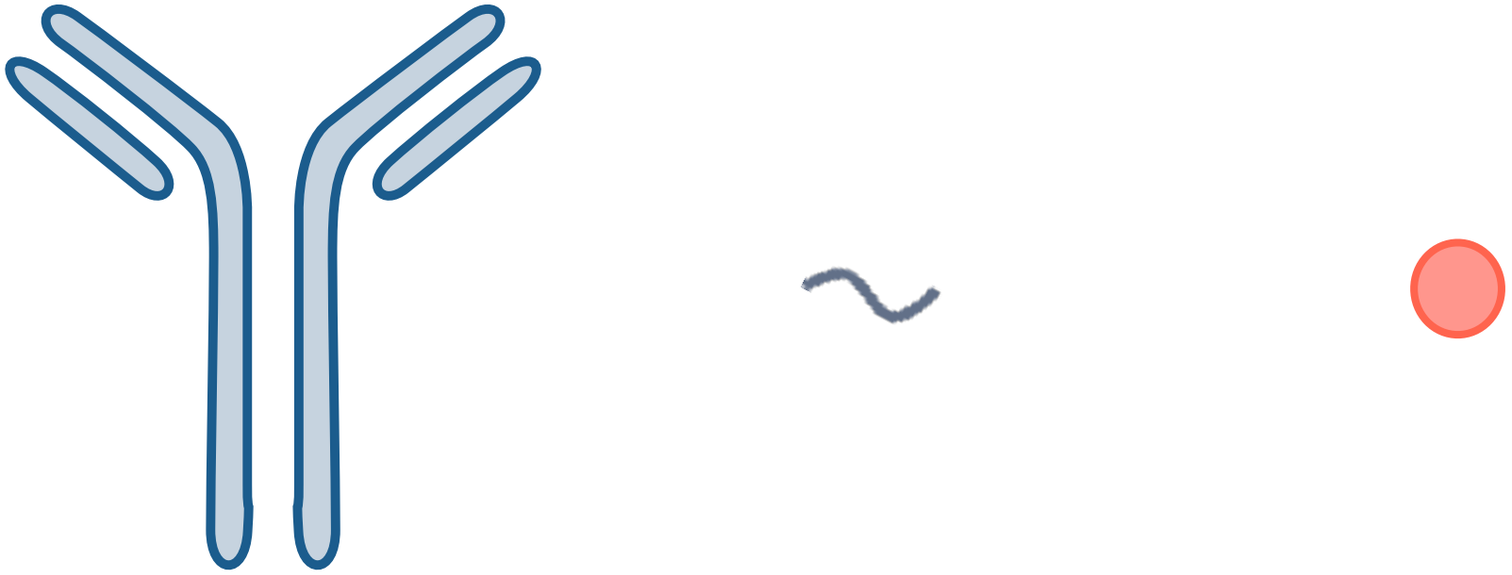
What is antibody-drug conjugate?



Monoclonal antibody (mAb)

Drug

What is antibody-drug conjugate?

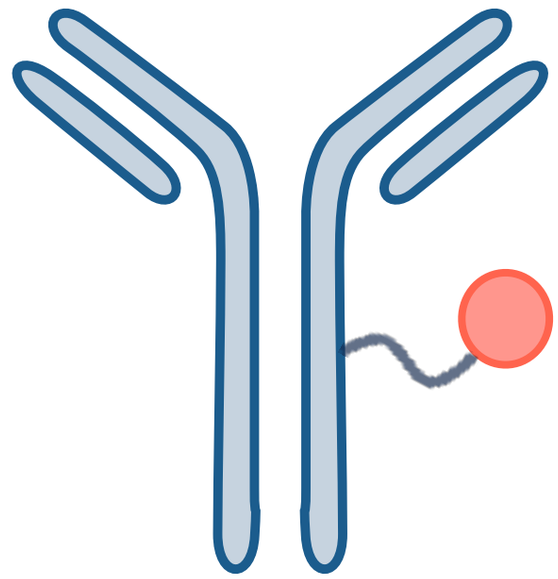


Monoclonal antibody (mAb)

Linker

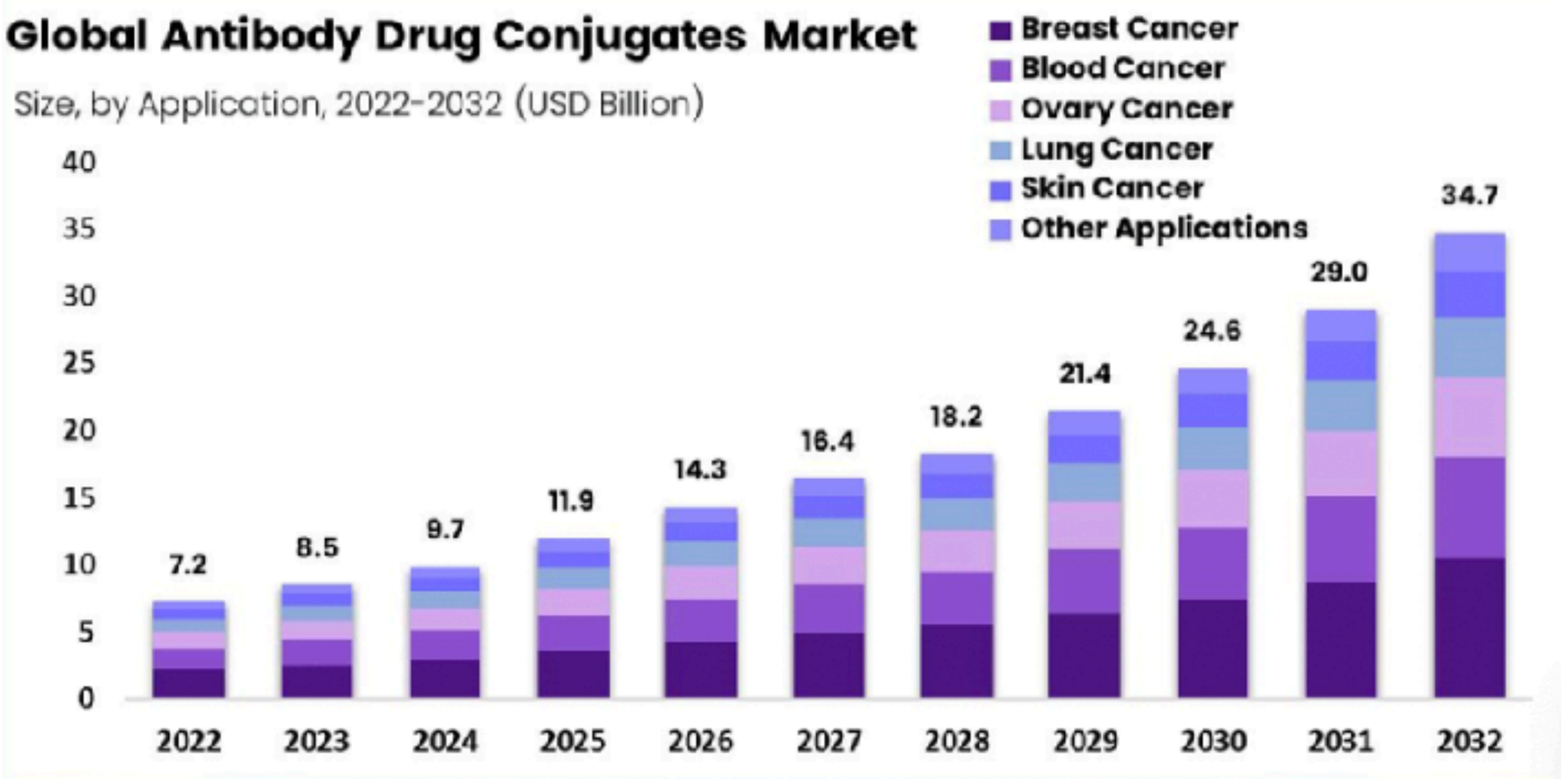
Drug

What is antibody-drug conjugate?



3 main components

- *Antibody*
- *Linker*
- *Drug*



Enhertu™

2023 net revenues

$2.57 bil

(Breast cancer)

Kadcyla™

2023 net revenues

$2.22 bil

(Breast cancer)

Market.us
Annual reports from Daiichi Sankyo and Genentech

Outline

- ***Introduction***
  - ***Structural features of ADC***
  - ***Mechanism of Action***
  - ***FDA-approved ADCs***

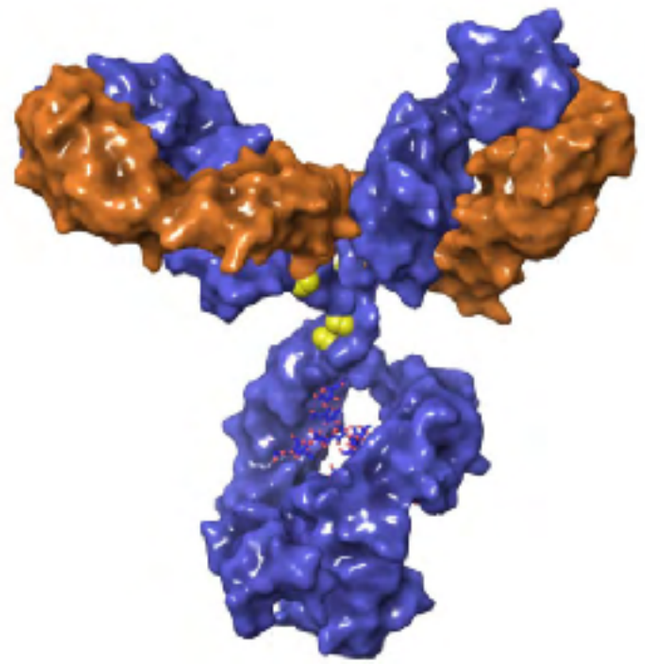
- ***'A' in ADC***
  - ***Antibody alone as a drug***
  - ***Characteristics of antibody drugs***
  - ***'A' versus 'ADC'***

- ***'C' in ADC***
  - ***Bioconjugation***
  - ***Cleavable or non-cleavable***

- ***'D' in ADC***
  - ***Types of 'D'***
  - ***Bystander effect***
  - ***Other types of 'D'***

Antibody-drug conjugate

Structural feature

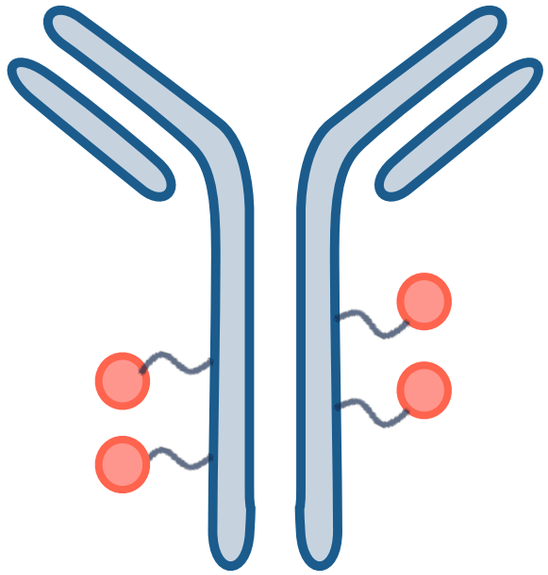


Antibody : Linker-Drug

150,000 Da : 1,000 Da

Antibody-drug conjugate

Structural feature: Drug-to-Antibody Ratio (DAR)

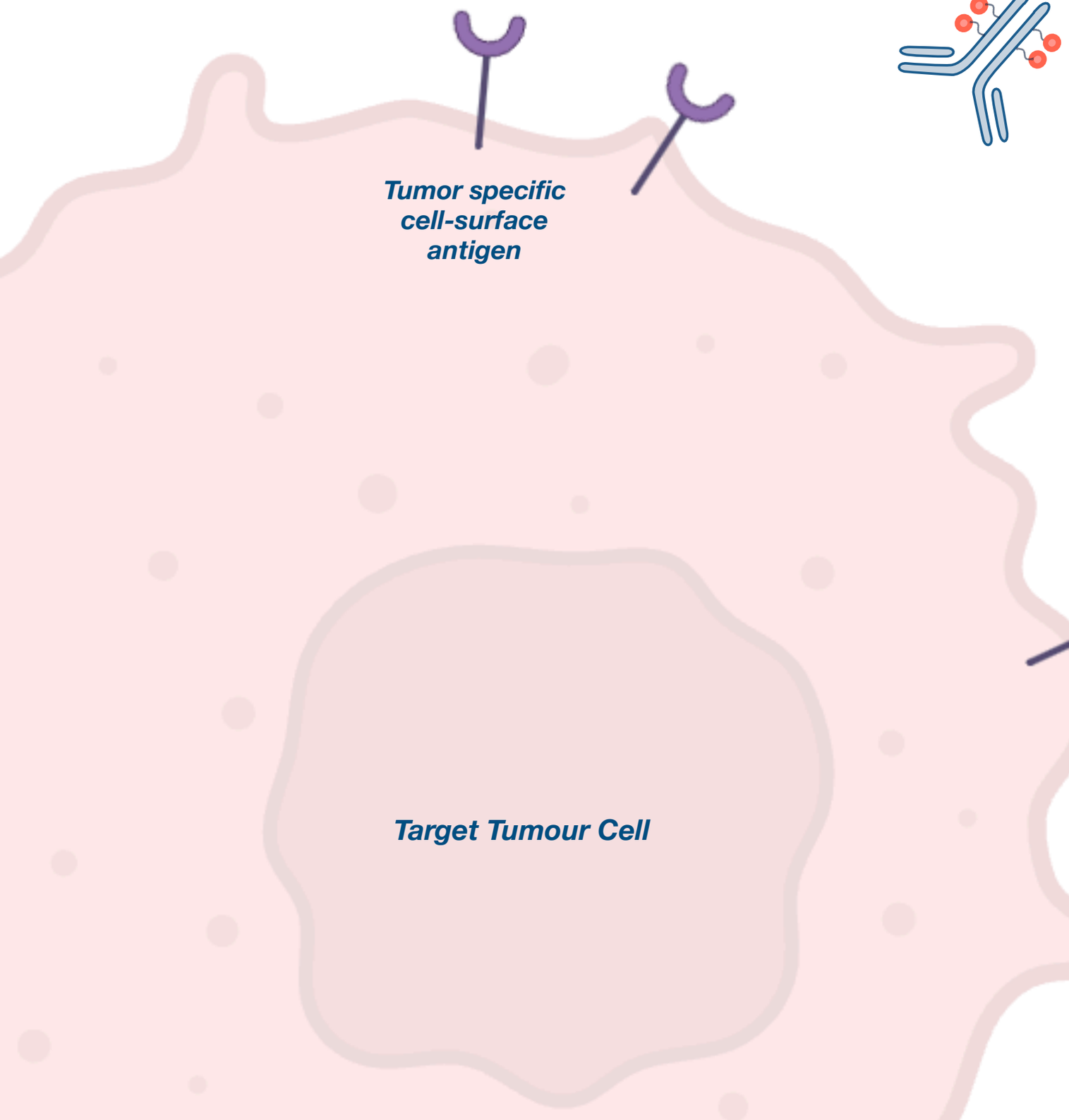


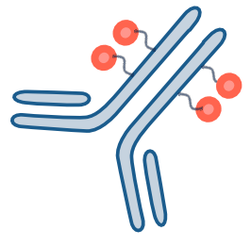
Drug-to-Antibody Ratio (DAR)

Average 3.5 - 4

Antibody-drug conjugate

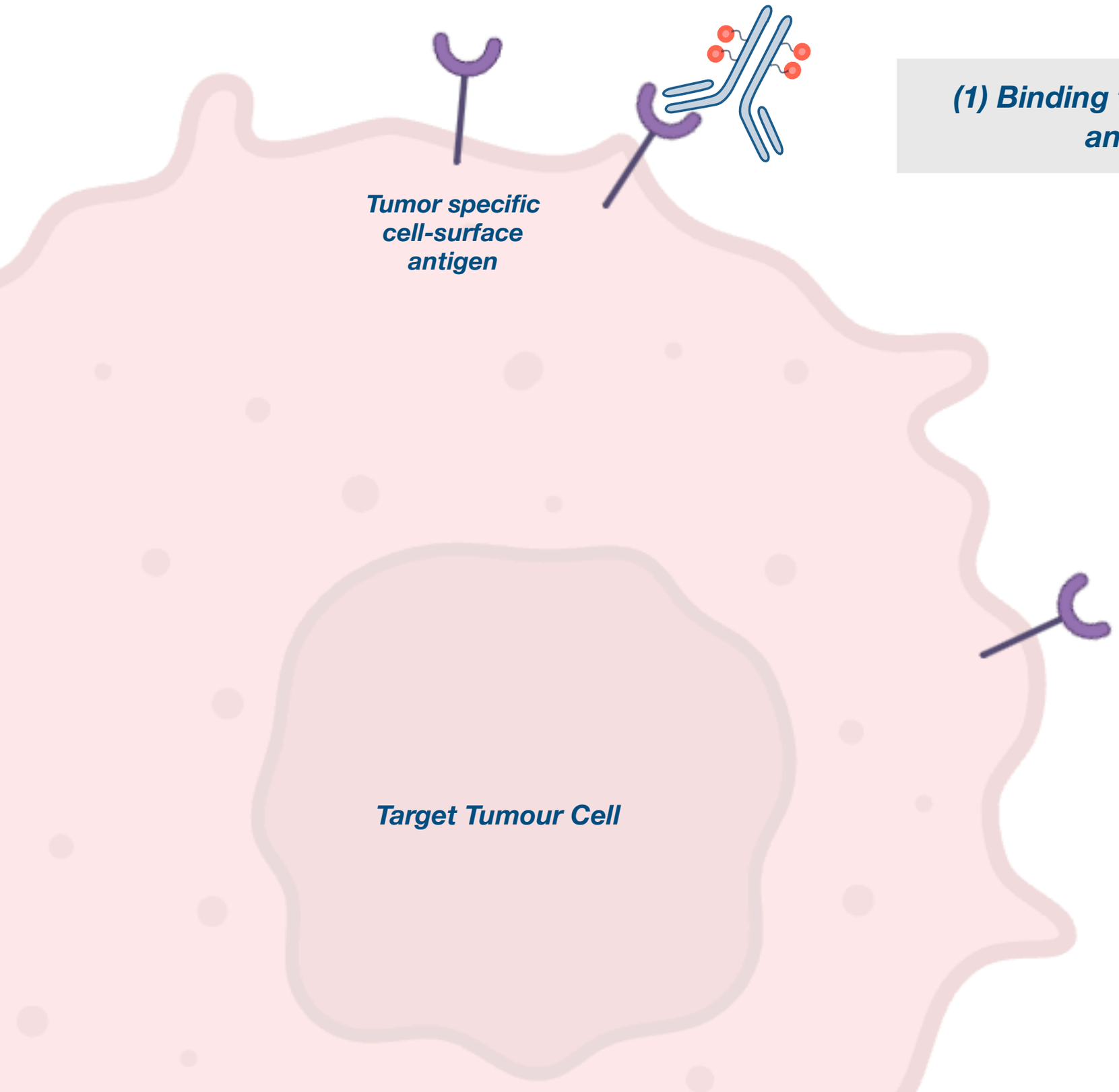
Mechanism of Action





Antibody-drug conjugate

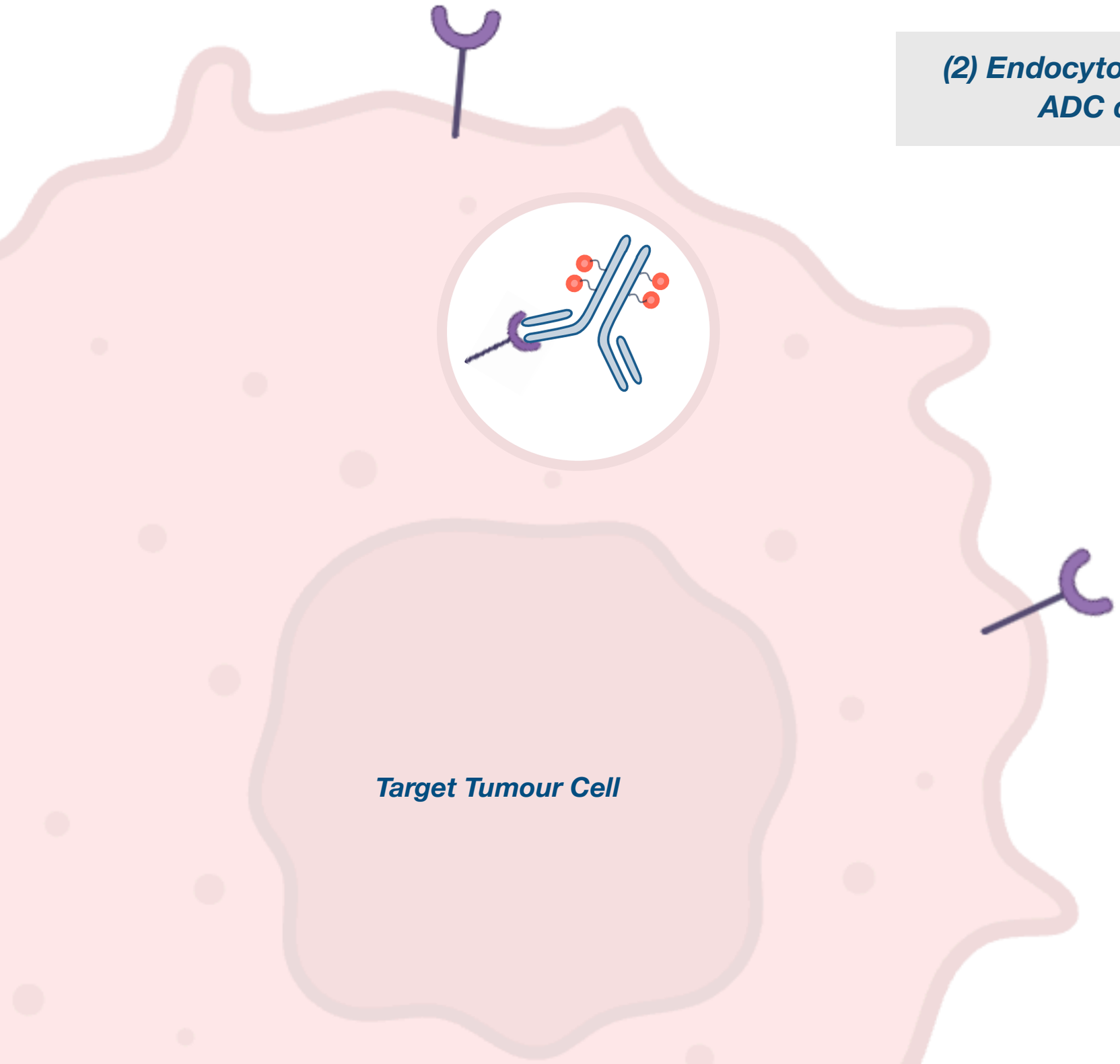
Mechanism of Action



(1) Binding to cell-surface antigen

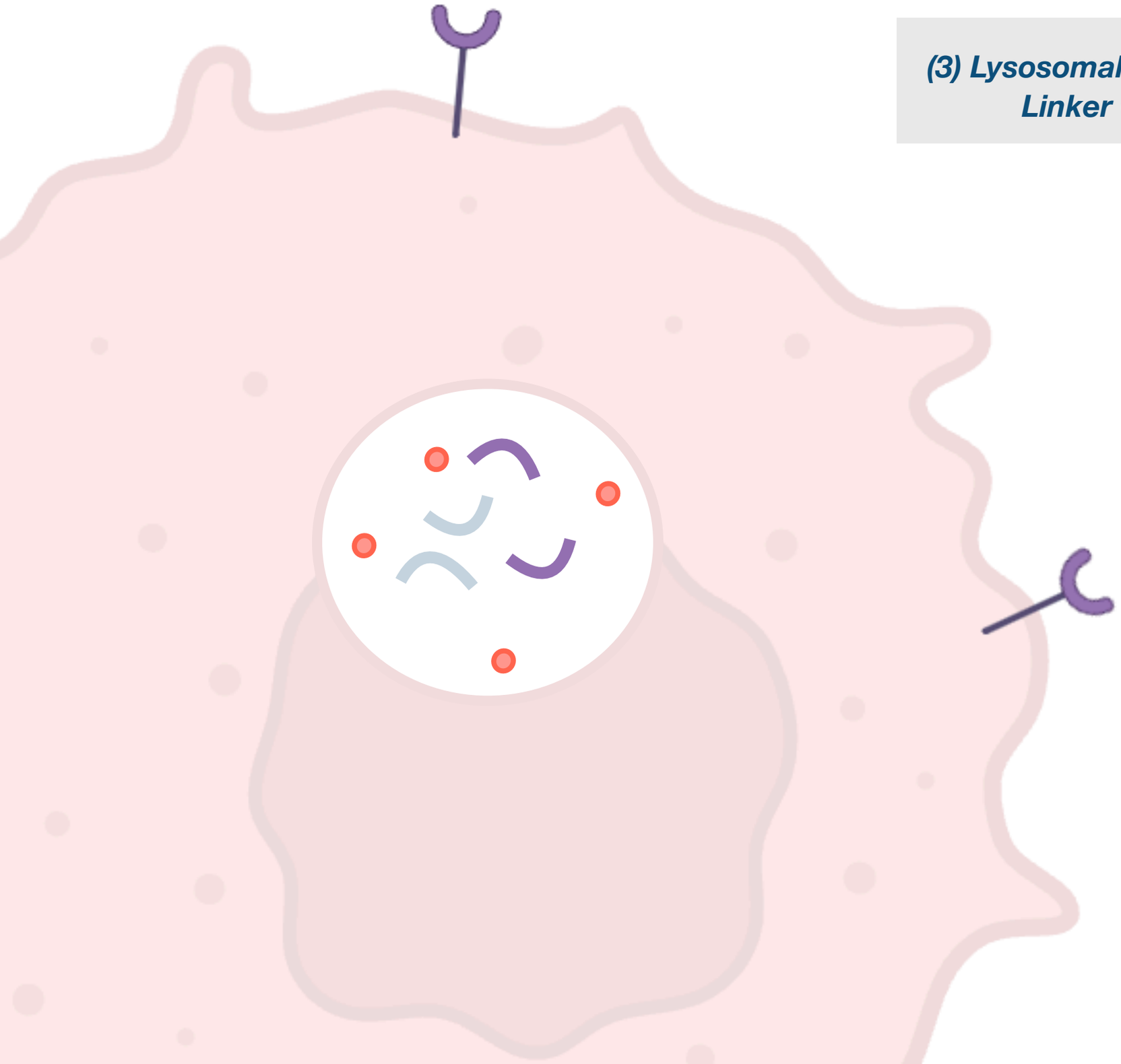
Antibody-drug conjugate

Mechanism of Action



Antibody-drug conjugate

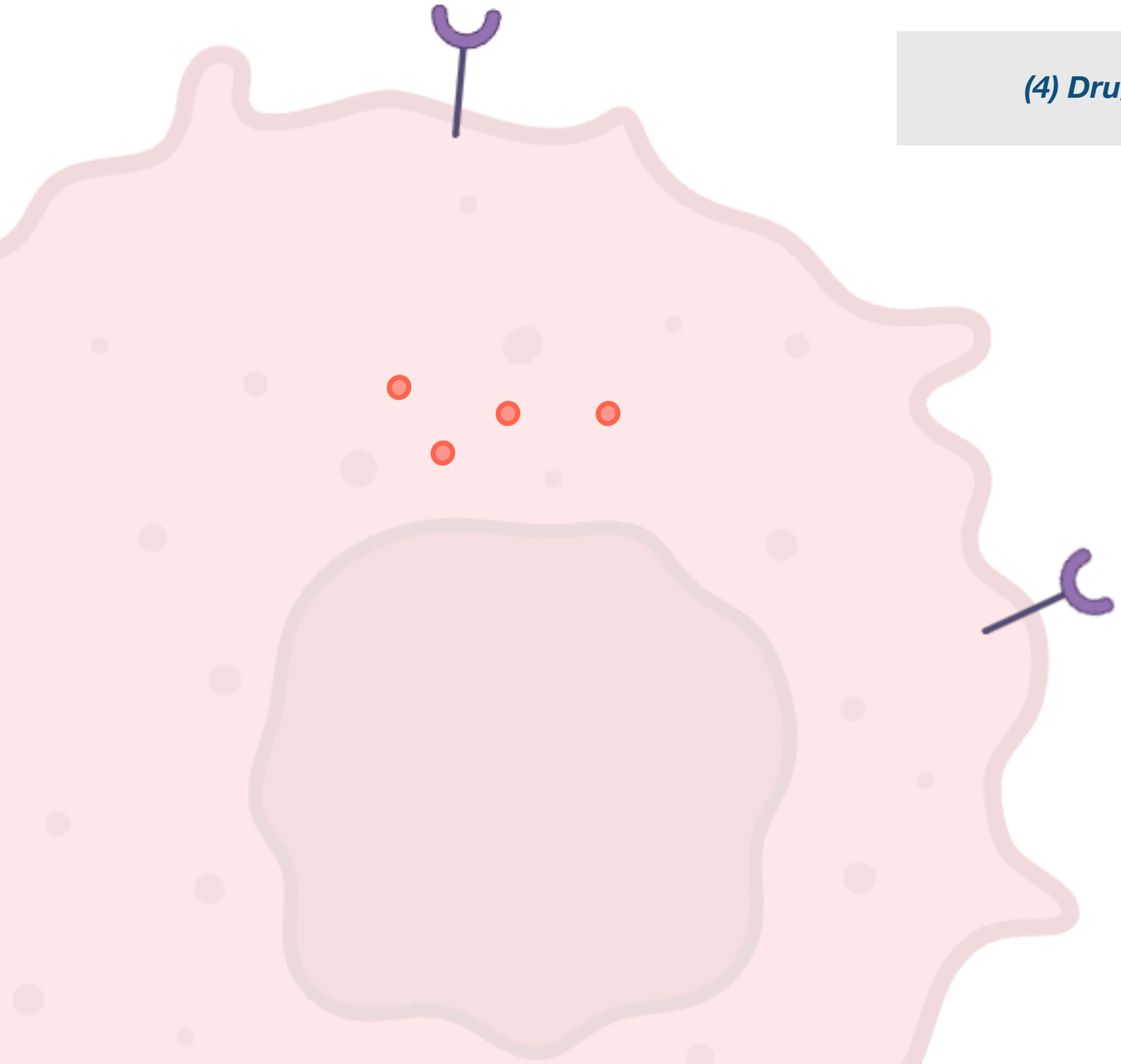
Mechanism of Action



(3) Lysosomal Degradation or Linker Cleavage

Antibody-drug conjugate

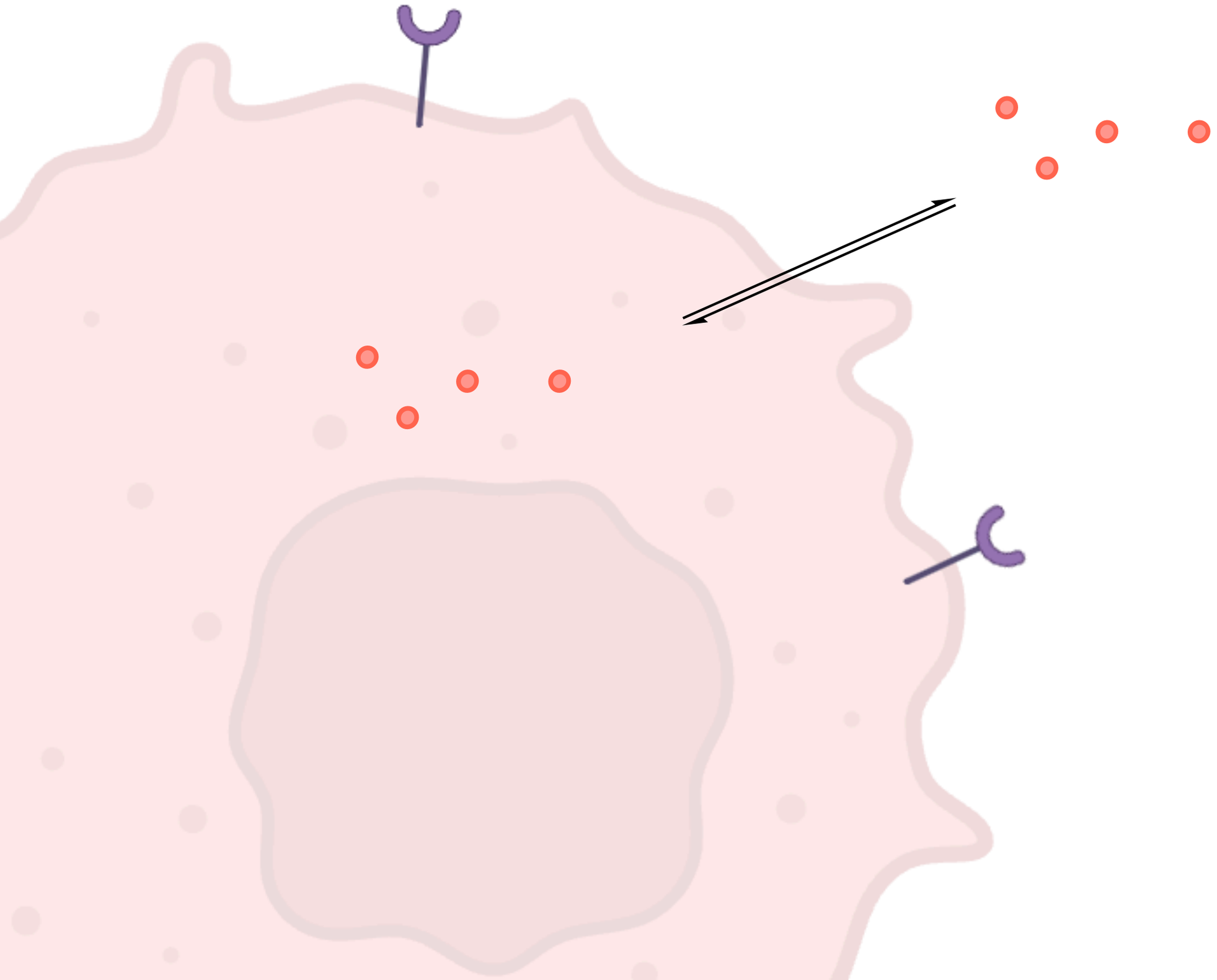
Mechanism of Action



(4) Drug release

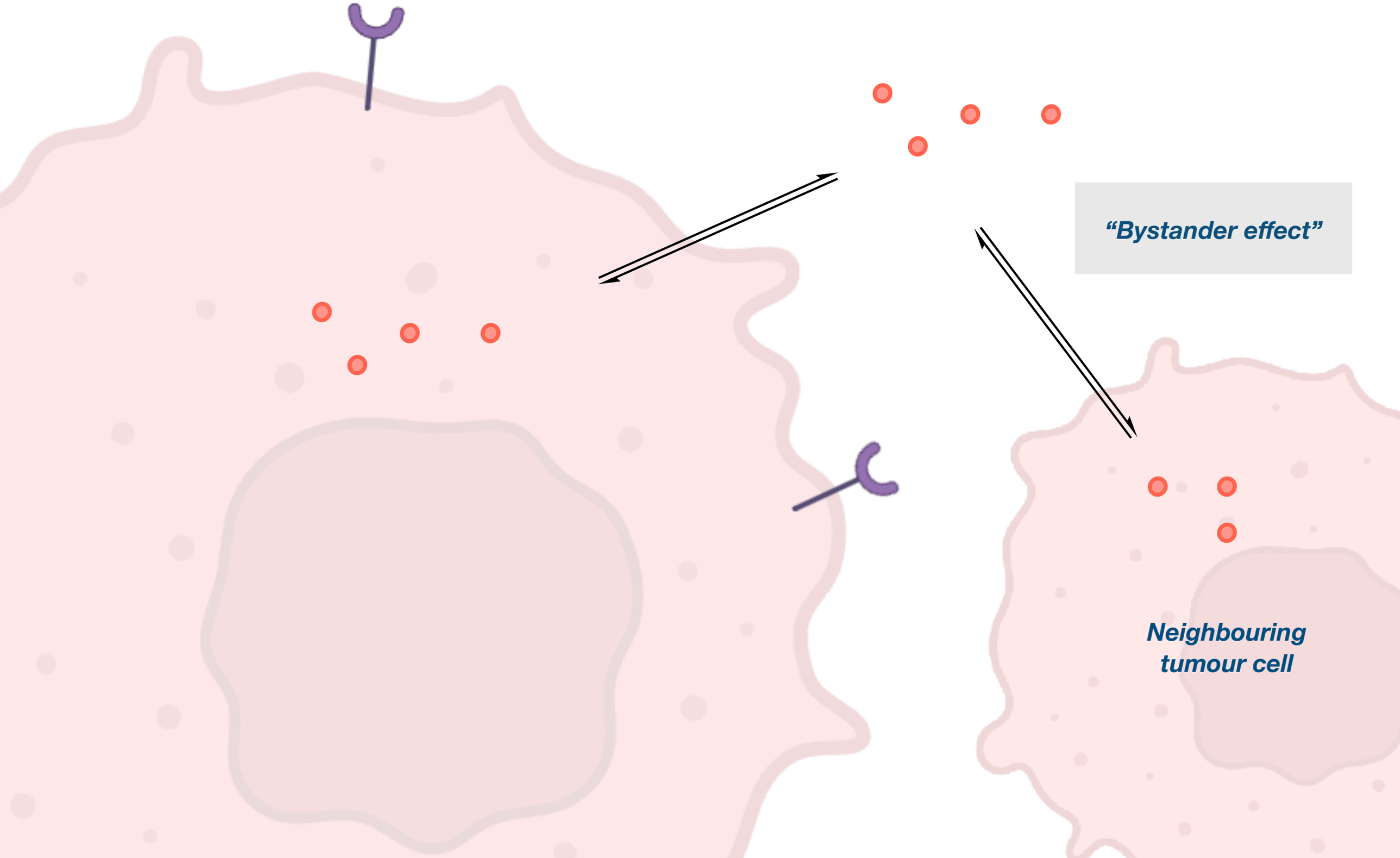
Antibody-drug conjugate

Mechanism of Action



Antibody-drug conjugate

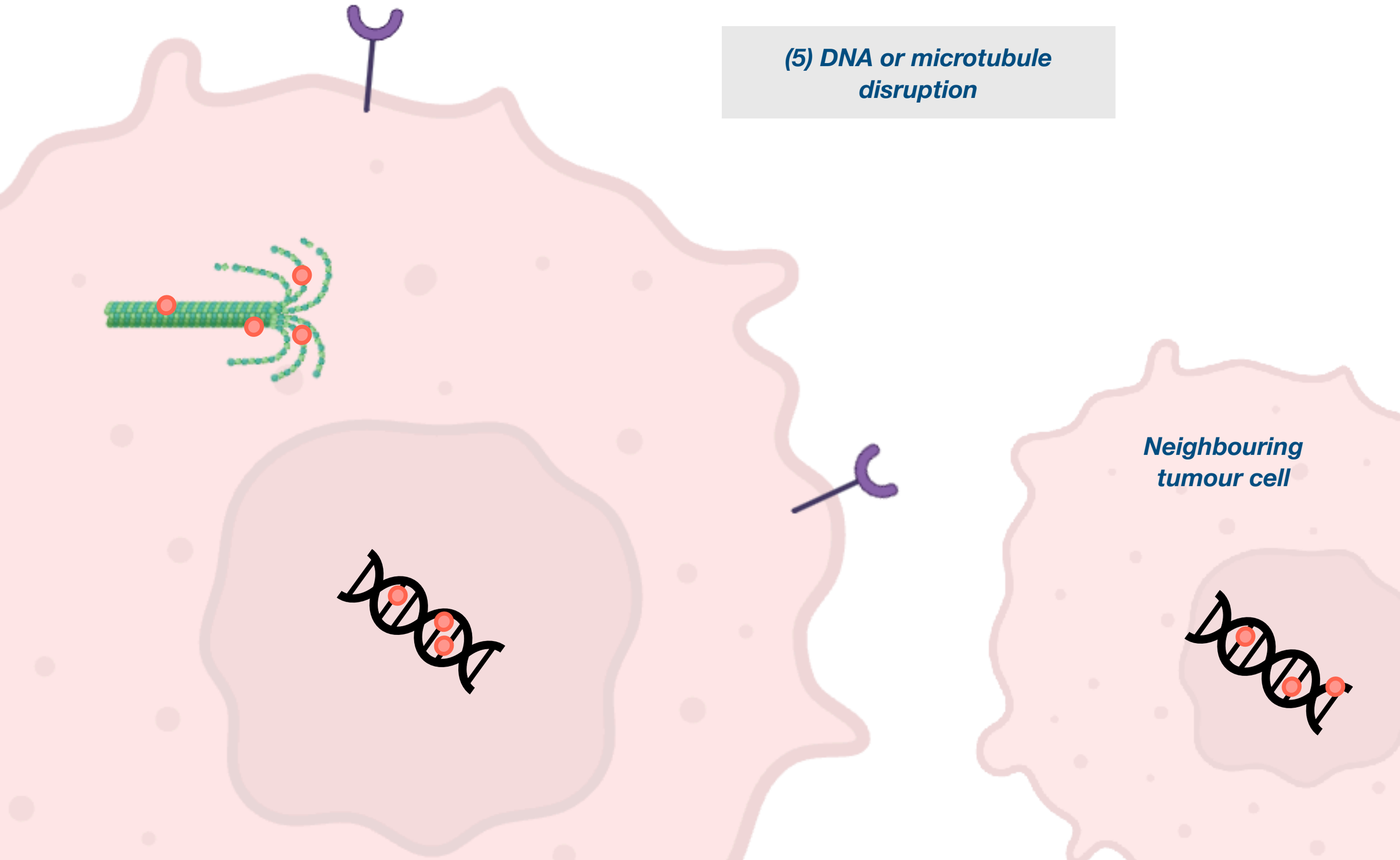
Mechanism of Action



Antibody-drug conjugate

Mechanism of Action

(5) DNA or microtubule disruption



Antibody-drug conjugate

Mechanism of Action

(6) Cell death

FDA-approved ADCs

12 in the market

Mylotarg™

Besponsa™

Adcetris™

Polivy™

Padcev™

Tivdak™

Kadcyla™

Enhertu™

Trodelvy™

Zynlota™

Blenrep™

Elahere™

FDA-approved ADCs

Antibody - Linker - Drug

Mylotarg™ **(First approval in 2000)**

Besponsa™

Adcetris™

Polivy™

Padcev™

Tivdak™

Kadcyla™

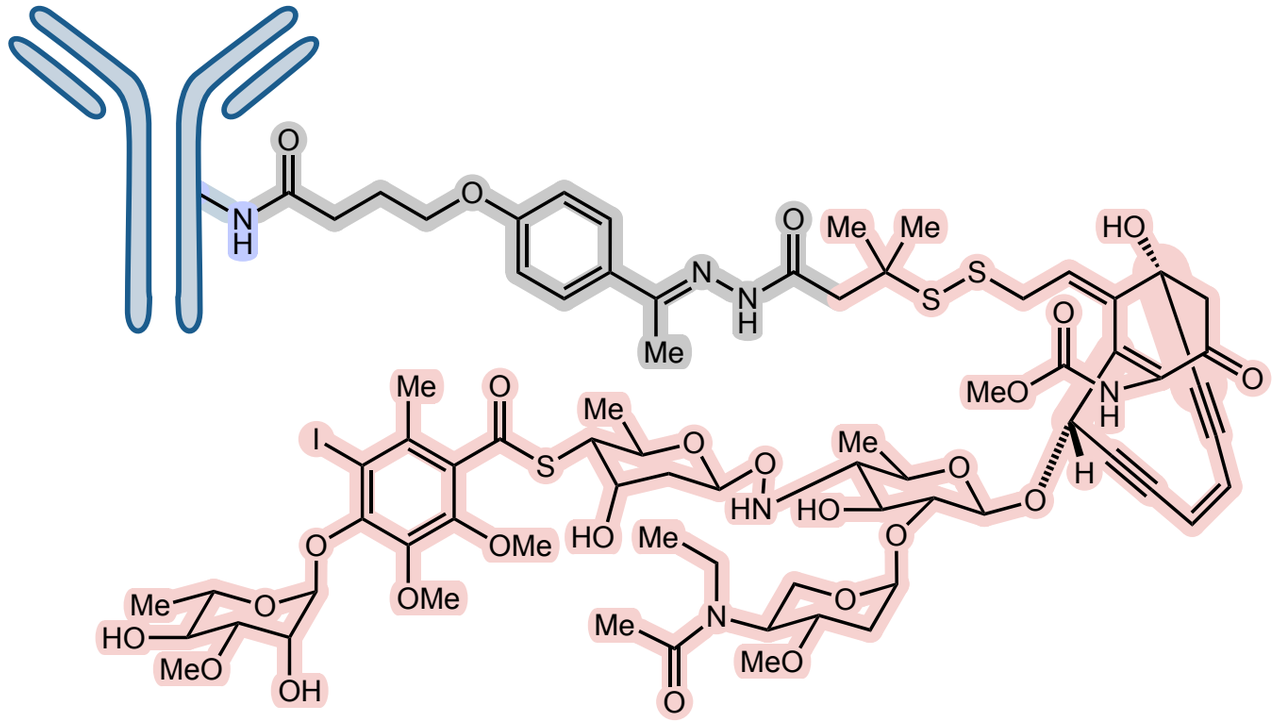
Enhertu™

Trodelvy™

Zynlota™

Blenrep™

Elahere™



	Antibody	***Indication***	***Developer***
Mylotarg™ *(Gemtuzumab ozogamicin)*	*anti-CD33 mAb*	*acute myeloid leukemia*	Pfizer
Besponsa™ *(inotuzumab ozogamicin)*	*anti-CD22 mAb*	*acute lymphoblastic leukemia*	Pfizer

FDA-approved ADCs

Antibody - Linker - Drug

Mylotarg™

Besponsa™

Adcetris™

Polivy™

Padcev™

Tivdak™

Kadcyla™

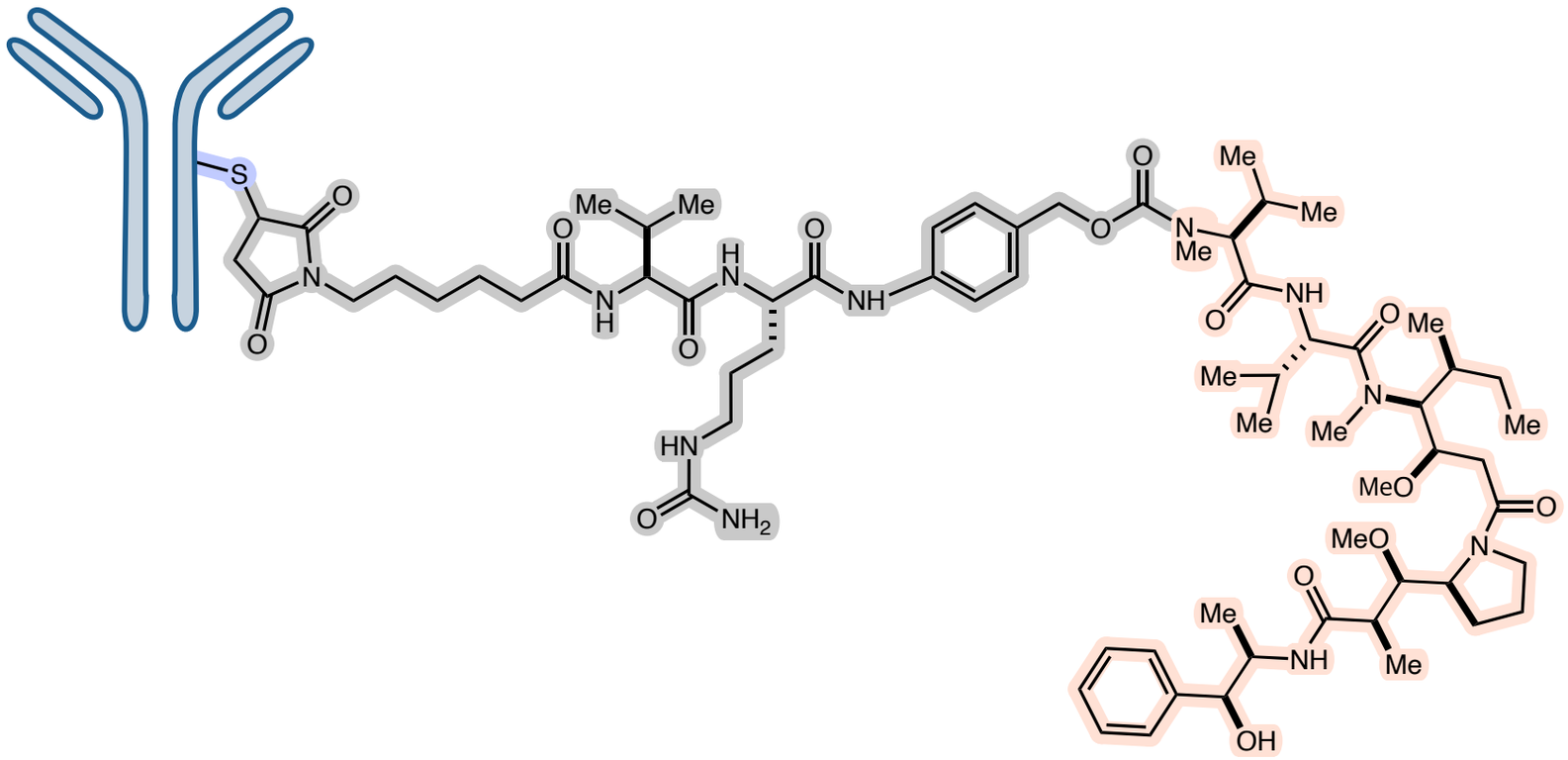
Enhertu™

Trodelvy™

Zynlota™

Blenrep™

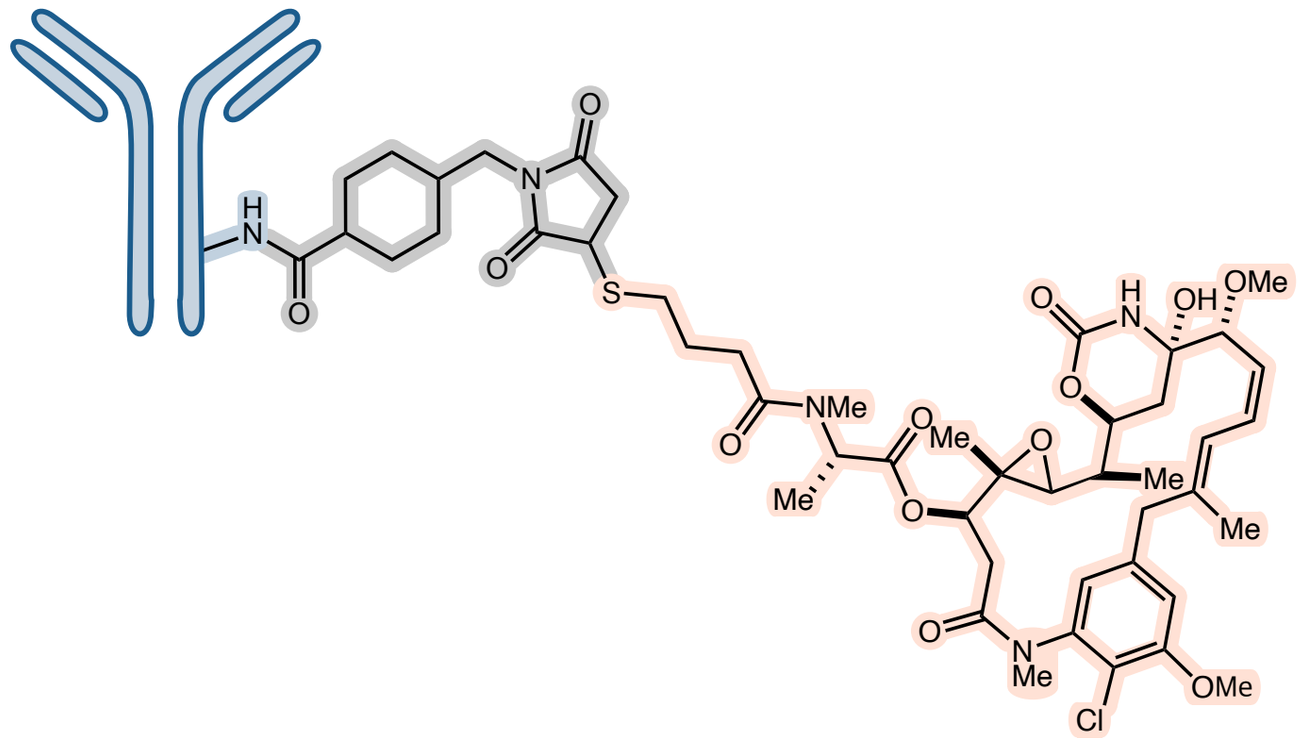
Elahere™



	Antibody	Indication	Developer
Adcetris™ *(Brentuximab vedotin)*	*anti-CD30 mAb*	*Hodgkin's Lymphoma*	Pfizer
Polivy™ *(Polatuzumab vedotin)*	*anti-CD79b mAb*	*Diffuse large B-cell lymphoma*	Genentech
Padcev™ *(Enfortumab vedotin)*	*anti-Nectin4 mAb*	*Urothelial Cancer*	astellas
Tivdak™ *(Tisotumab vedotin)*	*anti-TF mAb*	*Cervical Cancer*	Pfizer

FDA-approved ADCs

Antibody - Linker - Drug



Mylotarg™

Besponsa™

Adcetris™

Polivy™

Padcev™

Tivdak™

Kadcyla™

Enhertu™

Trodelvy™

Zynlota™

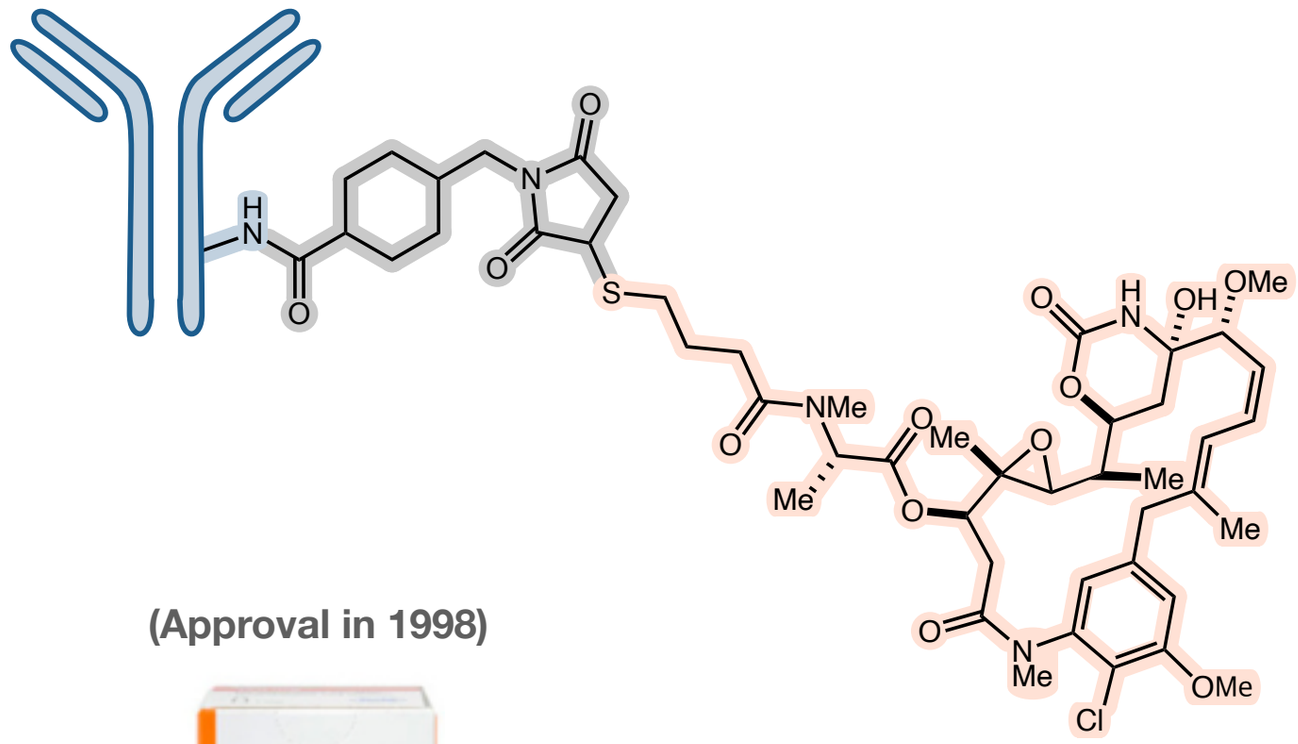
Blenrep™

Elahere™

	Antibody	***Indication***	***Developer***
Kadcyla™ *(Trastuzumab emtansine)*	*Trastuzumab*	*Breast cancer*	Genentech

FDA-approved ADCs

Antibody - Linker - Drug



Mylotarg™

Besponsa™

Adcetris™

Polivy™

Padcev™

Tivdak™

Kadcyla™ **(Approval in 2013)**

Enhertu™

Trodelvy™

Zynlota™

Blenrep™

Elahere™

(Approval in 1998)

	Antibody	***Indication***	***Developer***
Kadcyla™ *(Trastuzumab emtansine)*	*Trastuzumab*	*Breast cancer*	Genentech

FDA-approved ADCs

Antibody - Linker - Drug

Mylotarg™

Besponsa™

Adcetris™

Polivy™

Padcev™

Tivdak™

Kadcyla™

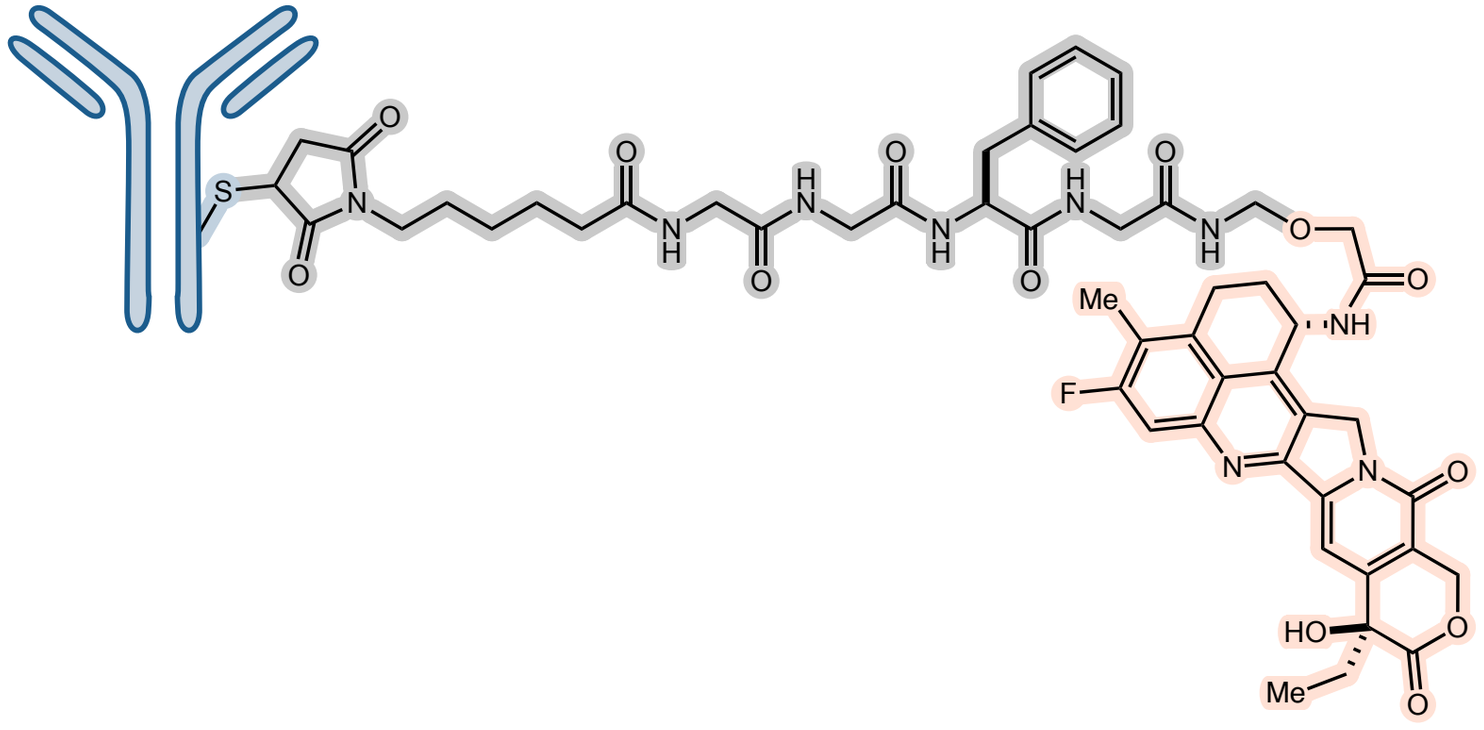
Enhertu™

Trodelvy™

Zynlota™

Blenrep™

Elahere™



	Antibody	Indication	Developer
Enhertu™ *(Trastuzumab deruxtecan)*	*Trastuzumab*	*Breast cancer* *Solid cancer*	Daiichi-Sankyo

FDA-approved ADCs

Antibody - Linker - Drug

Mylotarg™

Besponsa™

Adcetris™

Polivy™

Padcev™

Tivdak™

Kadcyla™

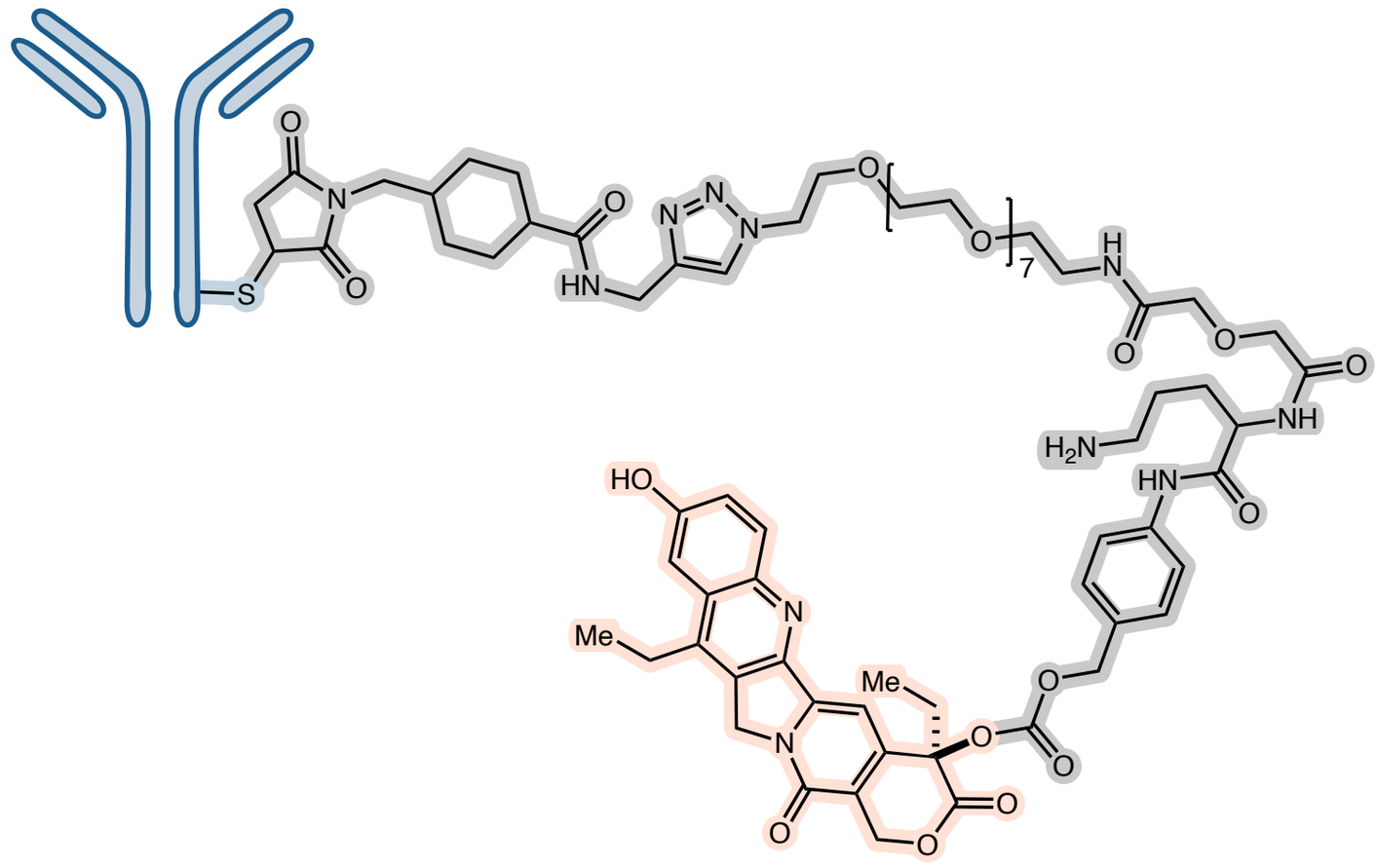
Enhertu™

Trodelvy™

Zynlota™

Blenrep™

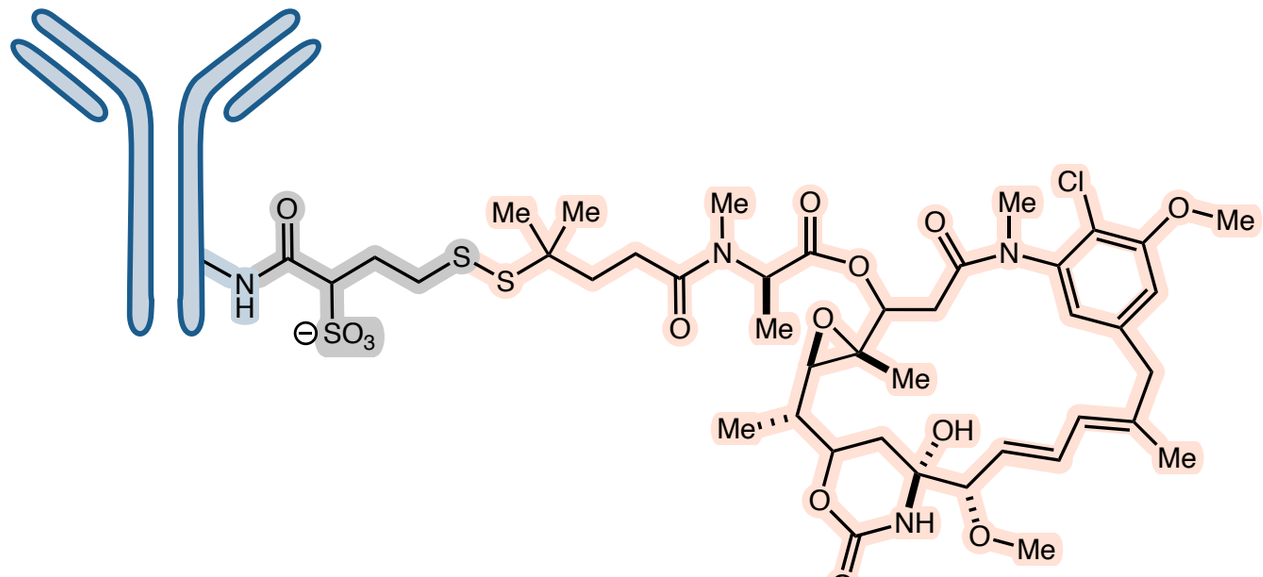
Elahere™



	Antibody	***Indication***	***Developer***
Trodevly™ *(Sacituzumab govitecan)*	*anti-Trop2 mAb*	*Triple-negative breast cancer*	GILEAD

FDA-approved ADCs

Antibody - Linker - Drug



Mylotarg™

Besponsa™

Adcetris™

Polivy™

Padcev™

Tivdak™

Kadcyla™

Enhertu™

Trodelvy™

Zynlota™

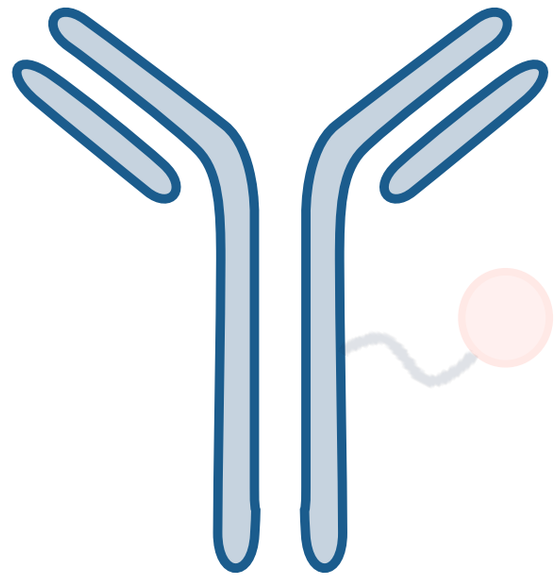
Blenrep™

Elahere™ **(Approval in 2022)**

	Antibody	***Indication***	***Developer***
Elahere™ *(Mirvetuximab soravtansine)*	*anti-Folate Receptor alpha mAb*	*Ovarian cancer*	immunogen

‘A’ in ADC

Antibody



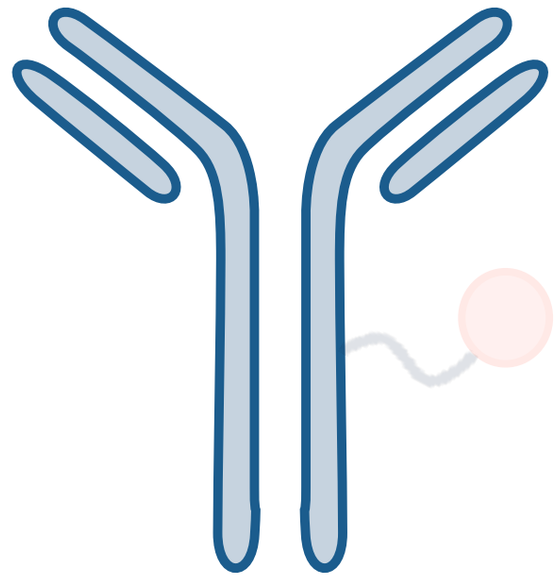
- ***Antibody alone as a drug***

- ***Characteristics of antibody drugs***

- ***‘A’ versus ‘ADC’***

‘A’ in ADC

Antibody



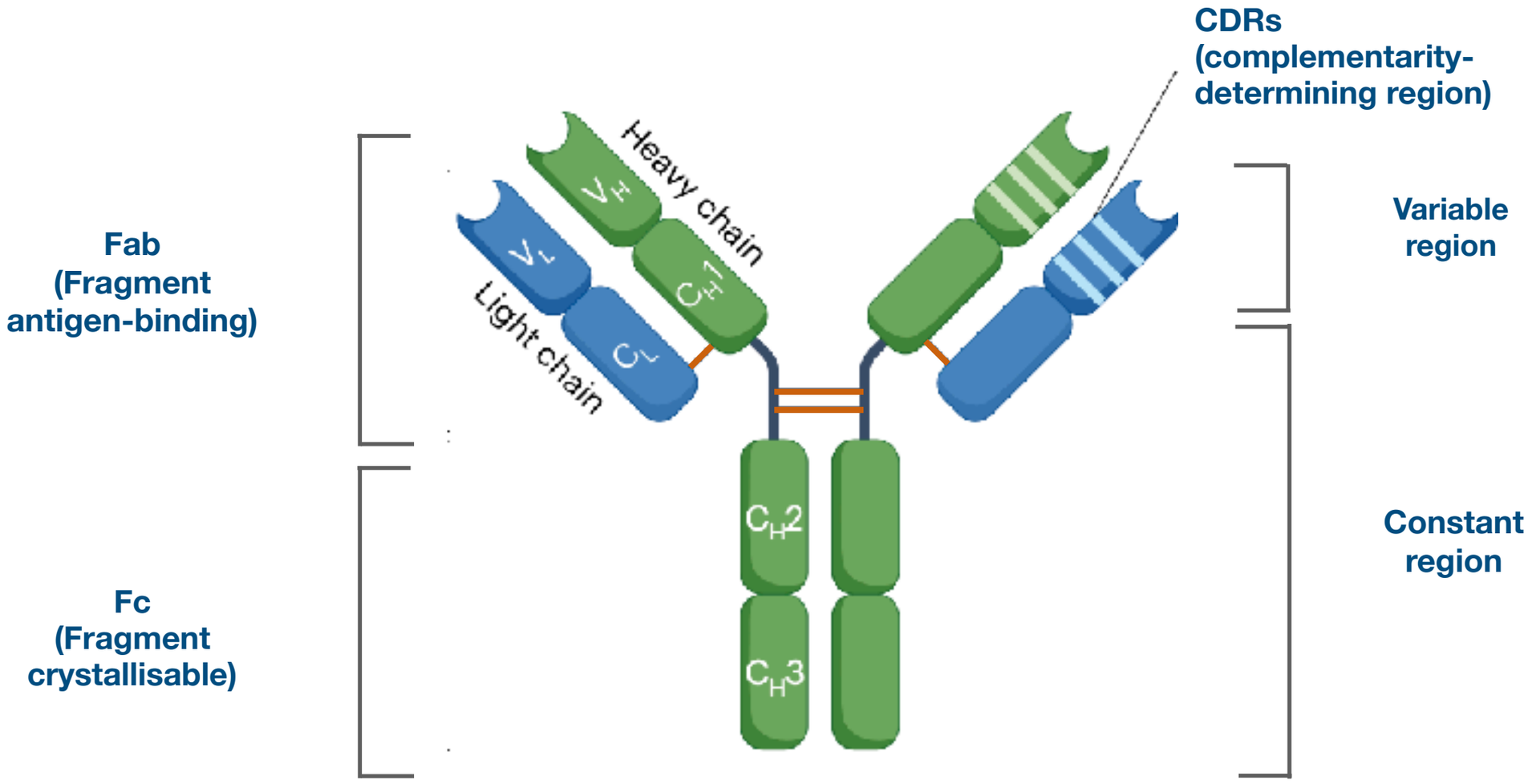
- ***Antibody alone as a drug***

- ***Characteristics of antibody drugs***

- ***‘A’ versus ‘ADC’***

'A' in ADC

Structural features of antibody

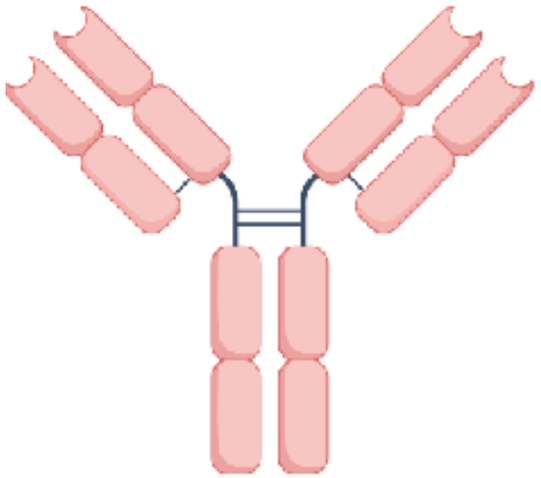


K. M.; Gabelli, S. B.; Ho, M.; Van Elsas, A.; Zhou, S. *Nat. Rev. Cancer* **2024**, *24* (6), 399–426

Antibody alone as a drug

How antibody could become a therapeutic modality

Mouse
(-momab)



- *Hybridoma system (developed in 1970s) allowed for production and selection of highly specific mouse mAb*

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Antibody alone as a drug

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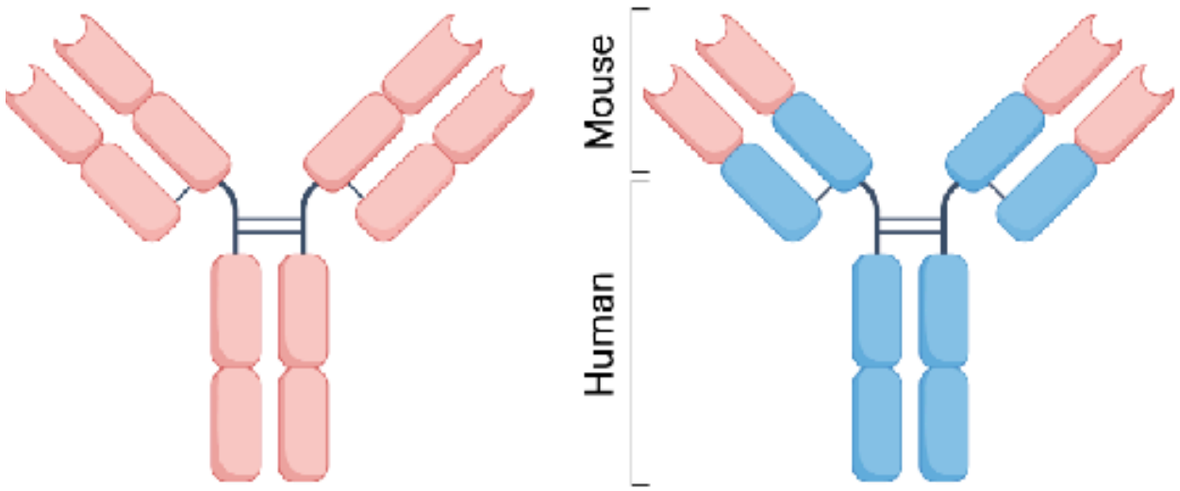
Mouse
(-<u>mo</u>mab)

Chimeric
(-<u>xi</u>mab)



- *Hybridoma system (developed in 1970s) allowed for production and selection of highly specific mouse mAb*
- *Antibody engineering enabled to graft a **human antibody constant region** to a **mouse antibody variable region***

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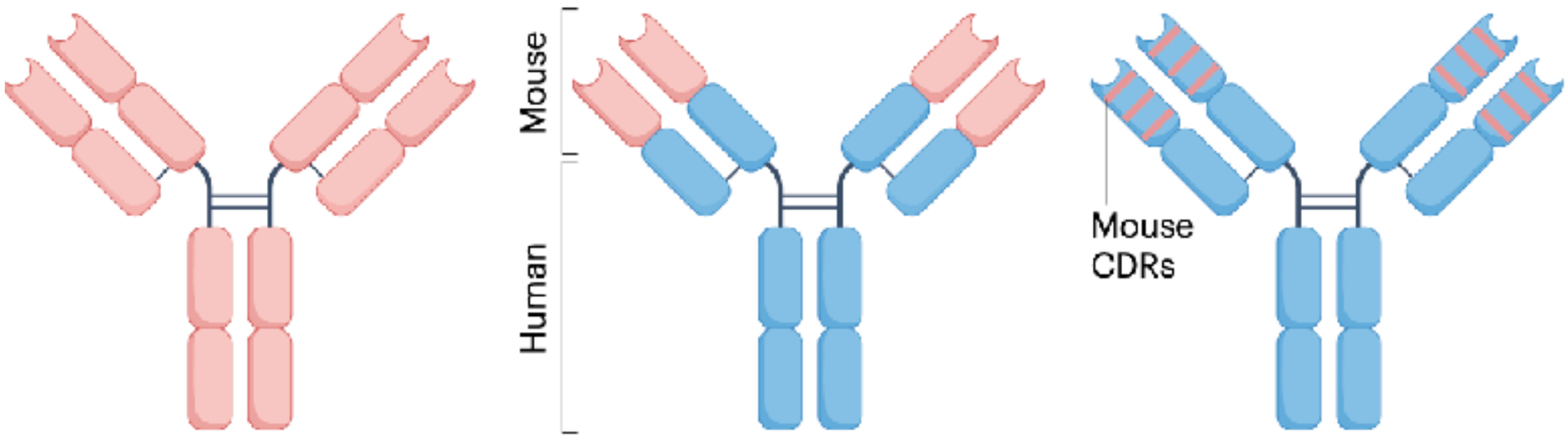
Humanised
(-<u>zu</u>mab)



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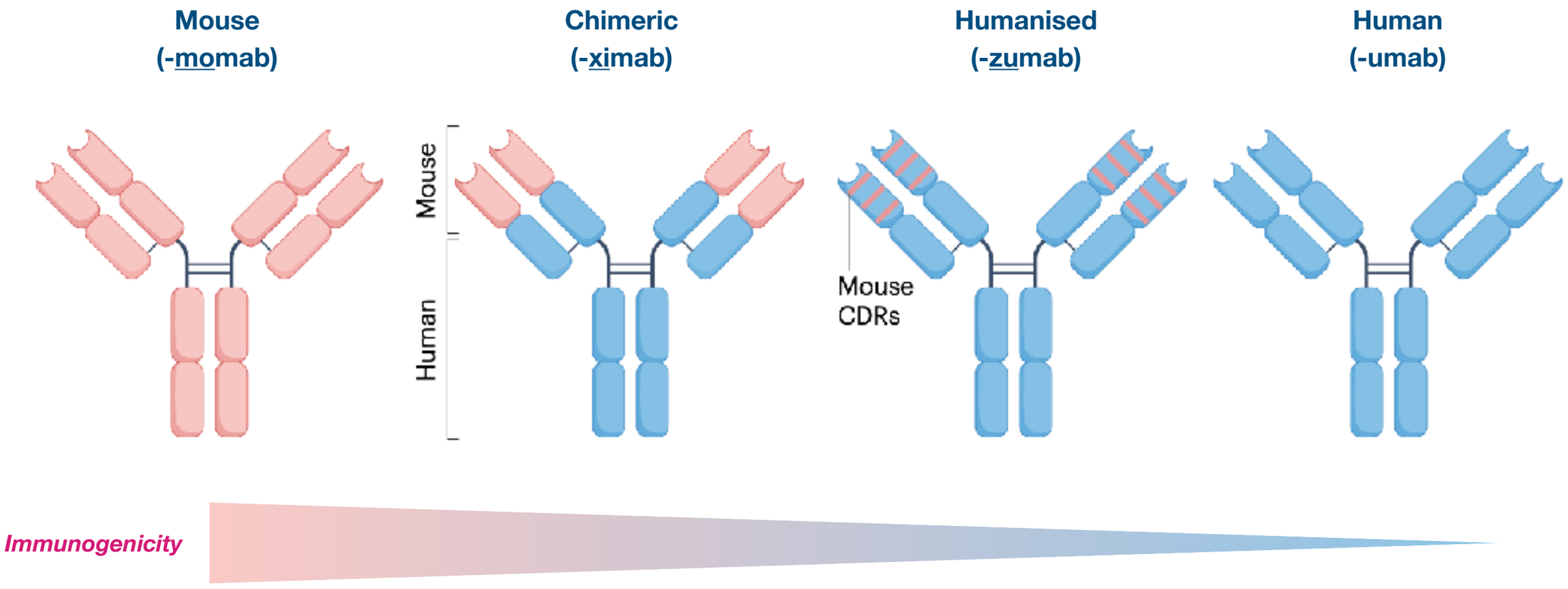

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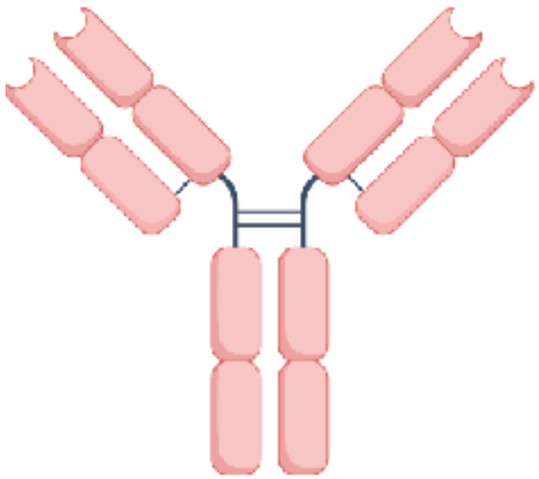
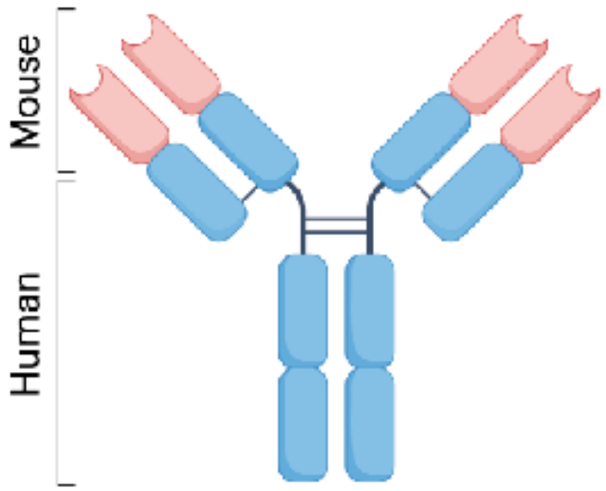


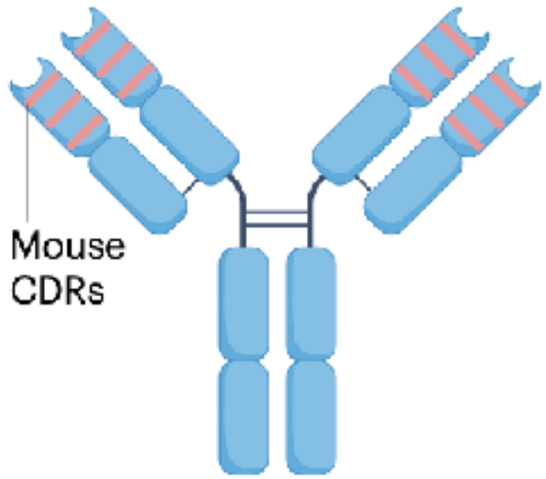
5 approvals
4 withdrawn

Chimeric
(-<u>xi</u>mab)



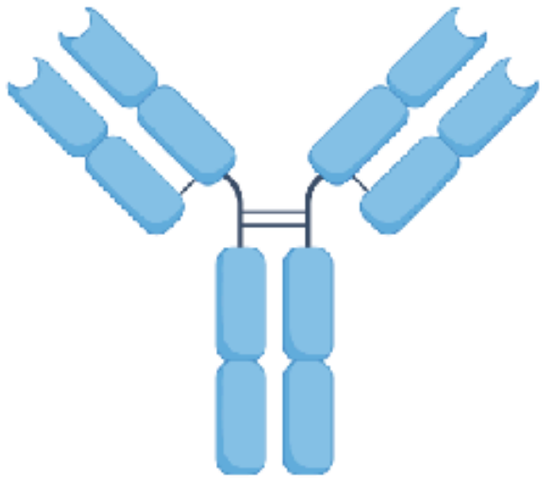
6 approvals

Humanised
(-<u>zu</u>mab)



31 approvals
1 withdrawn

Human
(-umab)



15 approvals

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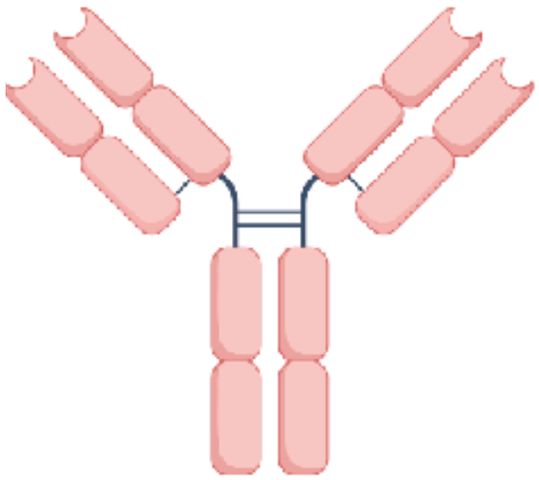

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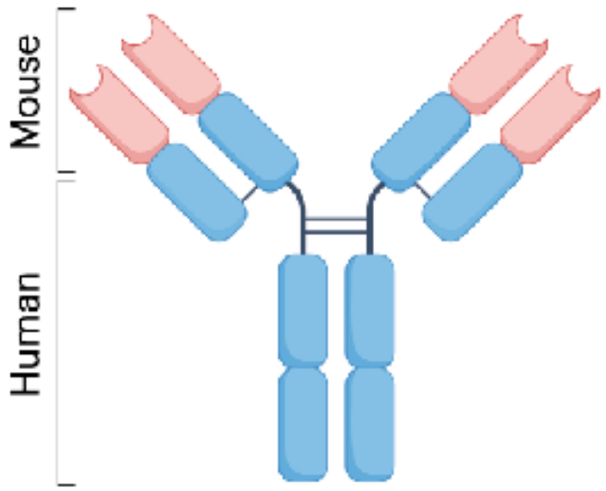
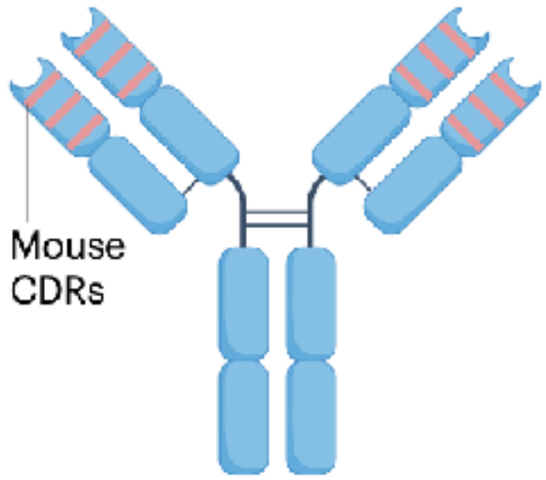
(-<u>xi</u>mab)



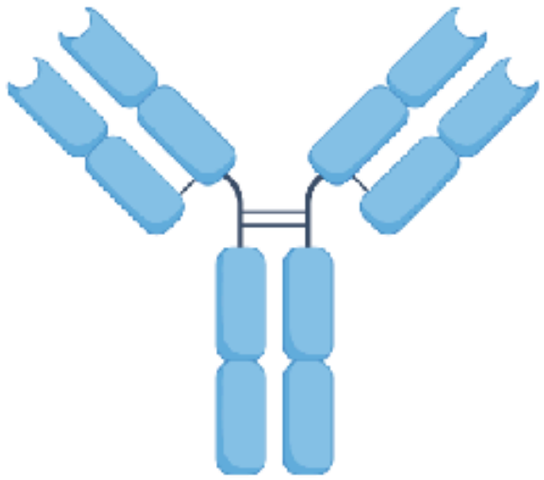
6 approvals

Humanised
(-<u>zu</u>mab)



31 approvals
1 withdrawn

Human
(-umab)



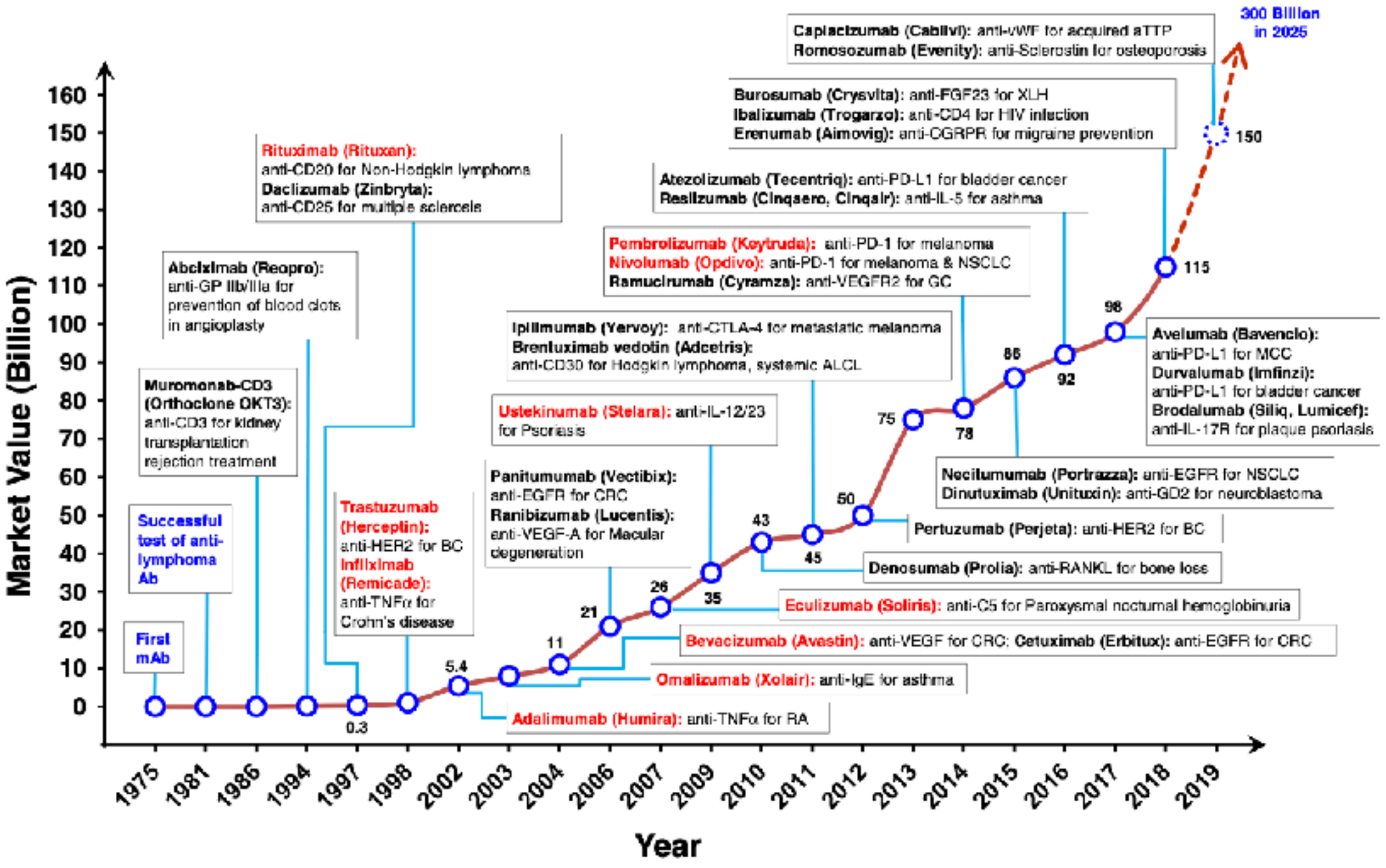
15 approvals

- *Hybridoma system (developed in 1970s) allowed for production and selection of highly specific mouse mAb*
- *Antibody engineering enabled to graft a **human antibody constant region** to a **mouse antibody variable region***
- *Transgenic mouse models and phage display systems in 1990s enabled the generation of **fully human antibodies***
- ***For ADCs, 10 out of 12 ADCs use humanized antibodies***

K. M.; Gabelli, S. B.; Ho, M.; Van Elsas, A.; Zhou, S. *Nat. Rev. Cancer* **2024**, *24* (6), 399–426

Antibody alone as a drug

Successful development of therapeutic antibodies and their applications



As of 2024 2Q, more than 130 mAb therapies are approved

Lu, R.-M.; Hwang, Y.-C.; Liu, I.-J.; Lee, C.-C.; Tsai, H.-Z.; Li, H.-J.; Wu, H.-C. *J. Biomed. Sci.* **2020**, *27* (1), 1

Antibody alone as a drug

Successful development of therapeutic antibodies and their applications

Top 10 drugs by revenue forecasts

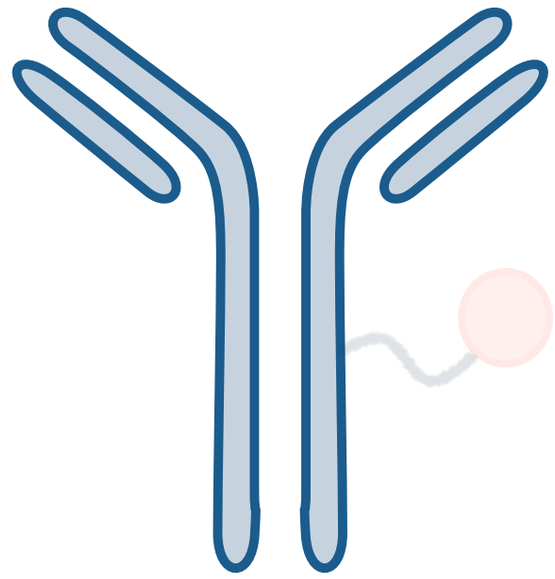
Rank	Product	Company	Pharmacological class	2024 worldwide sales forecast (US$)
1	Keytruda	Merck & Co.	Anti-PD1 mAb	27.19 billion
2	Ozempic	Novo Nordisk	GLP1 receptor agonist	16.13 billion
3	Dupixent	Sanofi/Regeneron	Anti-IL-4/IL-13 mAb	13.45 billion
4	Eliquis	Bristol Myers Squibb/Pfizer	Factor Xa inhibitor	13.31 billion
5	Biktarvy	Gilead Sciences/Yuhan	HIV INSTI/NRTI/NtRTI	12.57 billion
6	Darzalex	Johnson & Johnson	Ant-CD38 mAb	11.98 billion
7	Opdivo	Bristol Myers Squibb/Ono	Anti-PD1 mAb	11.33 billion
8	Comirnaty	Pfizer/BioNTech	SARS-CoV-2 vaccine	10.79 billion
9	Gardasil	Merck & Co./CSL	HPV vaccine	10.03 billion
10	Skyrizi	AbbVie	Anti-IL-23 mAb	9.93 billion

5 out of 10 are mAb therapies

Verdin, P. *Nat. Rev. Drug Discov.* **2024**, *23*

‘A’ in ADC

Antibody



- *Antibody alone as a drug*

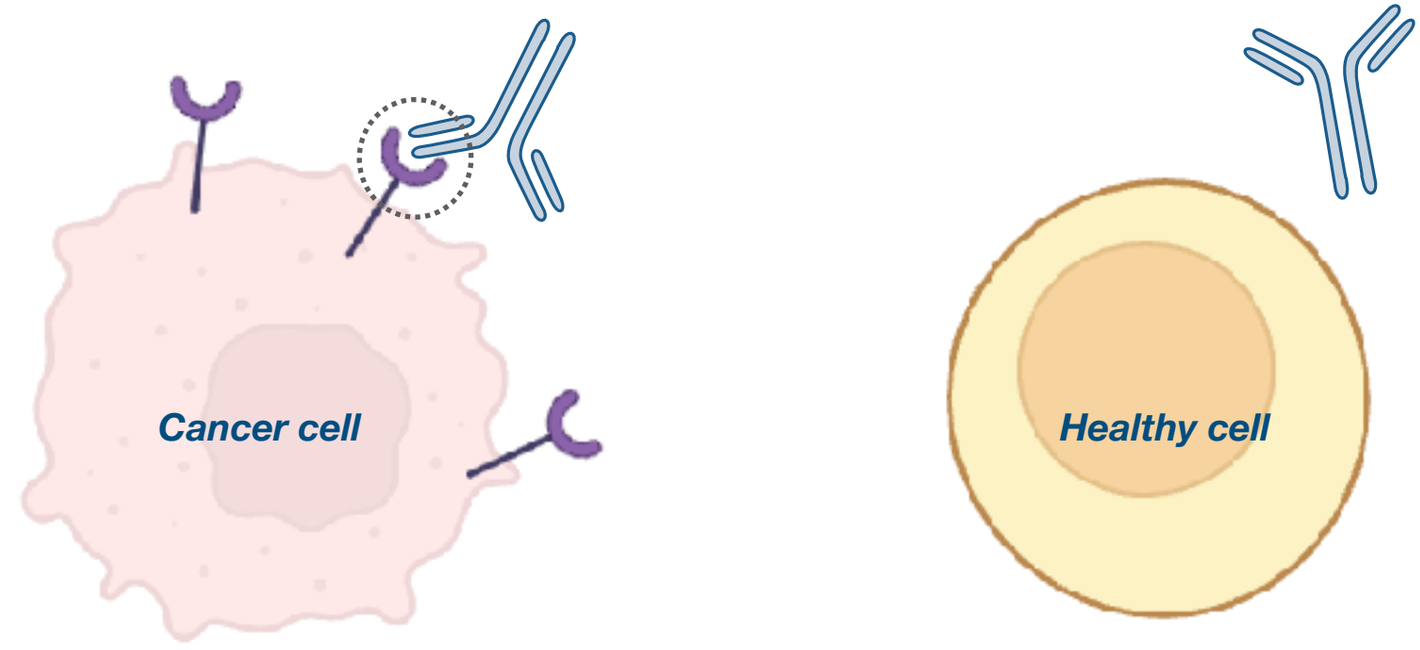
- ***Characteristics of antibody drugs***

- *‘A’ versus ‘ADC’*

Characteristics of antibody drugs

Target specificity

$K_d = 10^{-9} - 10^{-12}$ *M*

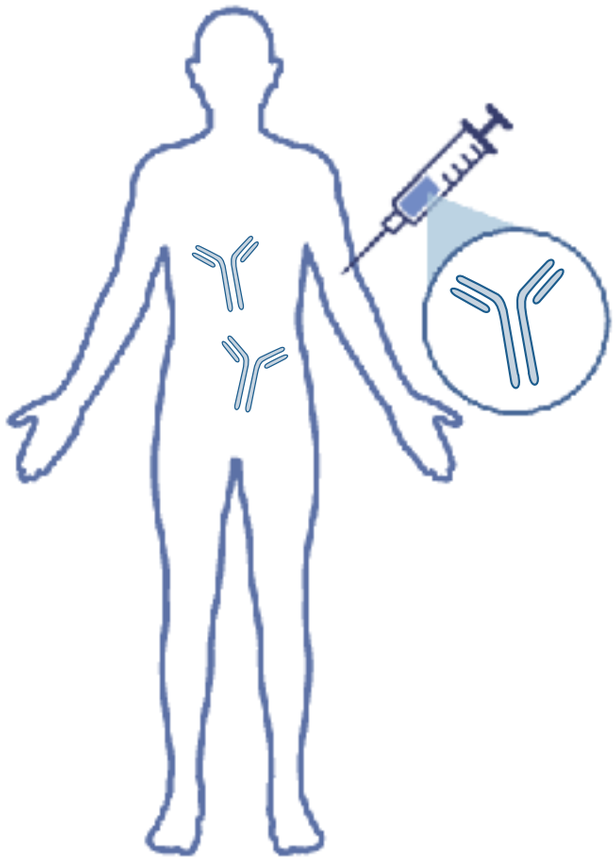


Targets only pathological cells while sparing healthy cells

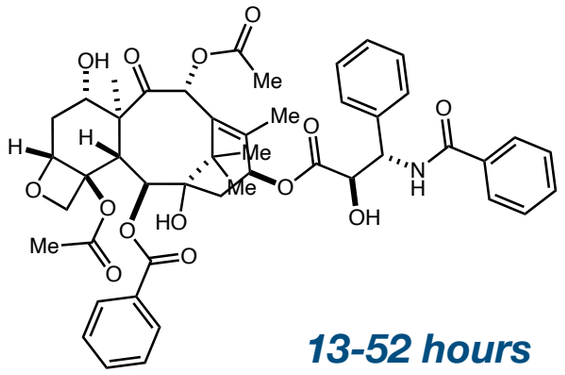
Miller, N. L.; Clark, T.; Raman, R.; Sasisekharan, R. *Front. Mol. Biosci.* **2023**, *10*

Characteristics of antibody drugs

Less adverse events and longer half-life



c.f. Half-life of small-molecule drugs (e.g. Taxol)



13-52 hours

Significantly reduced immunogenicity

Less likely to be cleared by our body's immune system

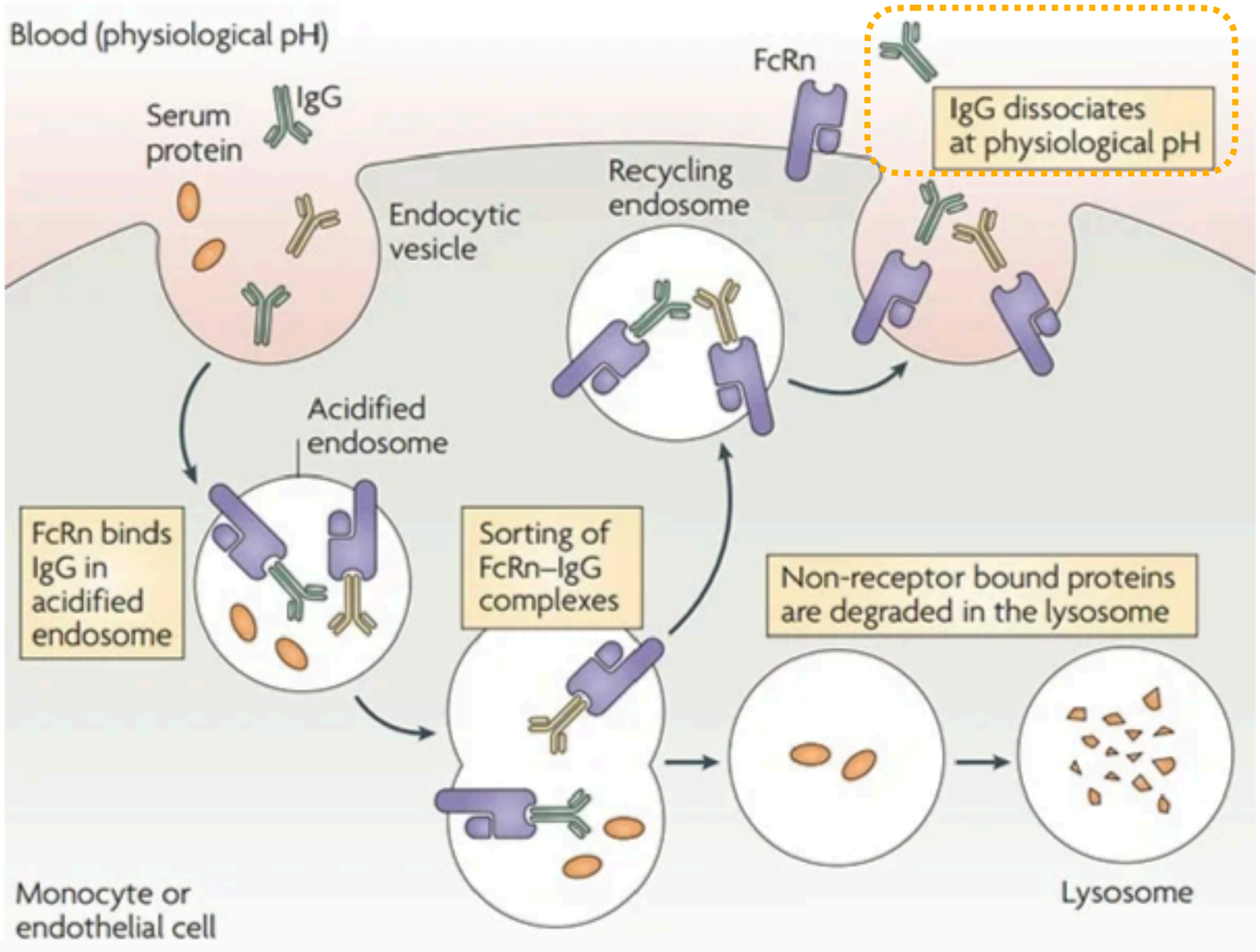
Longer half-life: 2-4 weeks

Less dosing frequency, fewer side-effects

Characteristics of antibody drugs

Less adverse events and longer half-life

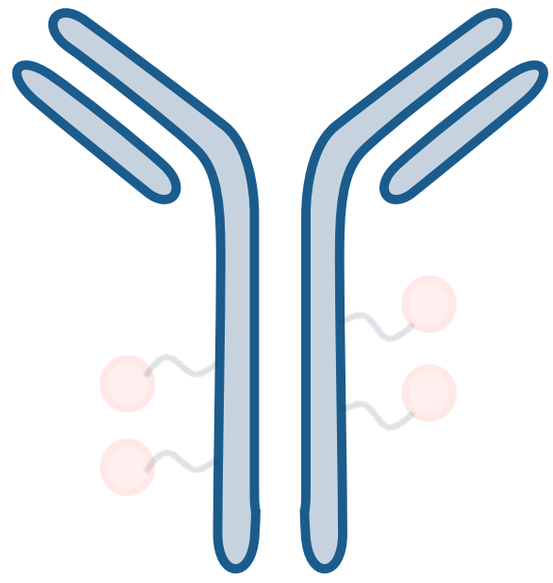
FcRn (neonatal Fc receptor)-mediated recycling



FcRn-mediated recycling can extend the half-life antibody (2-4 weeks)

Verdin, P. *Nat. Rev. Drug Discov.* **2024**, *23* (1), 13–13

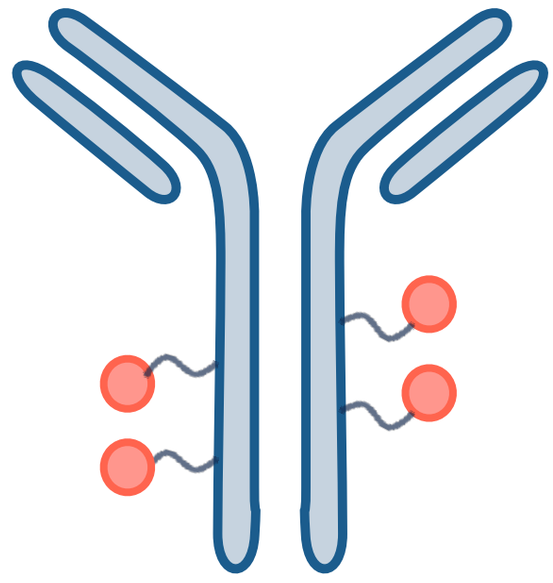
Characteristics of antibody drugs



Target specificity

Less adverse events and longer half-life

Characteristics of antibody drugs



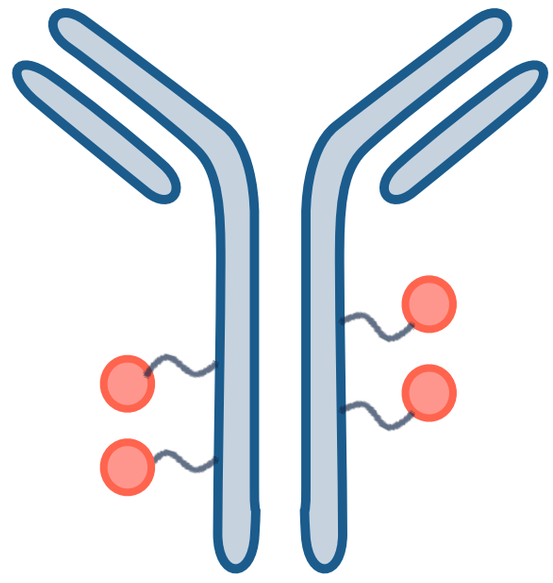
Target specificity

Less adverse events and longer half-life

Enables the delivery of highly cytotoxic drugs to target sites

Extends the half-life of small-molecule drugs attached to mAb

Characteristics of antibody drugs



Target specificity

Less adverse events and longer half-life

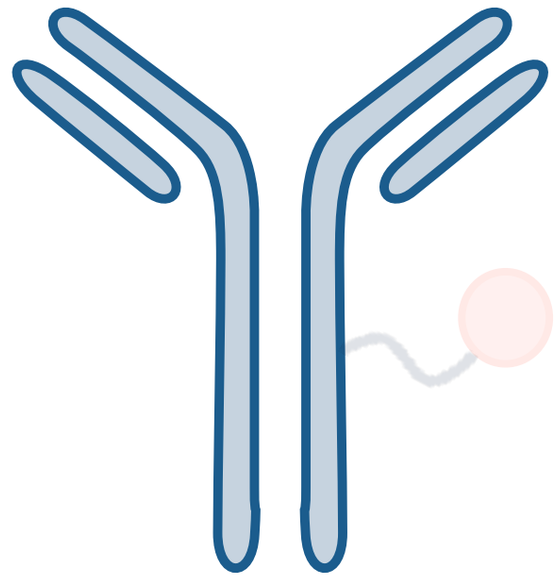
Enables the delivery of highly cytotoxic drugs to target sites
Extends the half-life of small-molecule drugs attached to mAb

	MMAE alone	***Antibody-MMAE***
Half-life *of MMAE (‘**D**’ of 4 FDA-approved ADCs)*	*2.5 h*	*3 days*

Chang, H.P.; Cheung, Y.K.; Shah, D.K. *J. Clin. Med.* **2021**, *10*, 1332

Characteristics of antibody drugs

What ‘A’ is suitable for ‘ADC’

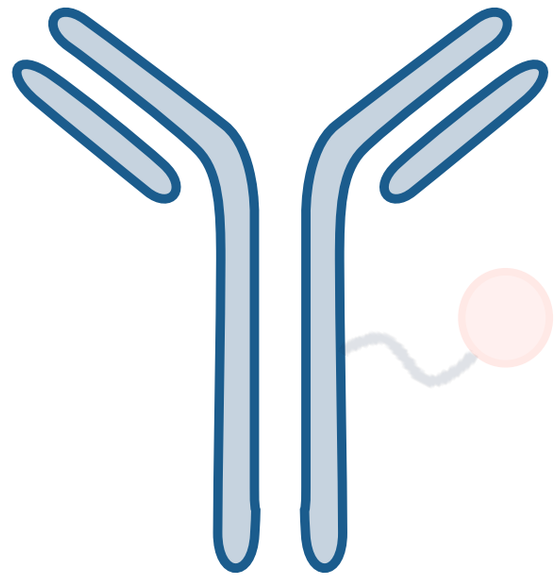


✓ *Well-elucidated MOA:* ***endocytosis (internalisation)*** *is necessary*

✓ *Its target should be* ***cell surface antigens*** *that are abundantly expressed on cancer cells, and absent on healthy cells*

‘A’ in ADC

Antibody

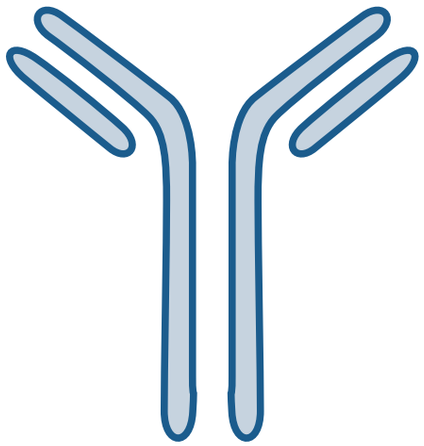


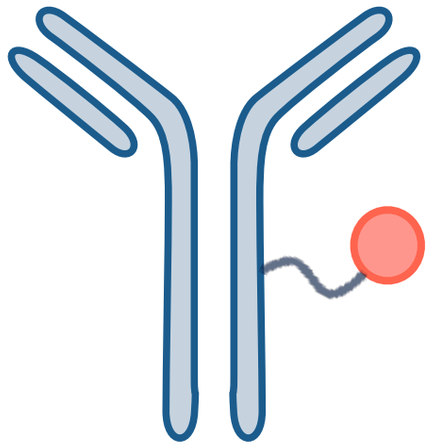
- *Antibody alone as a drug*

- *Characteristics of antibody drugs*

- ***‘A’ versus ‘ADC’***

‘A’ alone versus ‘ADC’



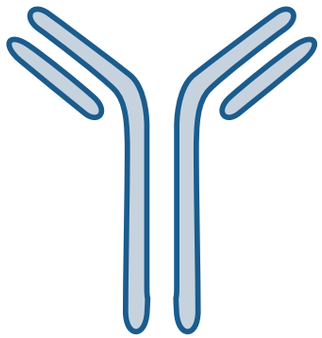


'A' alone versus 'ADC'

Herceptin™ versus Kadcyla™

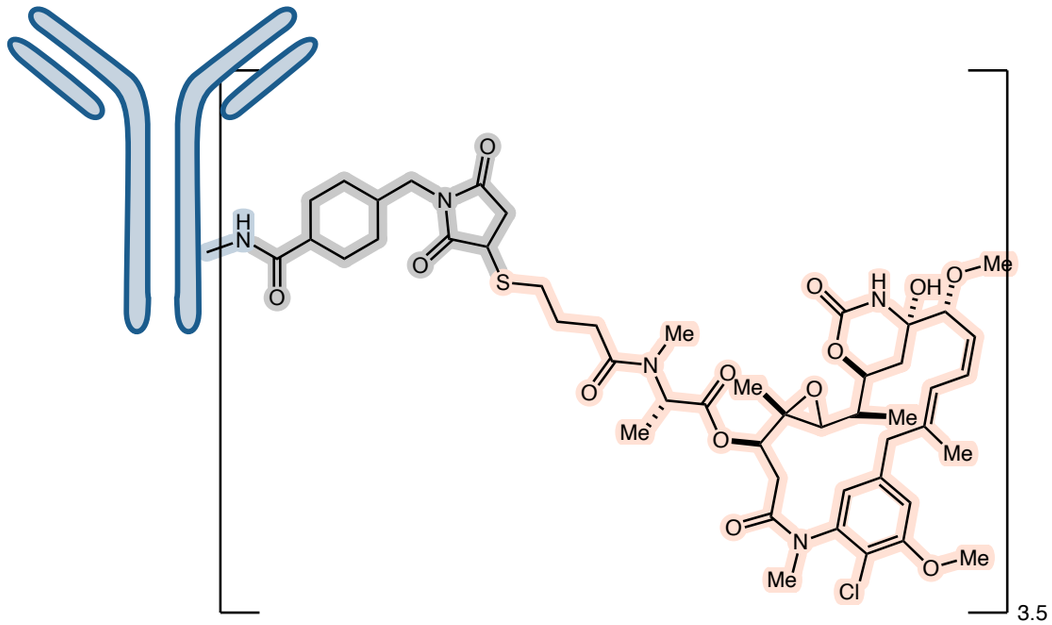
Herceptin™
(Trastuzumab, T)

Genentech



Kadcyla™
(T-DM1)

Genentech



Same 'A' (trastuzumab)

N. Engl. J. Med. **2019**, *380* (7), 617–628

Herceptin™ versus Kadcyla™

KATHERINE trial

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 FEBRUARY 14, 2019 VOL. 380 NO. 7

Trastuzumab Emtansine for Residual Invasive HER2-Positive Breast Cancer

G. von Minckwitz, C.-S. Huang, M.S. Mano, S. Loibl, E.P. Mamounas, M. Untch, N. Wolmark, P. Rastogi, A. Schneeweiss, A. Redondo, H.H. Fischer, W. Jacot, A.K. Conlin, C. Arce-Salinas, I.L. Wapnir, C. Jackisch, M.P. DiGiovanna, P.A. Fasching, J.P. Crown, P. Wülfing, Z. Shao, E. Rota Caremoli, H. Wu, L.H. Lam, D. Tesarowski, M. Smitt, H. Douthwaite, S.M. Singel, and C.E. Geyer, Jr., for the KATHERINE Investigators*

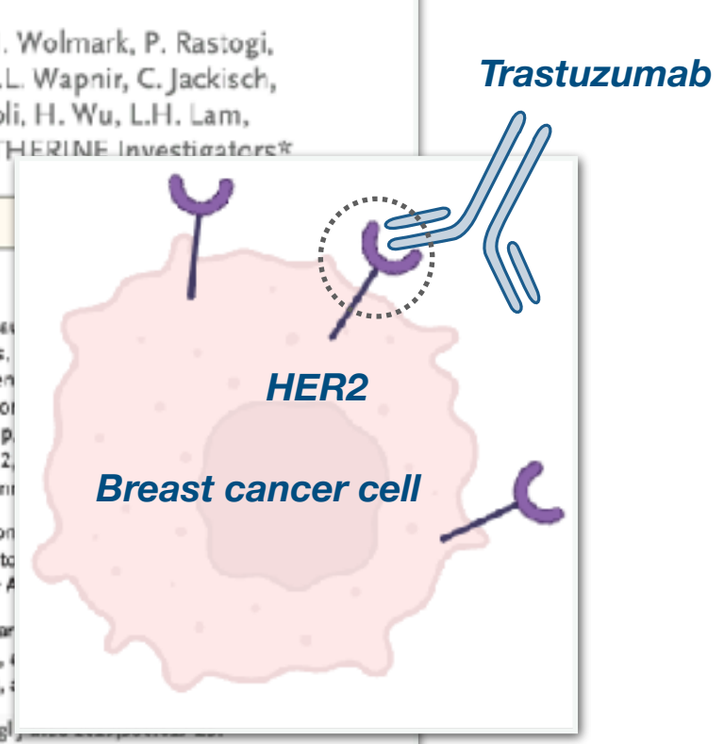
ABSTRACT

BACKGROUND

Patients who have residual invasive breast cancer after receiving neoadjuvant chemotherapy plus human epidermal growth factor receptor 2 (HER2)–targeted therapy have a worse prognosis than those who have no residual cancer. Trastuzumab emtansine (T-DM1), an antibody–drug conjugate of trastuzumab and the cytotoxic agent emtansine (DM1), a maytansine derivative and microtubule inhibitor, provides benefit in patients with metastatic breast cancer that was previously treated with chemotherapy plus HER2-targeted therapy.

METHODS

We conducted a phase 3, open-label trial involving patients with HER2-positive early breast cancer who were found to have residual invasive disease in the breast or axilla at surgery after receiving neoadjuvant therapy containing a taxane (with or without anthracycline) and trastuzumab. Patients were randomly assigned to receive adjuvant T-DM1 or trastuzumab for 14 cycles. The primary end point was invasive disease–free



N. Engl. J. Med. **2019**, *380* (7), 617–628

Herceptin™ versus Kadcyla™

KATHERINE trial

*Phase 3, open-label, **HER2-positive patients (2L)***
Multi-centre (28 countries)
2013-2015

Herceptin™
(Trastuzumab, T)

743 patients

Kadcyla™
(T-DM1)

743 patients

Herceptin™ versus Kadcyla™

KATHERINE trial

*Phase 3, open-label, **HER2-positive patients (2L)***
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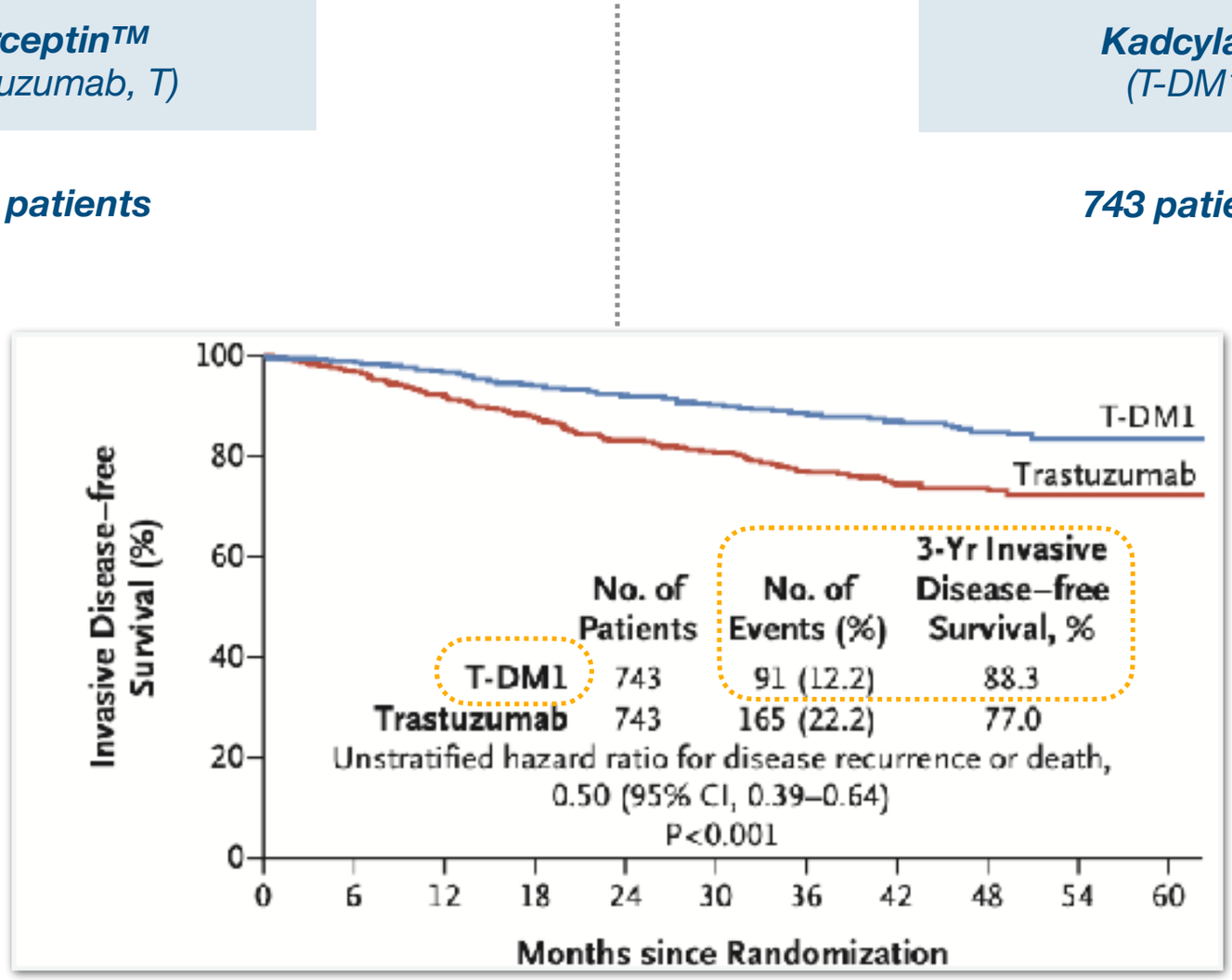
Herceptin™ *(Trastuzumab, T)*	***Kadcyla™*** *(T-DM1)*
743 patients	***743 patients***



Risk of recurrence of invasive breast cancer or death was 50% lower with T-DM1 than with trastuzumab alone

Herceptin™ versus Kadcyla™

KATHERINE trial

*Phase 3, open-label, **HER2-positive patients (2L)***
Multi-centre (28 countries)
2013-2015

Herceptin™
(Trastuzumab, T)

743 patients

Kadcyla™
(T-DM1)

743 patients

(8 years later…)

2023 San Antonio Breast Cancer Symposium; December 5-9, 2023; San Antonio, TX. Abstract GS03-12

Herceptin™ versus Kadcyla™

KATHERINE trial

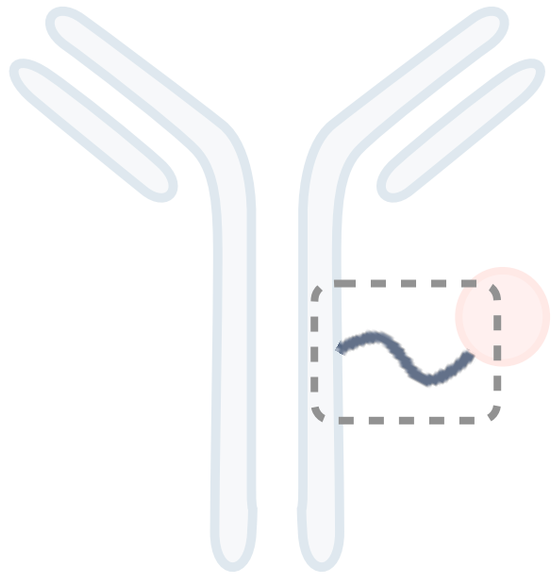
*Phase 3, open-label, **HER2-positive patients (2L)***
Multi-centre (28 countries)
2013-2015

Herceptin™ *(Trastuzumab, T)*	***Kadcyla™*** *(T-DM1)*
743 patients	***743 patients***
(8 years later…)	
461 (62.0%) patients were alive	***521 (70.1%) patients were alive***

2023 San Antonio Breast Cancer Symposium; December 5-9, 2023; San Antonio, TX. Abstract GS03-12

Antibody-drug conjugate

Linker

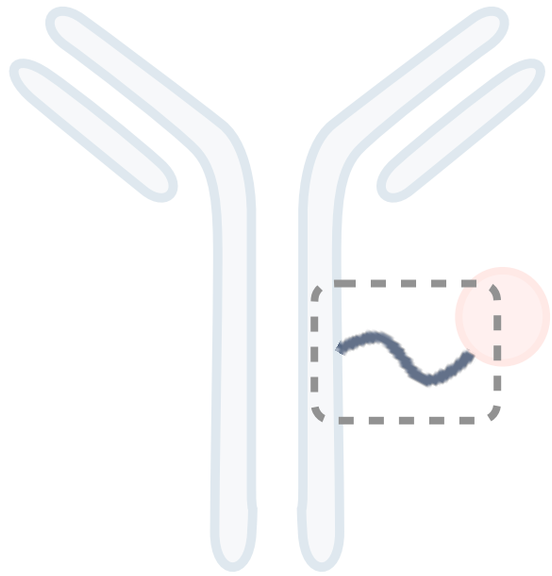


- ***Bioconjugation***

- ***Non-cleavable or cleavable***

Antibody-drug conjugate

Linker

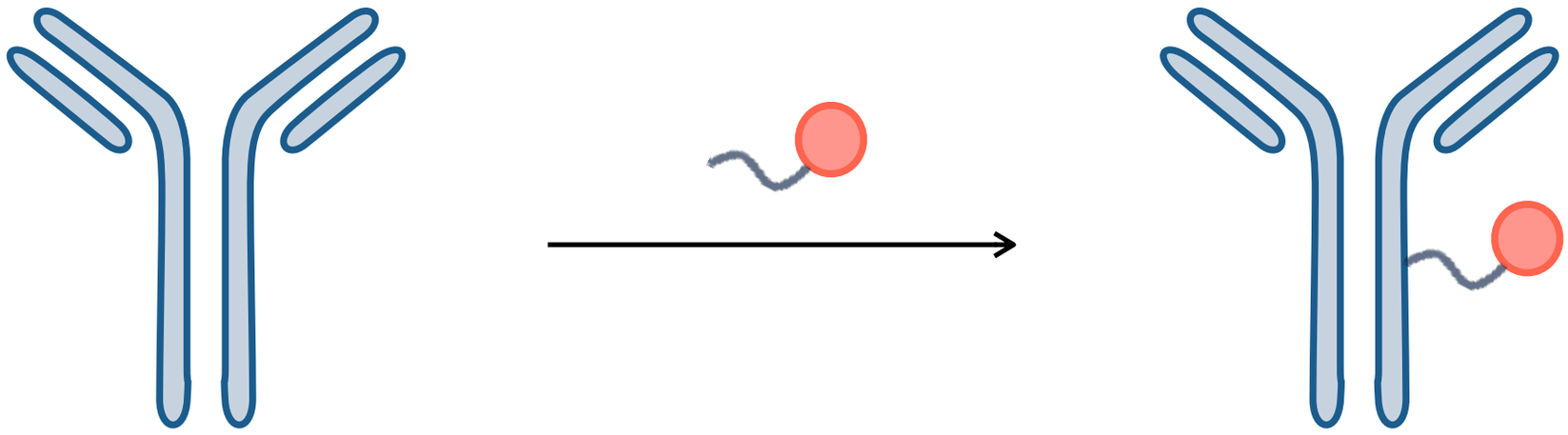


- ***Bioconjugation***

- *Non-cleavable or cleavable*

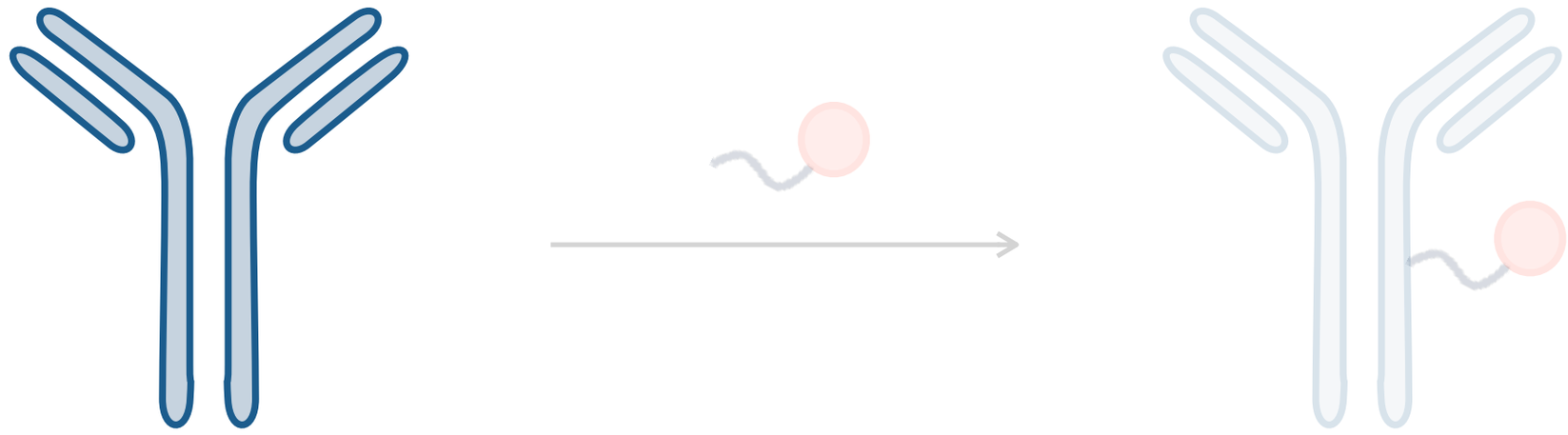
Linker

Bioconjugation



Linker

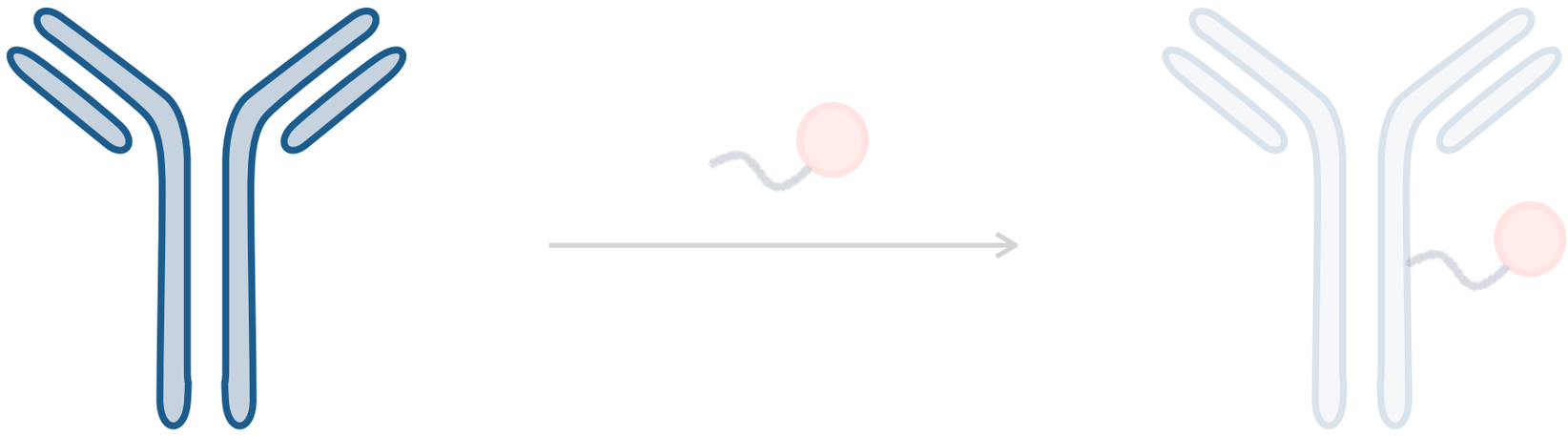
Bioconjugation



Biomolecules are sensitive

Linker

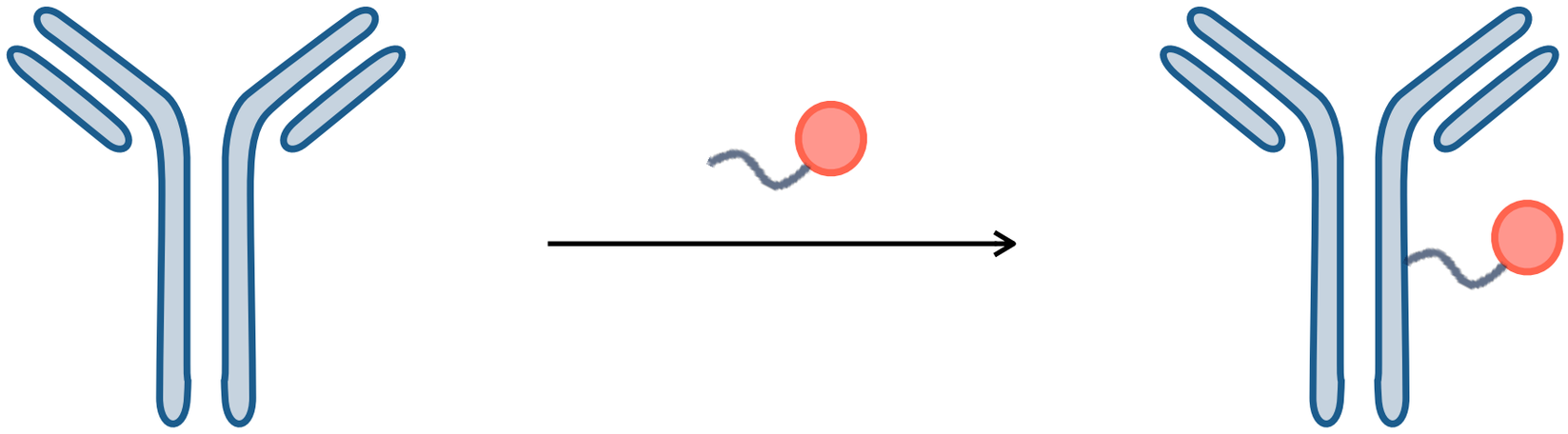
Bioconjugation



Biomolecules are sensitive

Linker

Bioconjugation

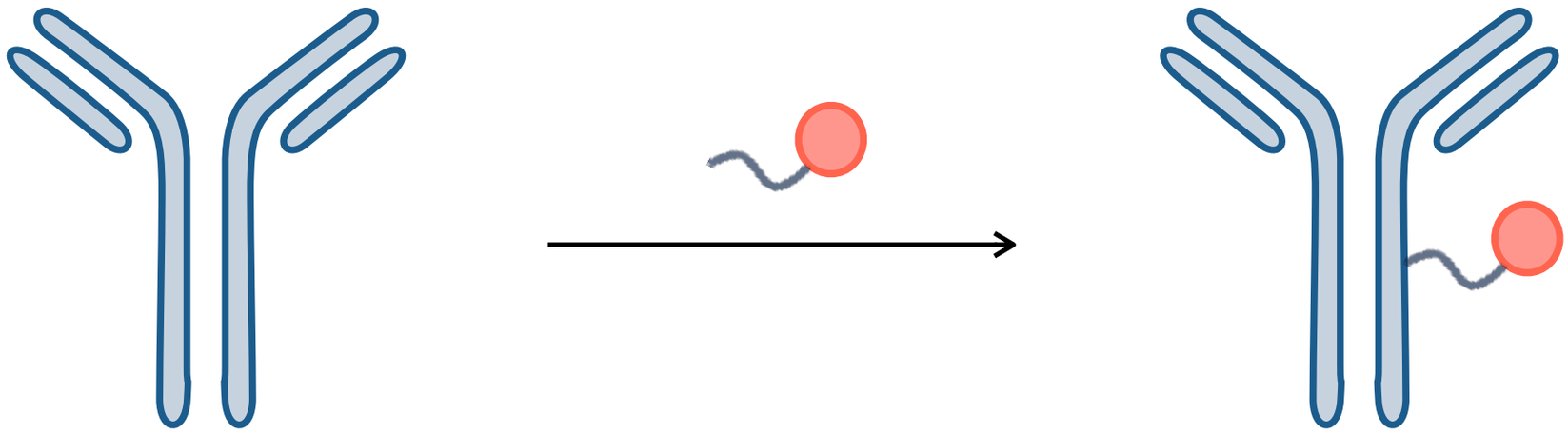


100 L ADC reactor (for conjugation)

“Shaken, not stirred”

Linker

Bioconjugation



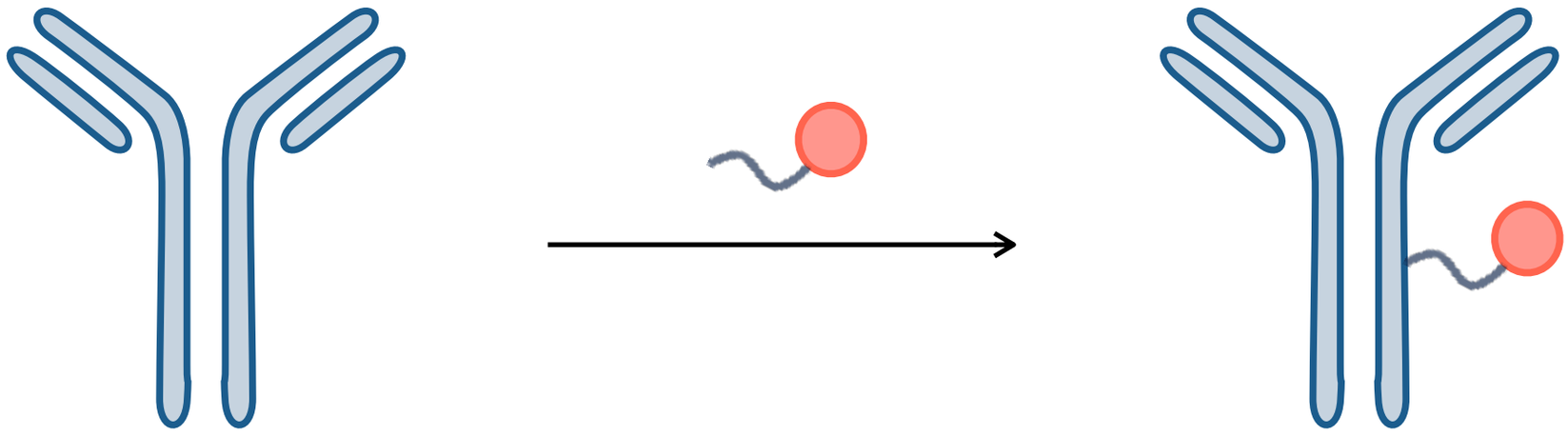
Bioconjugation Criteria

- ✓ *To avoid antibody aggregation*
- ✓ *To minimise antibody denaturation*
- ✓ *To minimise the degradation of linker-drug compounds*
- ✓ *To ensure product stability*

Kang, M. S.; Kong, T. W. S.; Khoo, J. Y. X.; Loh, T.-P. *Chem. Sci.* **2021**, *12* (41), 13613–13647

Linker

Bioconjugation



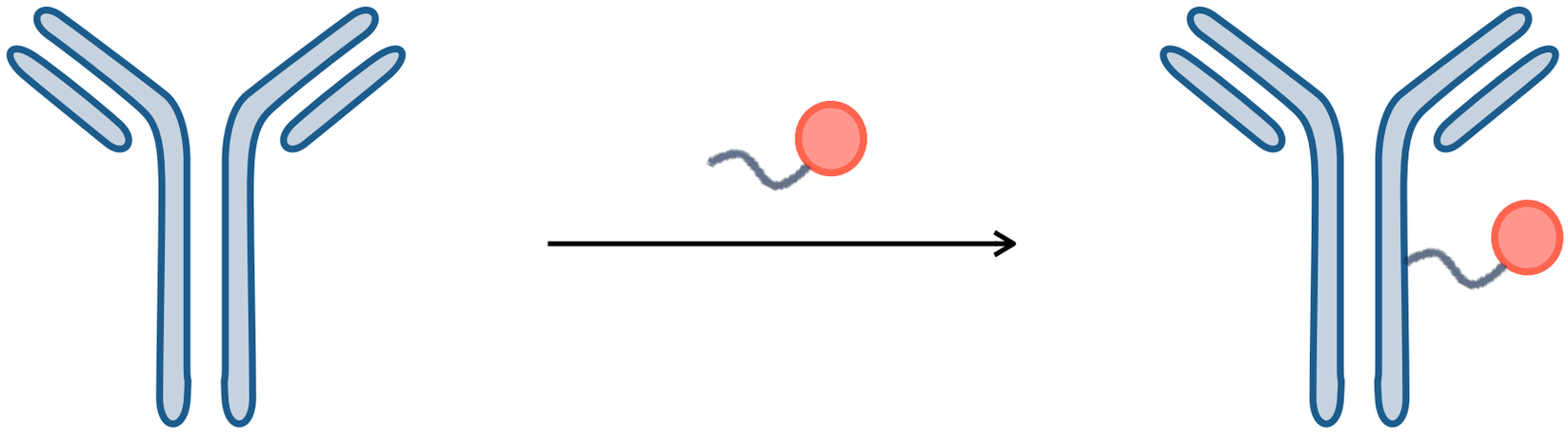
Bioconjugation Criteria

- ✓ *Aqueous*
- ✓ *pH neutral*
- ✓ *Below 37 °C*
- ✓ *Low concentration*
- ✓ *Atom-economic*
- ✓ *Rapid*

Kang, M. S.; Kong, T. W. S.; Khoo, J. Y. X.; Loh, T.-P. *Chem. Sci.* **2021**, *12* (41), 13613–13647

Linker

Bioconjugation



Bioconjugation Criteria

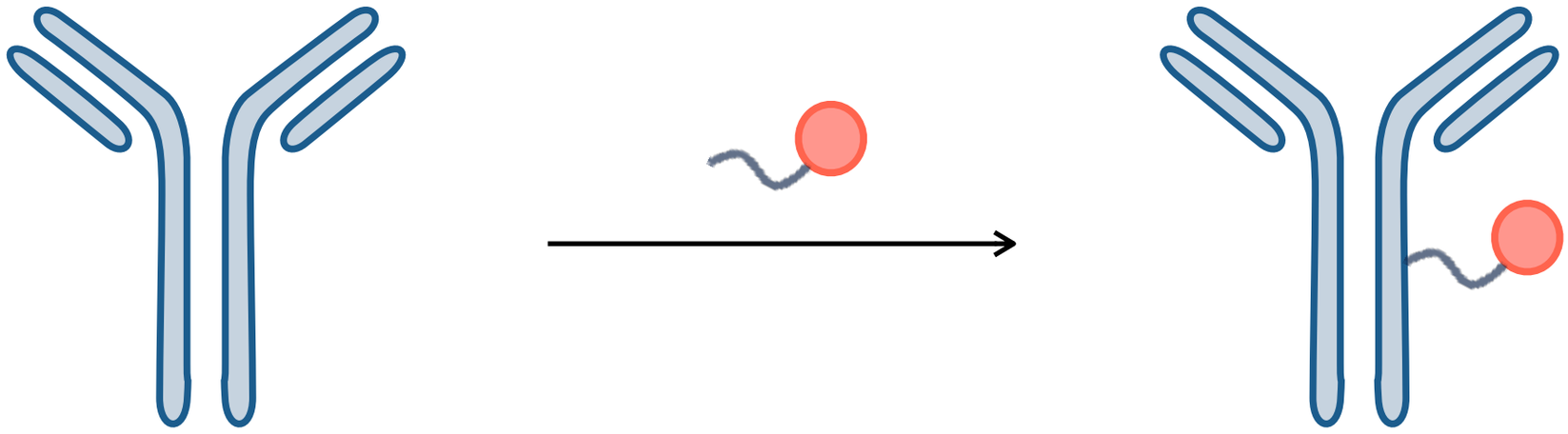
- ✓ *Aqueous*
- ✓ *pH neutral*
- ✓ *Below 37 °C*
- ✓ *Low concentration*
- ✓ *Atom-economic*
- ✓ *Rapid*

Mild, physiological condition that does not disturb the quaternary structure of antibody

Kang, M. S.; Kong, T. W. S.; Khoo, J. Y. X.; Loh, T.-P. *Chem. Sci.* **2021**, *12* (41), 13613–13647

Linker

Bioconjugation



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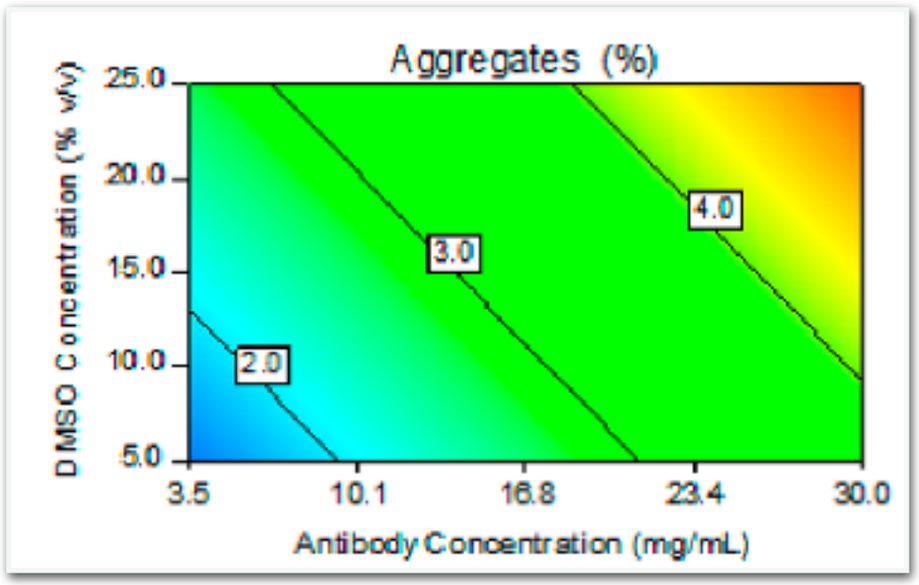
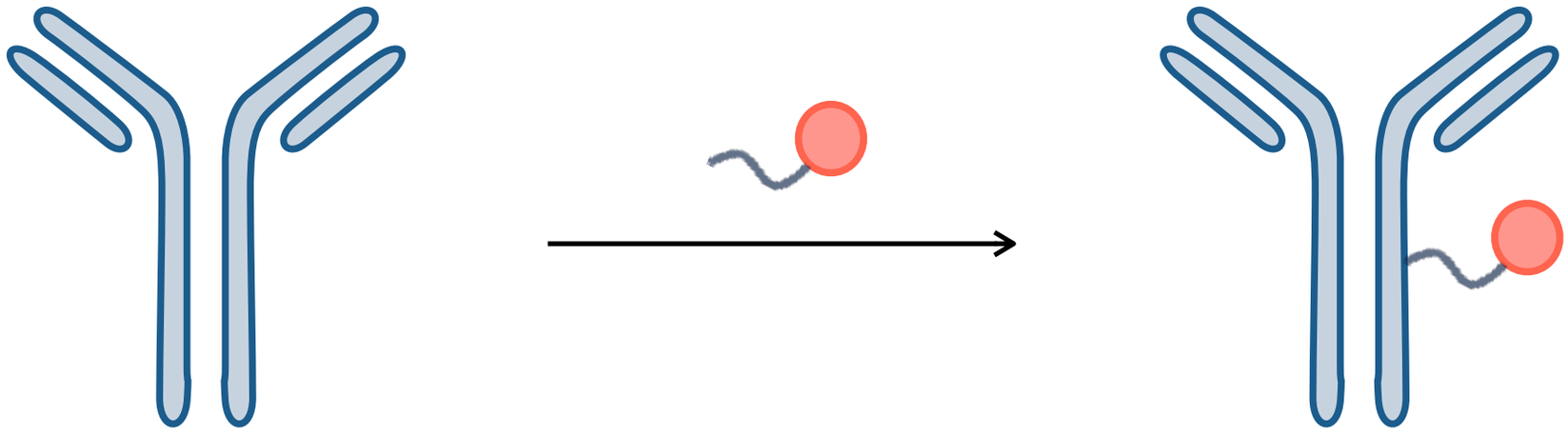


Aggregation(%) rises with organic solvent (% v/v) and Ab concentration (mg/mL)

Nadkarni, D. V.; Jiang, Q.; Friese, O.; Bazhina, N.; Meng, H.; Guo, J.; Kutlik, R.; Borgmeyer, J. *Org. Process Res. Dev.* **2018**, *22* (3), 286–295

Linker

Bioconjugation



Bioconjugation Criteria

- ✓ *Aqueous*
- ✓ *pH neutral*
- ✓ *Below 37 °C*
- ✓ *Low concentration*
- ✓ *Atom-economic*
- ✓ *Rapid*

Common production condition

1-5 mg/mL of mAb
(5-30 μM)

Nadkarni, D. V.; Jiang, Q.; Friese, O.; Bazhina, N.; Meng, H.; Guo, J.; Kutlik, R.; Borgmeyer, J. *Org. Process Res. Dev.* **2018**, *22* (3), 286–295

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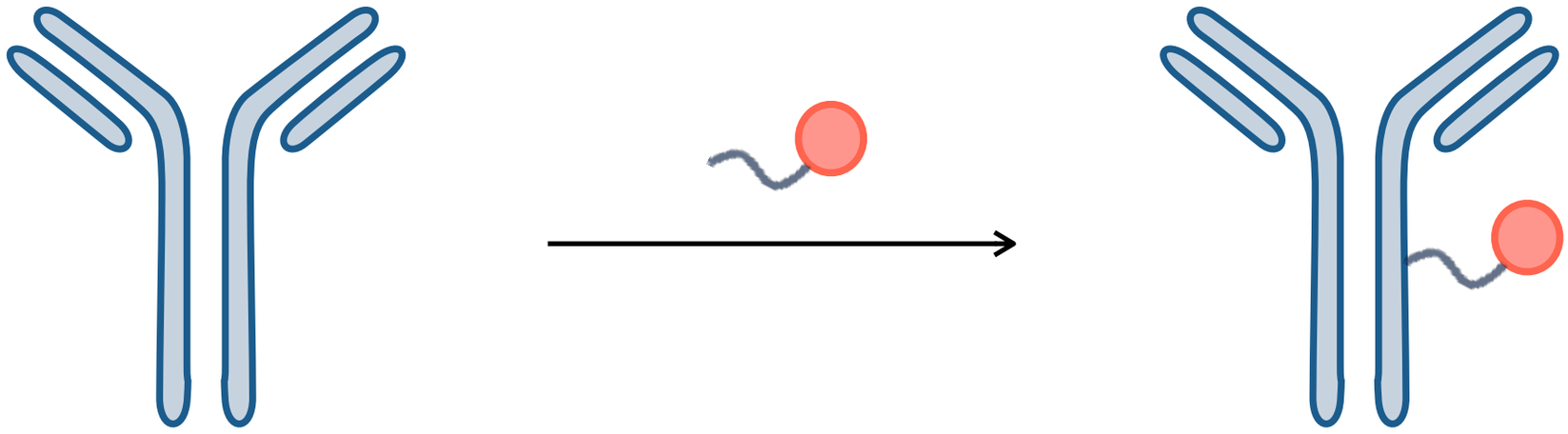
Bioconjugation



Bioconjugation Criteria

- ✓ *Aqueous*
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- ✓ *Below 37 °C*
- ✓ *Low concentration*
- ✓ *Atom-economic*
- ✓ *Rapid*

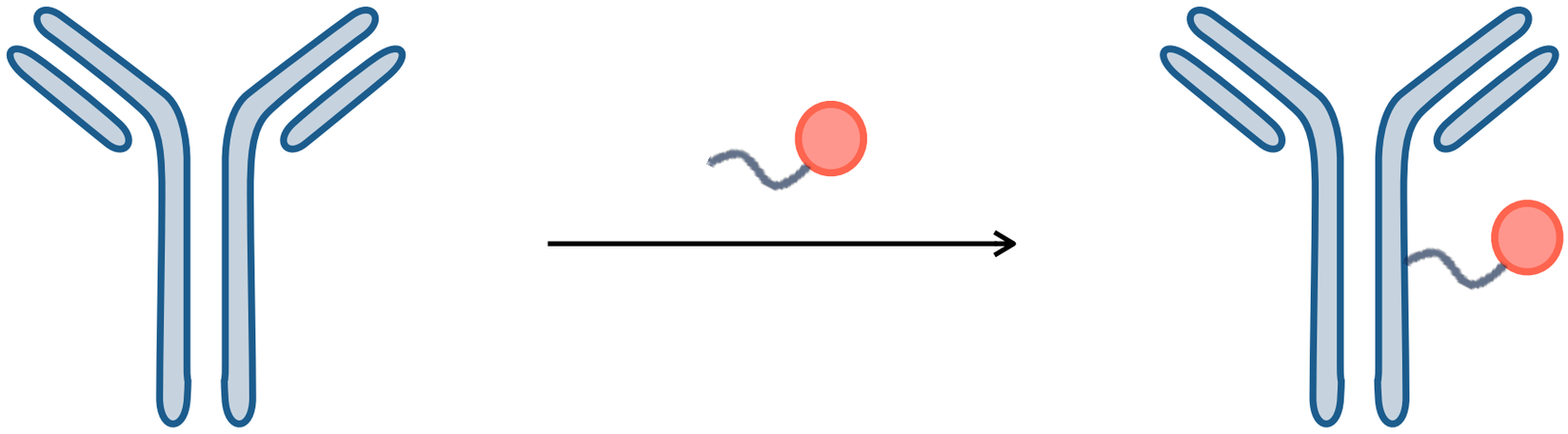
Molar equivalence

~ 1 : 50
(DAR ~ 4)

Nadkarni, D. V.; Jiang, Q.; Friese, O.; Bazhina, N.; Meng, H.; Guo, J.; Kutlik, R.; Borgmeyer, J. *Org. Process Res. Dev.* **2018**, *22* (3), 286–295

Linker

Bioconjugation



Bioconjugation Criteria

- ✓ *Aqueous*
- ✓ *pH neutral*
- ✓ *Below 37 °C*
- ✓ *Low concentration*
- ✓ *Atom-economic*
- ✓ *Rapid*

Rate constant = [mAb][Linker-Drug]

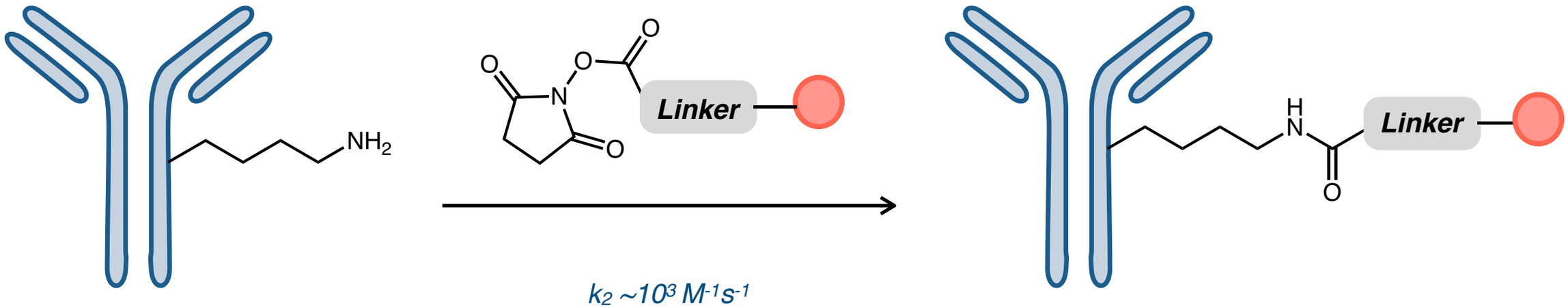
Second-order rate constant (k_2) > 10 $M^{-1}s^{-1}$

With this kinetics,
when [Antibody] = 10-50 μM,
97% conversion within 1 h,
with 10 equiv. of linker-drug to
achieve a DAR of 1

Chen, F.; Gao, J. *Chem. Eur. J.* **2022**, *28* (66)

'C' in ADC

Lysine-NHS ester conjugation

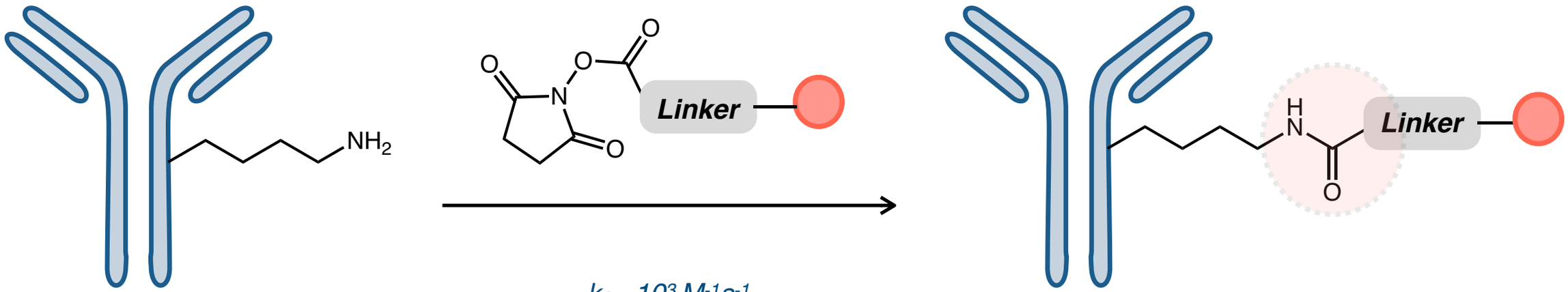


$k_2 \sim 10^3\ M^{-1}s^{-1}$

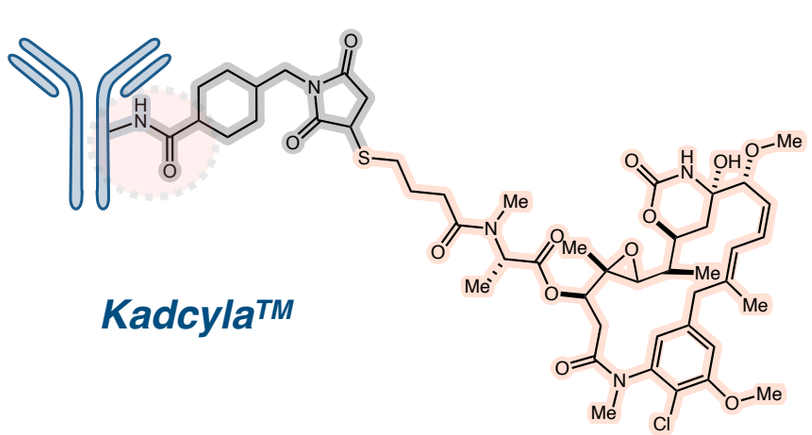
Cline, G. W.; Hanna, S. B. *J. Org. Chem.* **1988**, *53* (15), 3583–3586

'C' in ADC

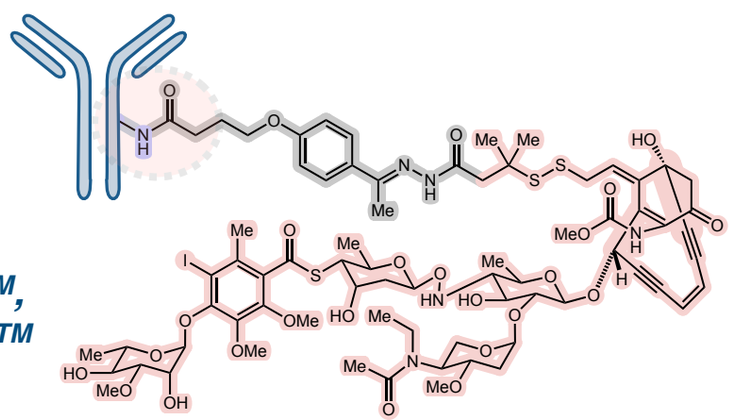
Lysine-NHS ester conjugation



$k_2 \sim 10^3\ M^{-1}s^{-1}$



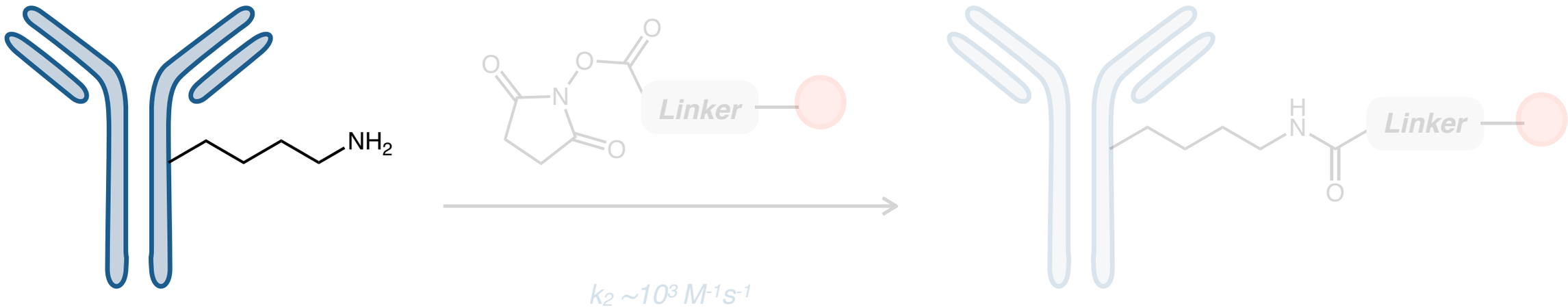
Kadcyla™



Mylotarg™, Besponsa™

Lysine-NHS ester conjugation

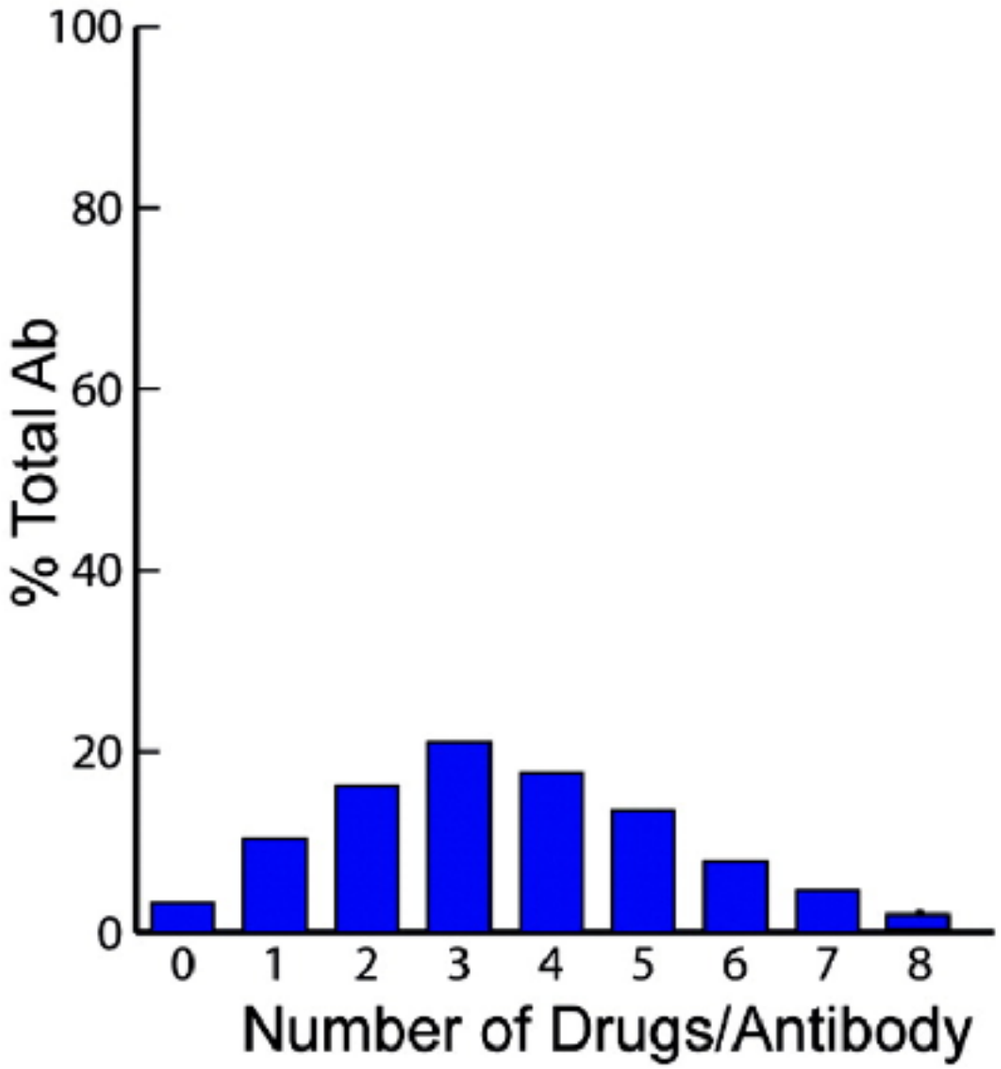
Regioselectivity and chemoselectivity

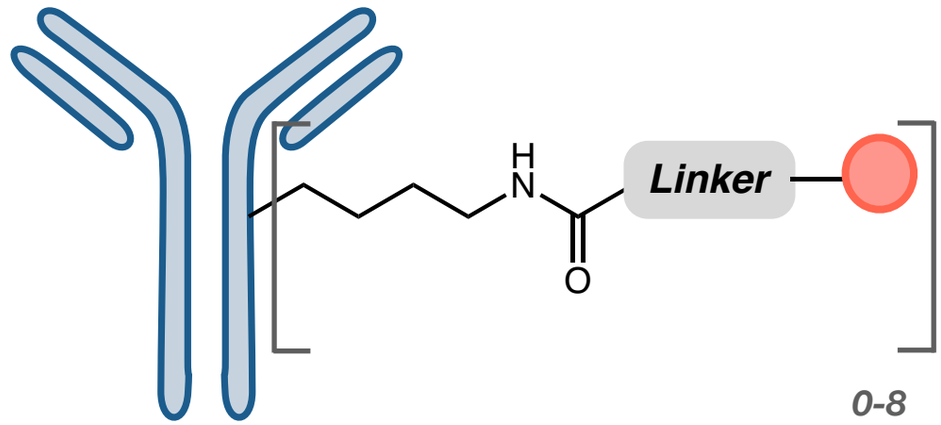


~ 80 accessible lysine sites

Lysine-NHS ester conjugation

Regioselectivity and chemoselectivity

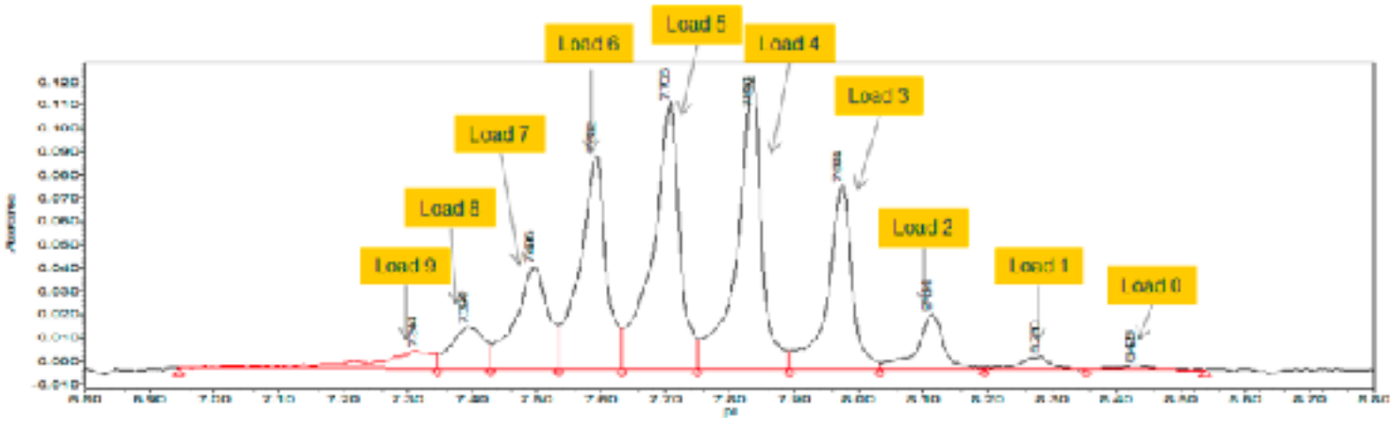


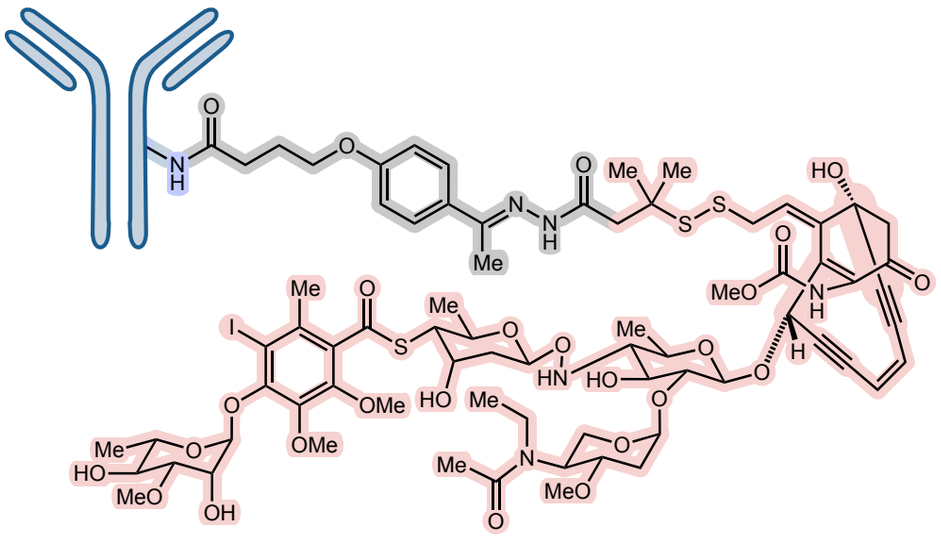


Heterogeneous mixtures with different DARs

Lysine-NHS ester conjugation

Regioselectivity and chemoselectivity





Mylotarg™ ***Average DAR = 1.5***

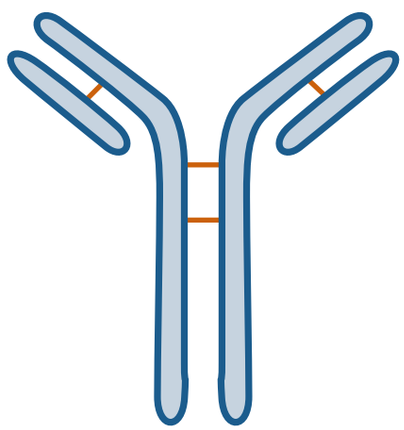
Besponsa™ ***Average DAR = 6***

ADCs with the same DAR are likely to be regioisomers

Nadkarni, D. V.; Jiang, Q.; Friese, O.; Bazhina, N.; Meng, H.; Guo, J.; Kutlik, R.; Borgmeyer, J. *Org. Process Res. Dev.* **2018**, *22* (3), 286–295

‘C’ in ADC

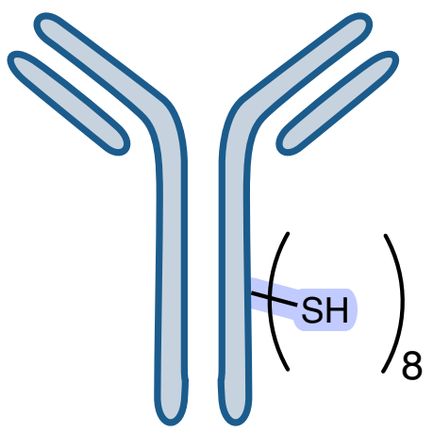
Cysteine-maleimide conjugation



4 Disulfide bonds (IgG1)

‘C’ in ADC

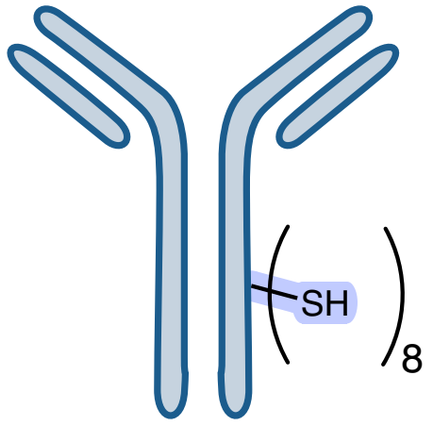
Cysteine-maleimide conjugation



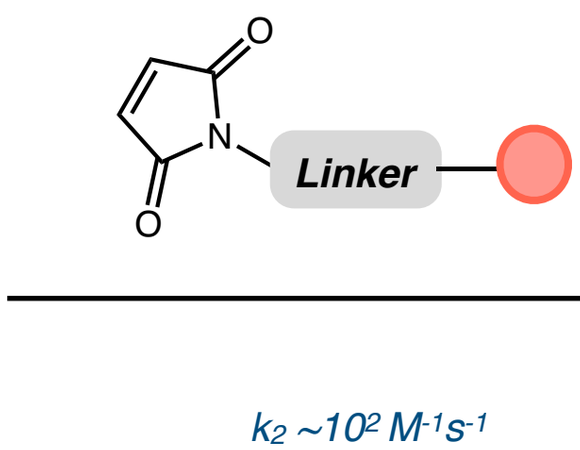
Reduced (with DTT/TCEP)

'C' in ADC

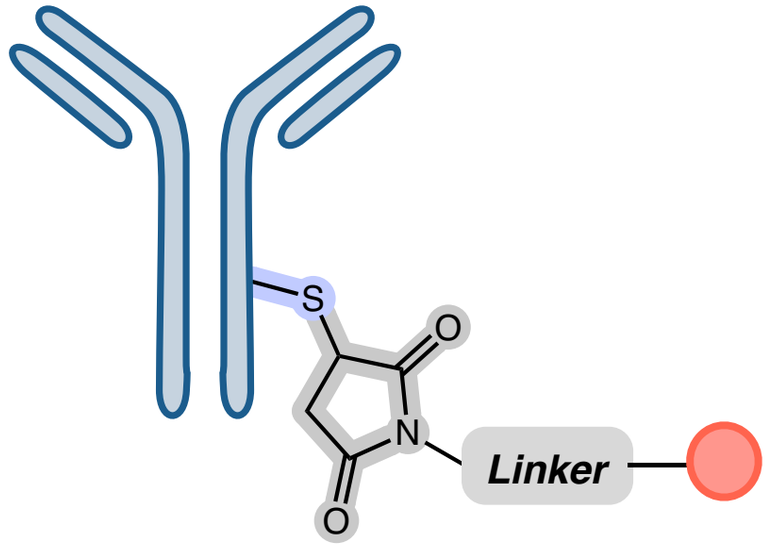
Cysteine-maleimide conjugation



Reduced (with DTT/TCEP)



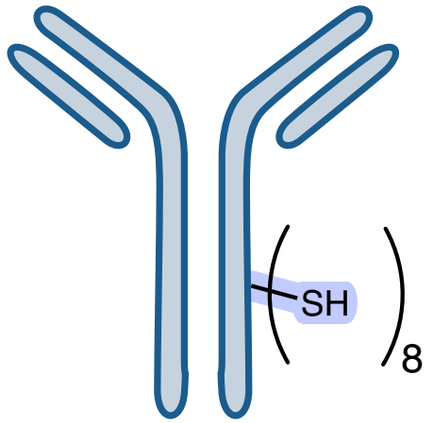
$k_2 \sim 10^2\ M^{-1}s^{-1}$



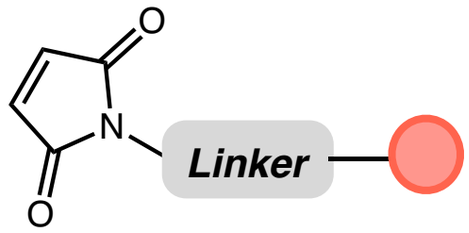
F.-J. Chen, J. Gao, *Chem. Eur. J.* **2022**, *28*, e202201843

'C' in ADC

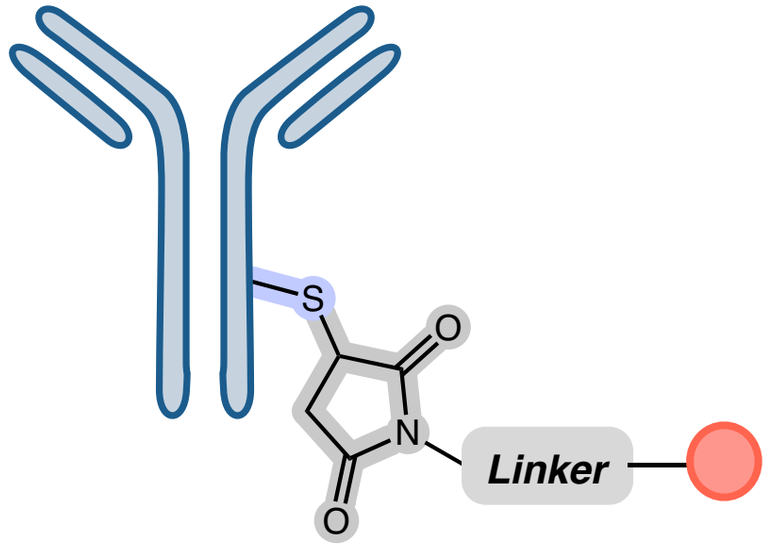
Cysteine-maleimide conjugation



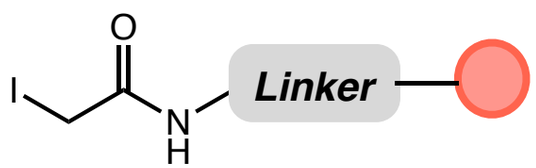
Reduced (with DTT/TCEP)



$k_2 \sim 10^2\ M^{-1}s^{-1}$



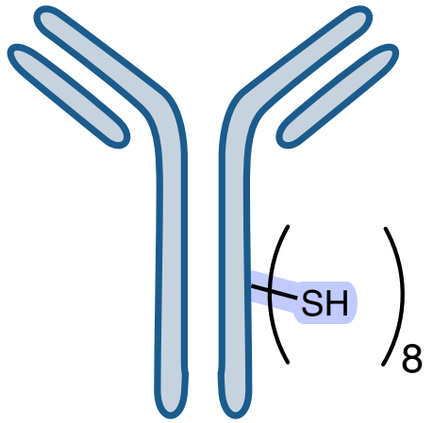
c.f. Iodoacetamide



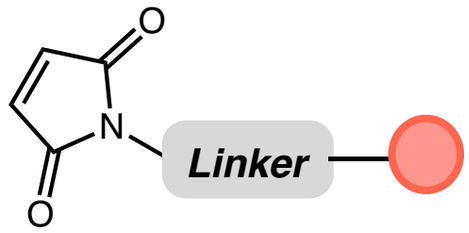
F.-J. Chen, J. Gao, *Chem. Eur. J.* **2022**, *28*, e202201843

'C' in ADC

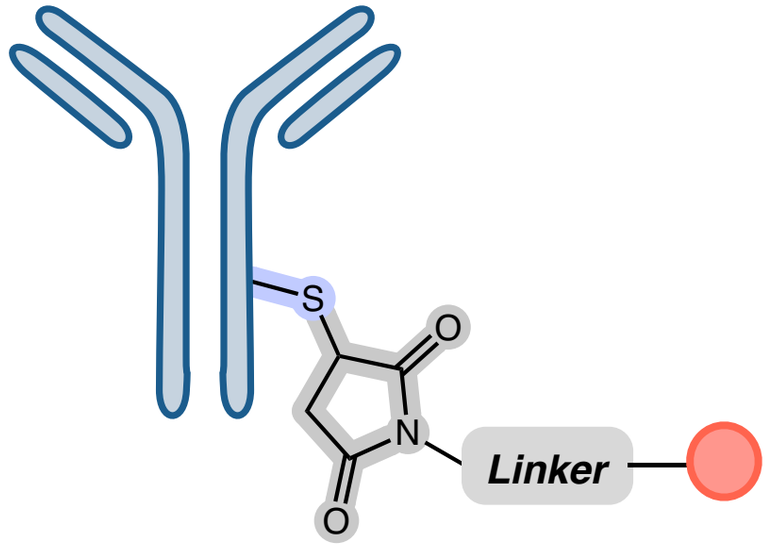
Cysteine-maleimide conjugation



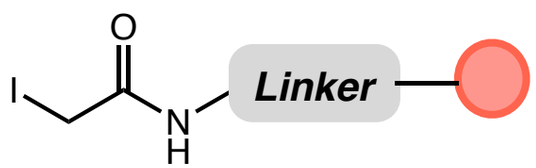
Reduced (with DTT/TCEP)



$k_2 \sim 10^2\ M^{-1}s^{-1}$



c.f. Iodoacetamide



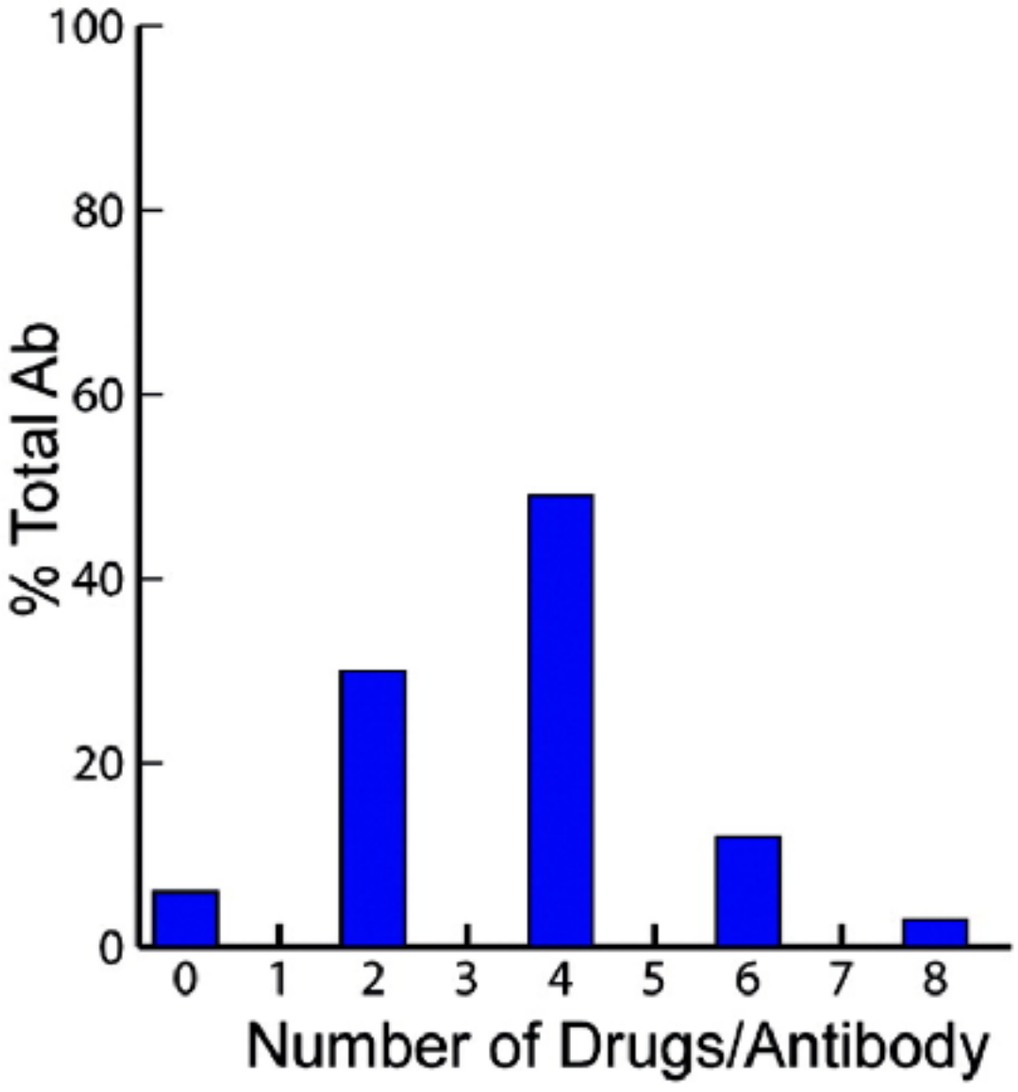
$k_2 \sim 0.6\ M^{-1}s^{-1}$

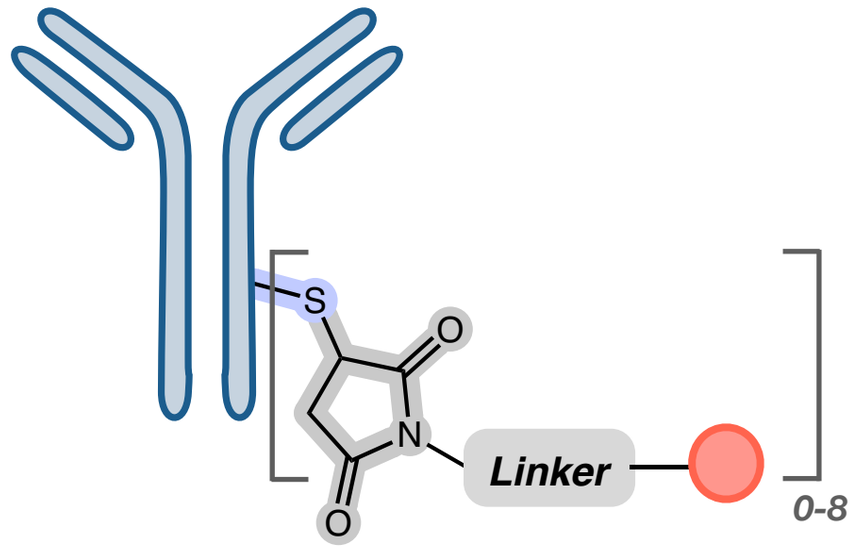
More than 160-fold slower

9 out of 12 FDA-approved ADCs use cysteine-maleimide conjugation

'C' in ADC

Cysteine-maleimide conjugation

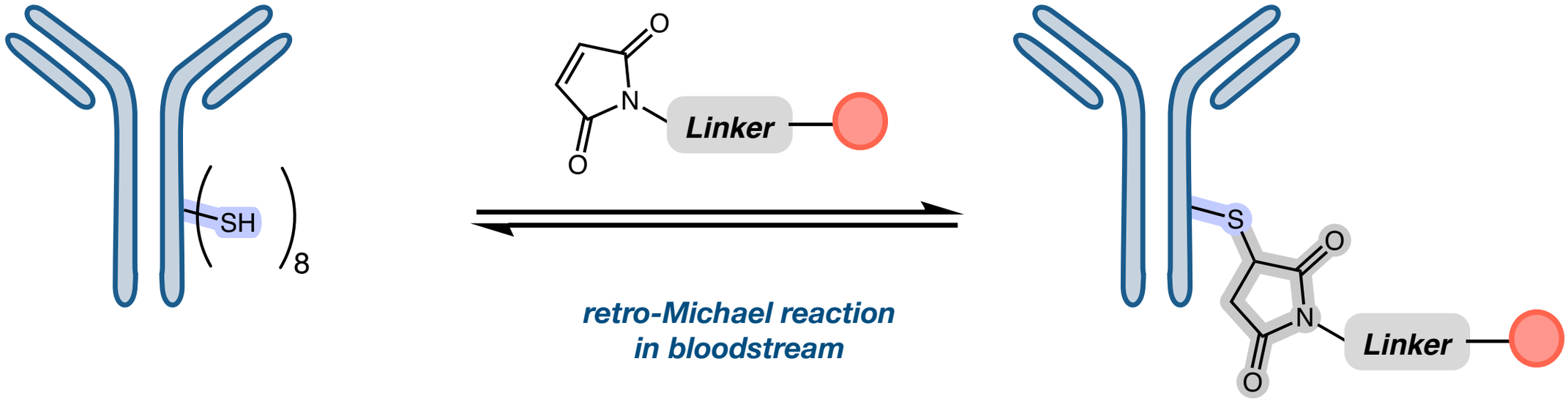




Less heterogeneity compared to the lysine conjugation method

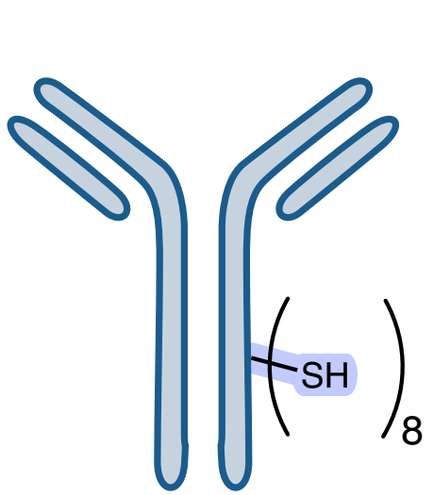
Cysteine-maleimide conjugation

Deconjugation via retro-Michael reaction

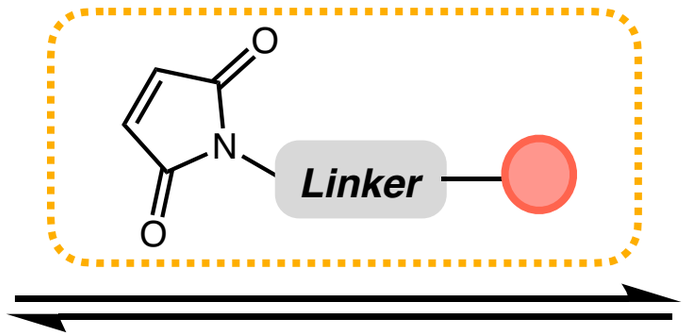


Cysteine-maleimide conjugation

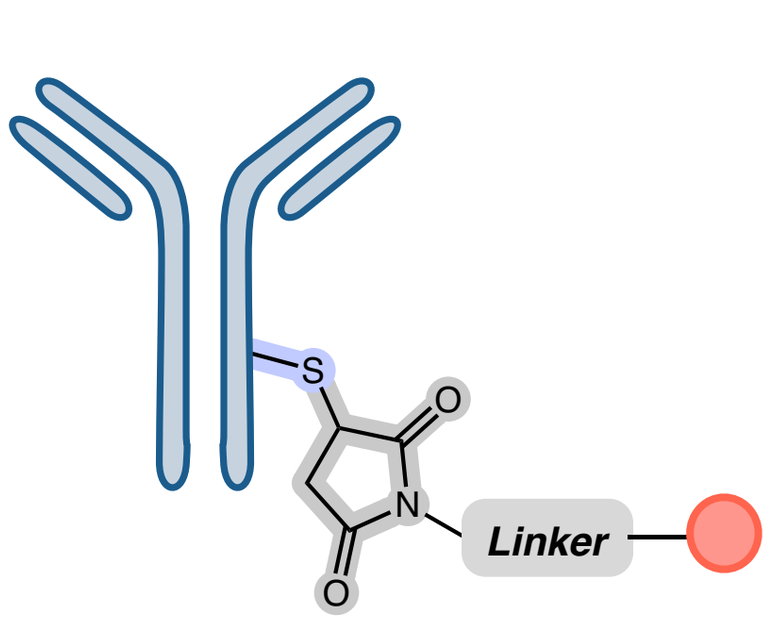
Deconjugation via retro-Michael reaction

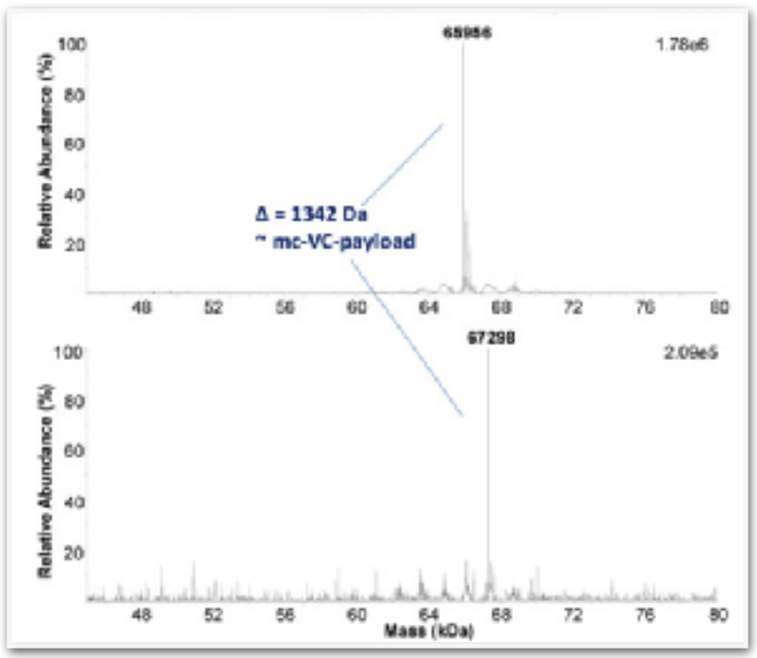


Off-target toxicity



retro-Michael reaction in bloodstream



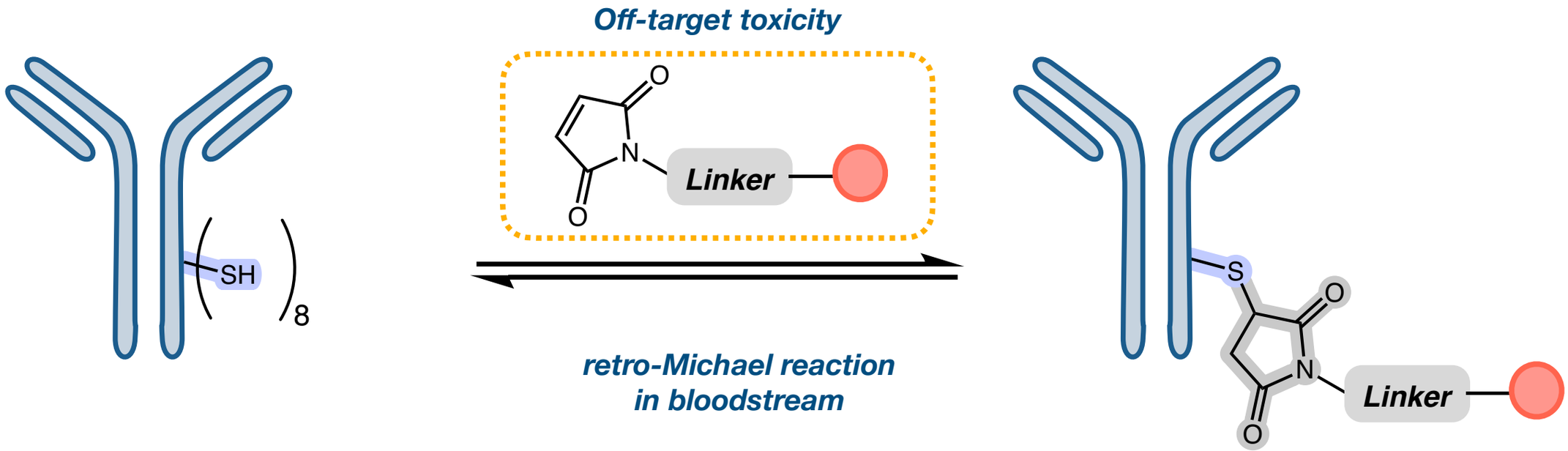


100% drug loss after 144 hours in human plasma

Anal. Chem. **2016**, *88* (9), 4979–4986

Cysteine-maleimide conjugation

Deconjugation via retro-Michael reaction

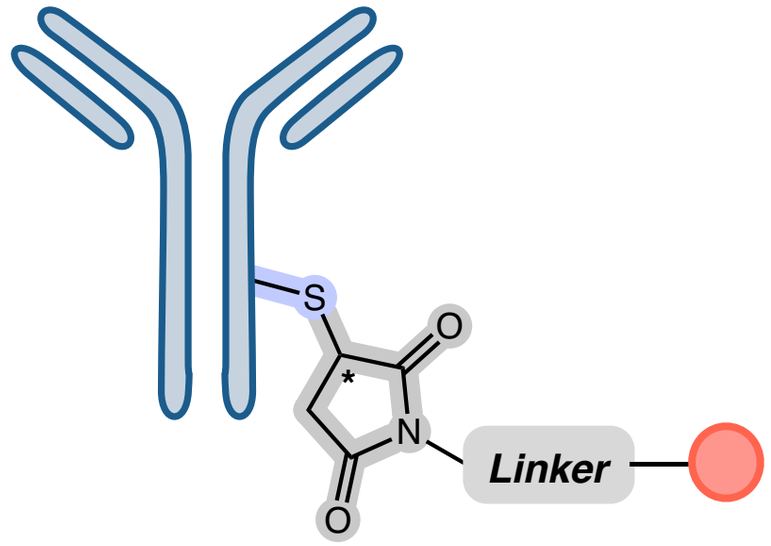


Key G3/4 toxicities of ADCs in the clinic are likely off-target and related to payload

Masters, J. C.; Nickens, D. J.; Xuan, D.; Shazer, R. L.; Amantea, M. *Invest. New Drugs* **2018**, *36* (1), 121–135

Cysteine-maleimide conjugation

Formation of diastereoisomers

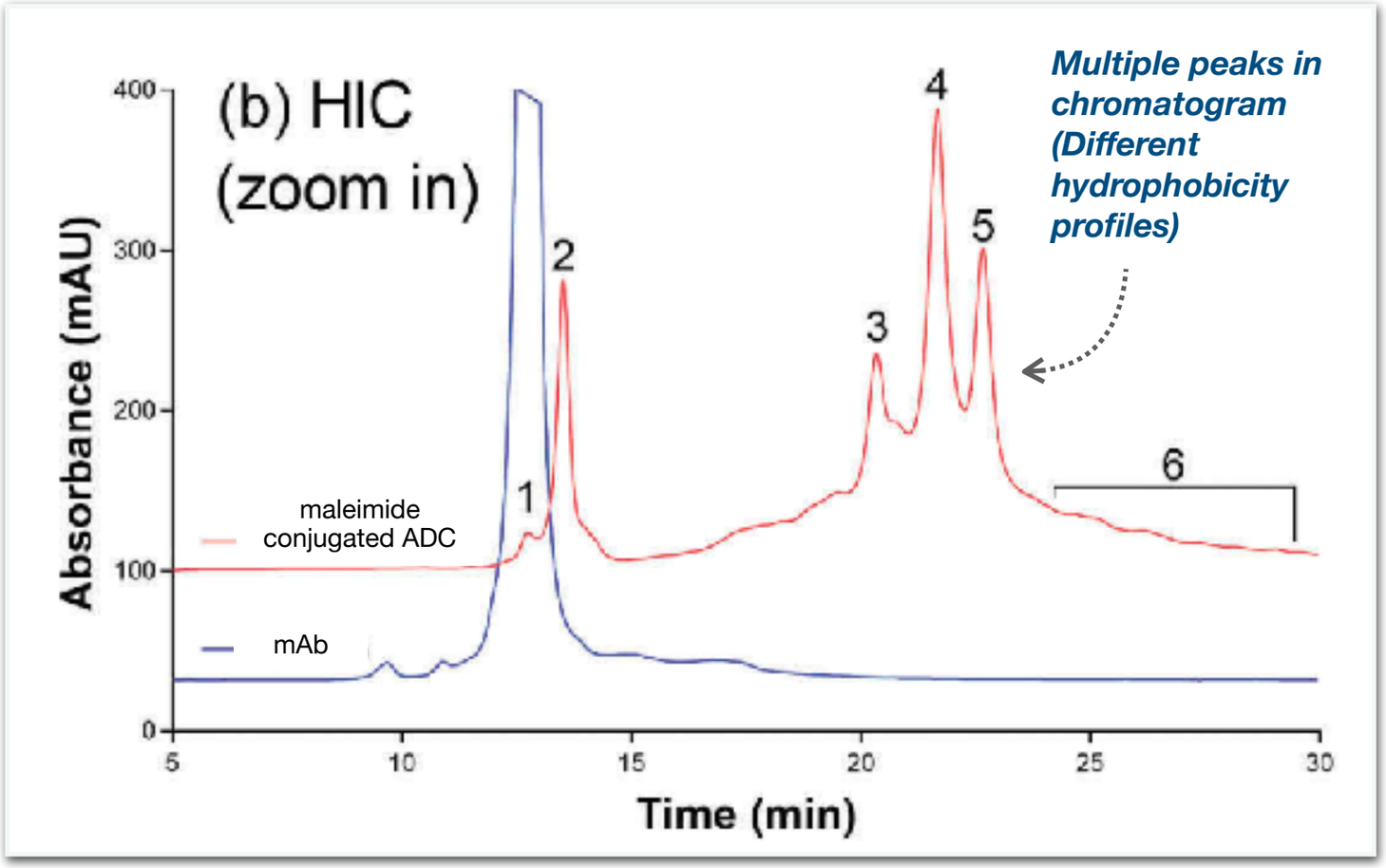


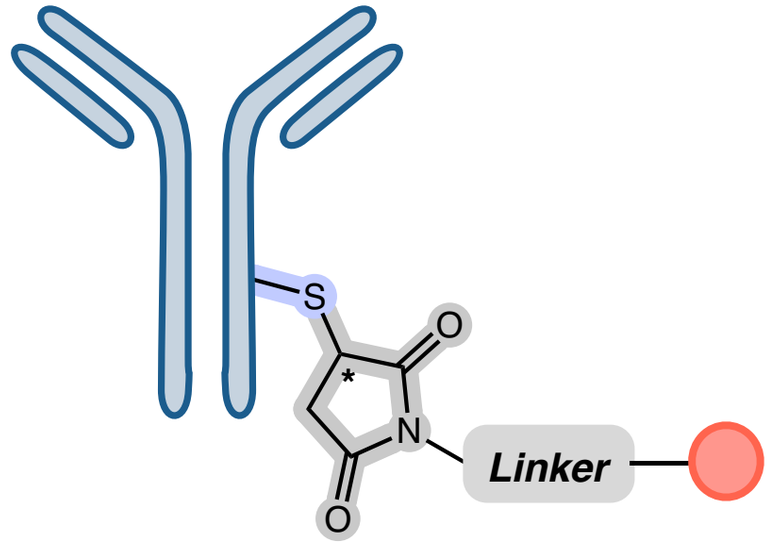
Cysteine-maleimide conjugation

Formation of diastereoisomers

Hydrophobic interaction chromatogram (HIC)

(analytical/purification technique in ADC)





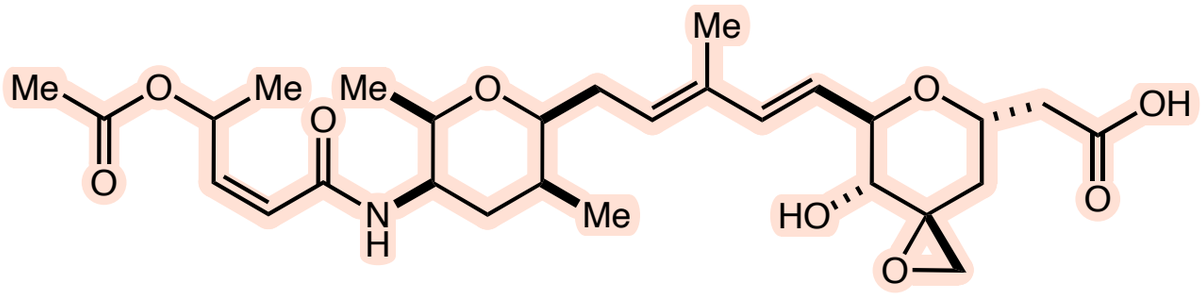
Stereoisomeric conjugation sites result in ***differences in the three-dimensional orientation*** *of the linker-drug payload on the antibody*

Complicates analysis/separation of ADCs

Parthemore, C.; Korman, S.; Thompson, C.; Wendeler, M.; Liu, D. *mAbs* **2019**, *11* (6), 1064–1076.

Cysteine-maleimide conjugation

Pfizer's development of Thailanstatin A-containing ADC



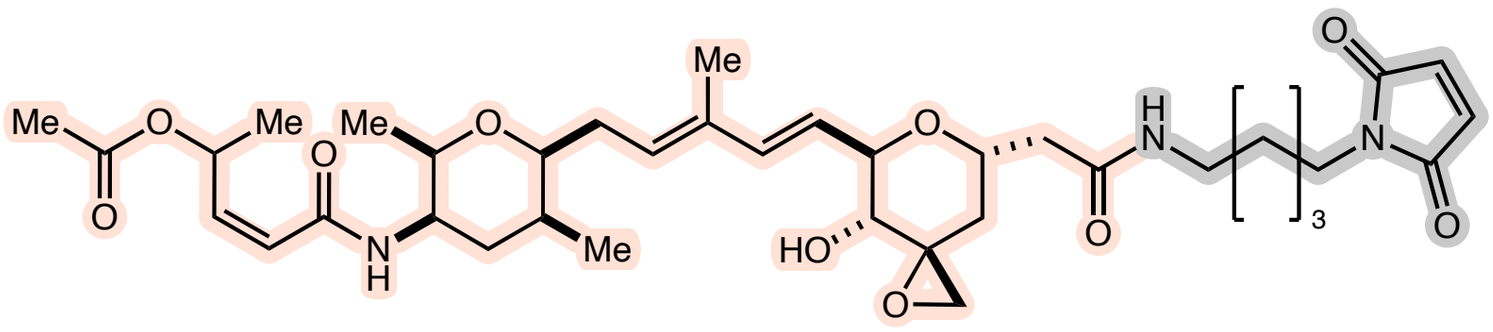
Thailanstatin A

IC_{50} = 650 nM

Puthenveetil, S.; Sapra, P.; Gerber, H. P.; Ma, D.; Graziani, E. I. *Bioconjug. Chem.* **2016**, *27* (8), 1880–1888

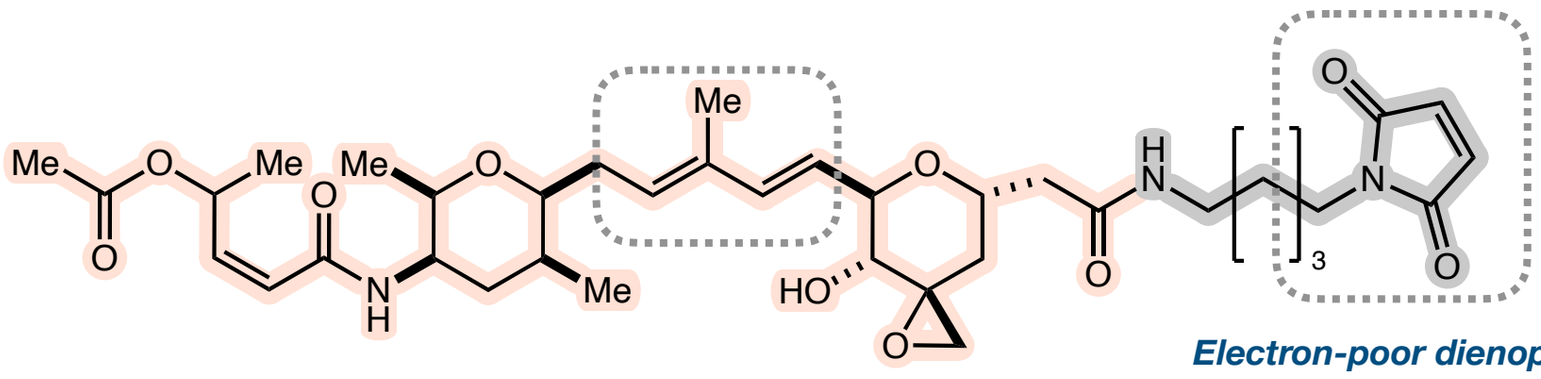
Cysteine-maleimide conjugation

Pfizer's development of Thailanstatin A-containing ADC



Cysteine-maleimide conjugation

Pfizer's development of Thailanstatin A-containing ADC

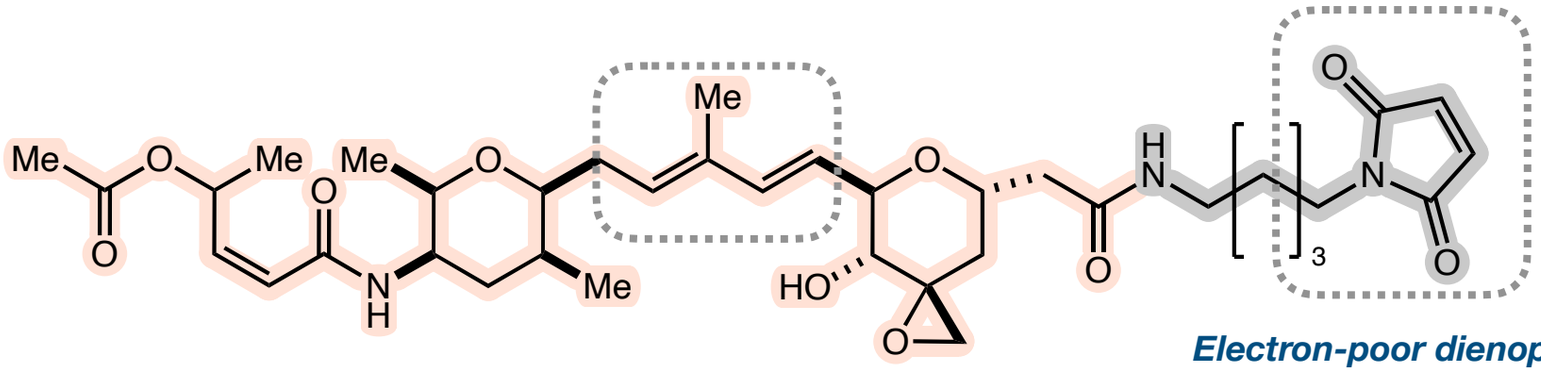


Electron-poor dienophile

Diels-Alder reaction

Cysteine-maleimide conjugation

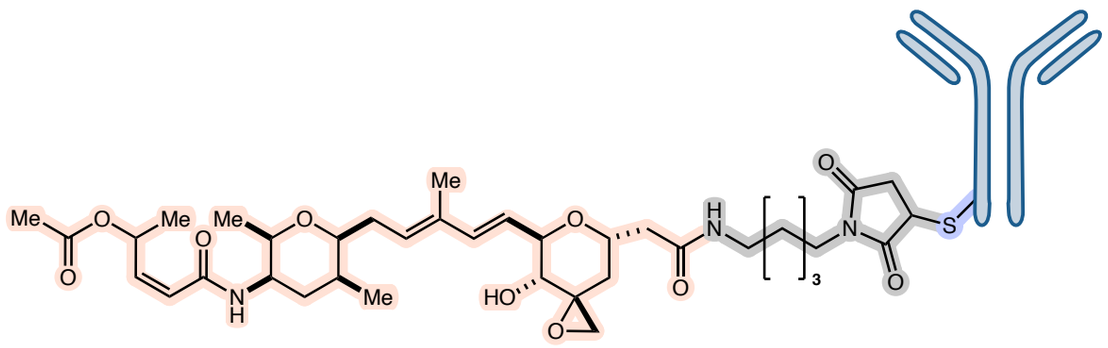
Pfizer's development of Thailanstatin A-containing ADC

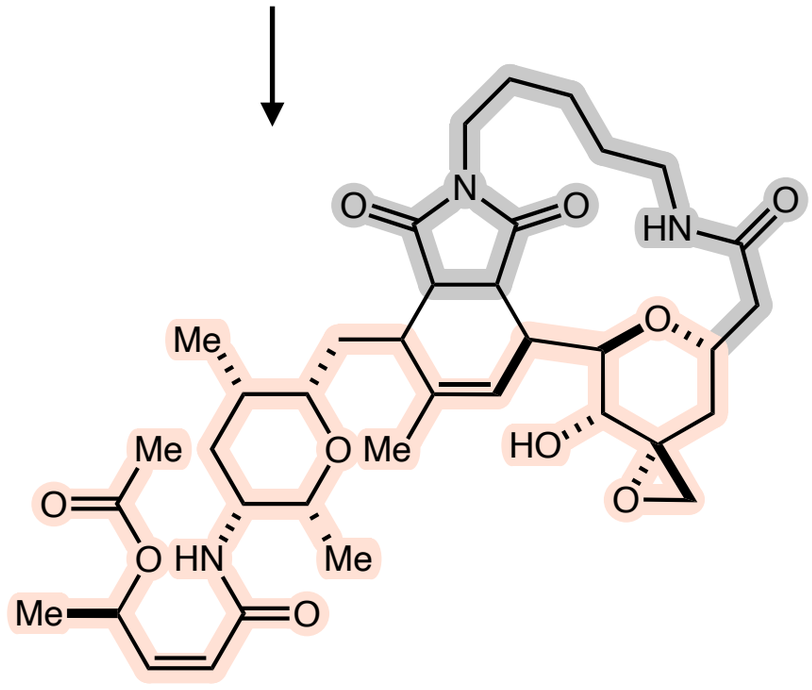


Electron-poor dienophile

Diels-Alder reaction

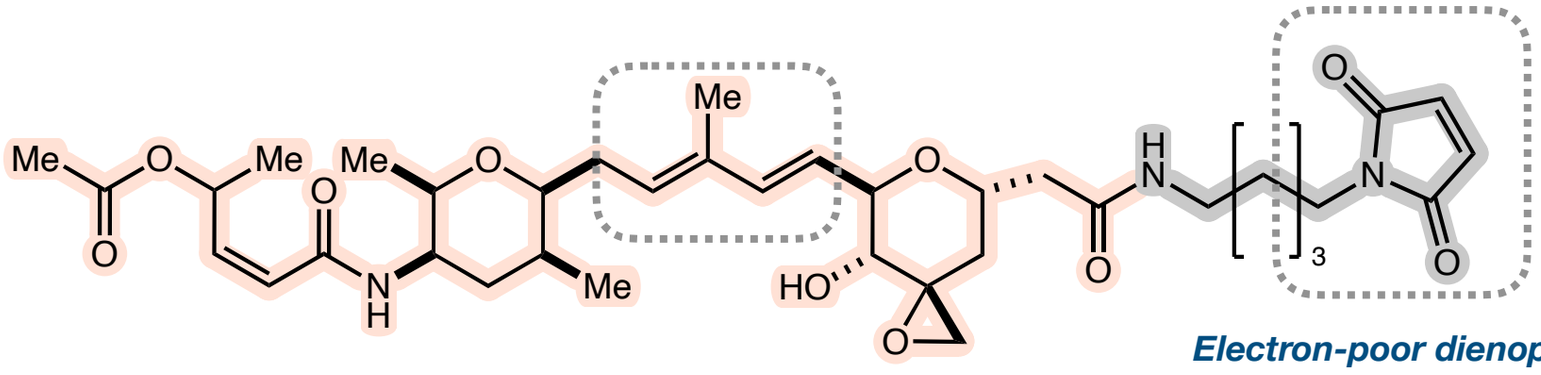
Desired product (ADC)





Cysteine-maleimide conjugation

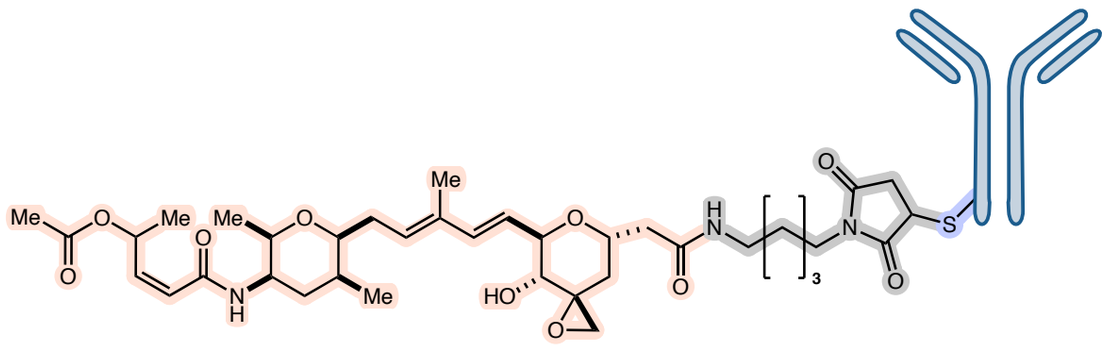
Pfizer's development of Thailanstatin A-containing ADC

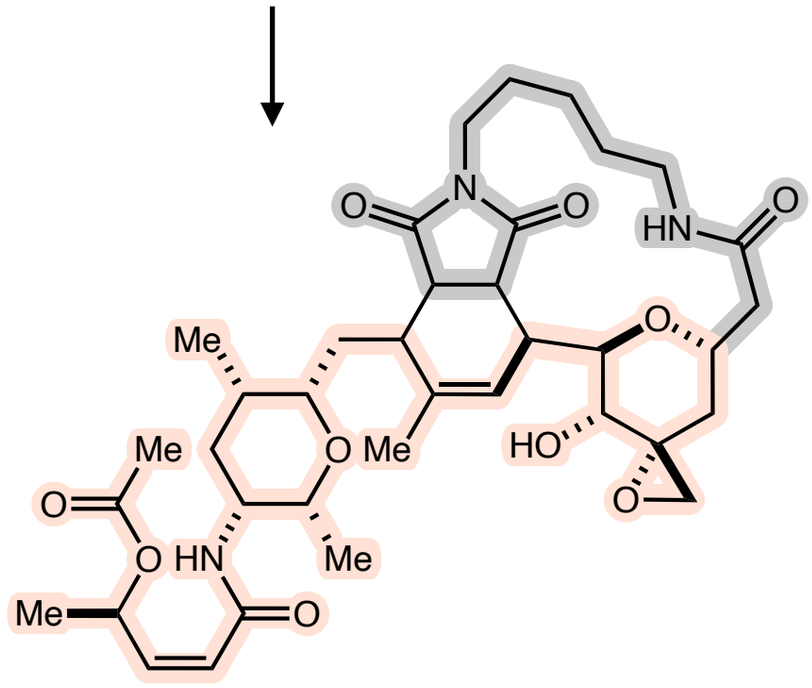


Electron-poor dienophile

Diels-Alder reaction

Desired product (ADC)



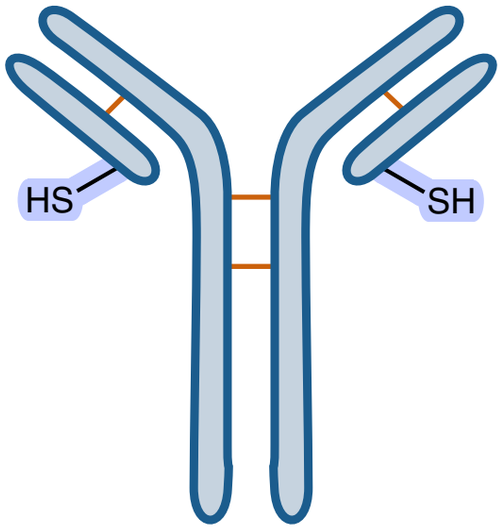


Maleimide bioconjugation linker is not compatible with diene-containing 'D'

'C' in ADC

Conjugation through antibody engineering

Genentech



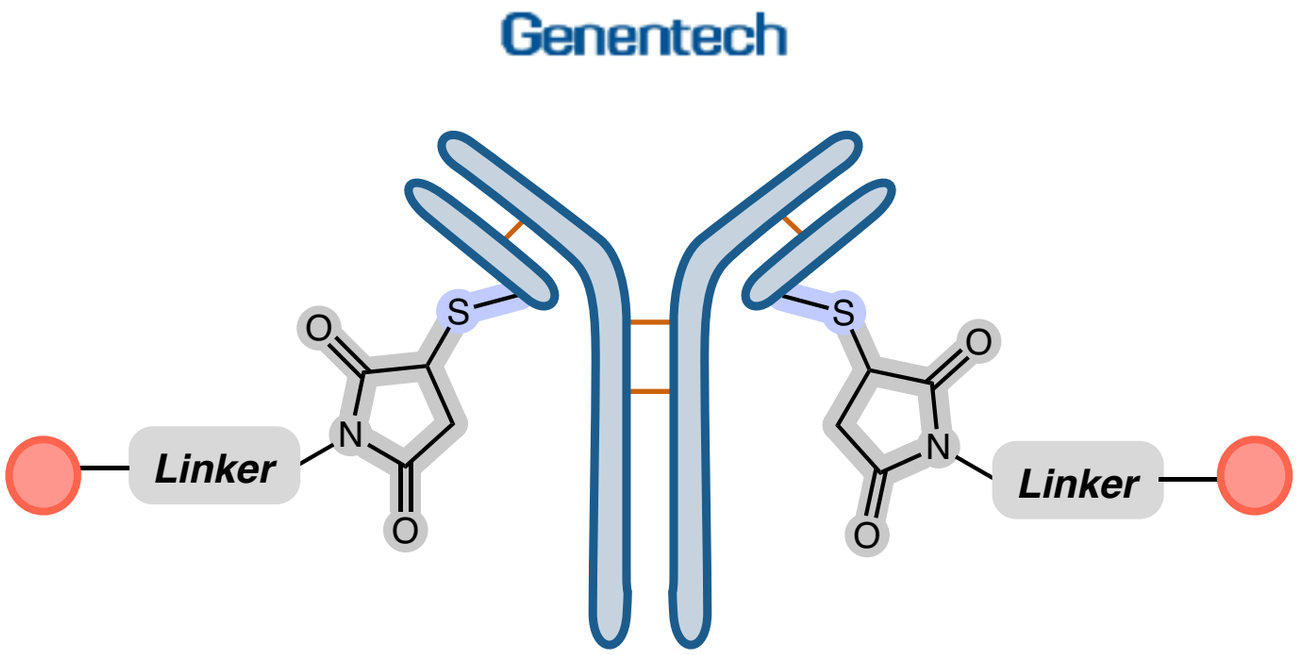
THIOMAB™ antibody

2 engineered cysteines

IP right US20070092940

Conjugation through antibody engineering

THIOMAB Technology

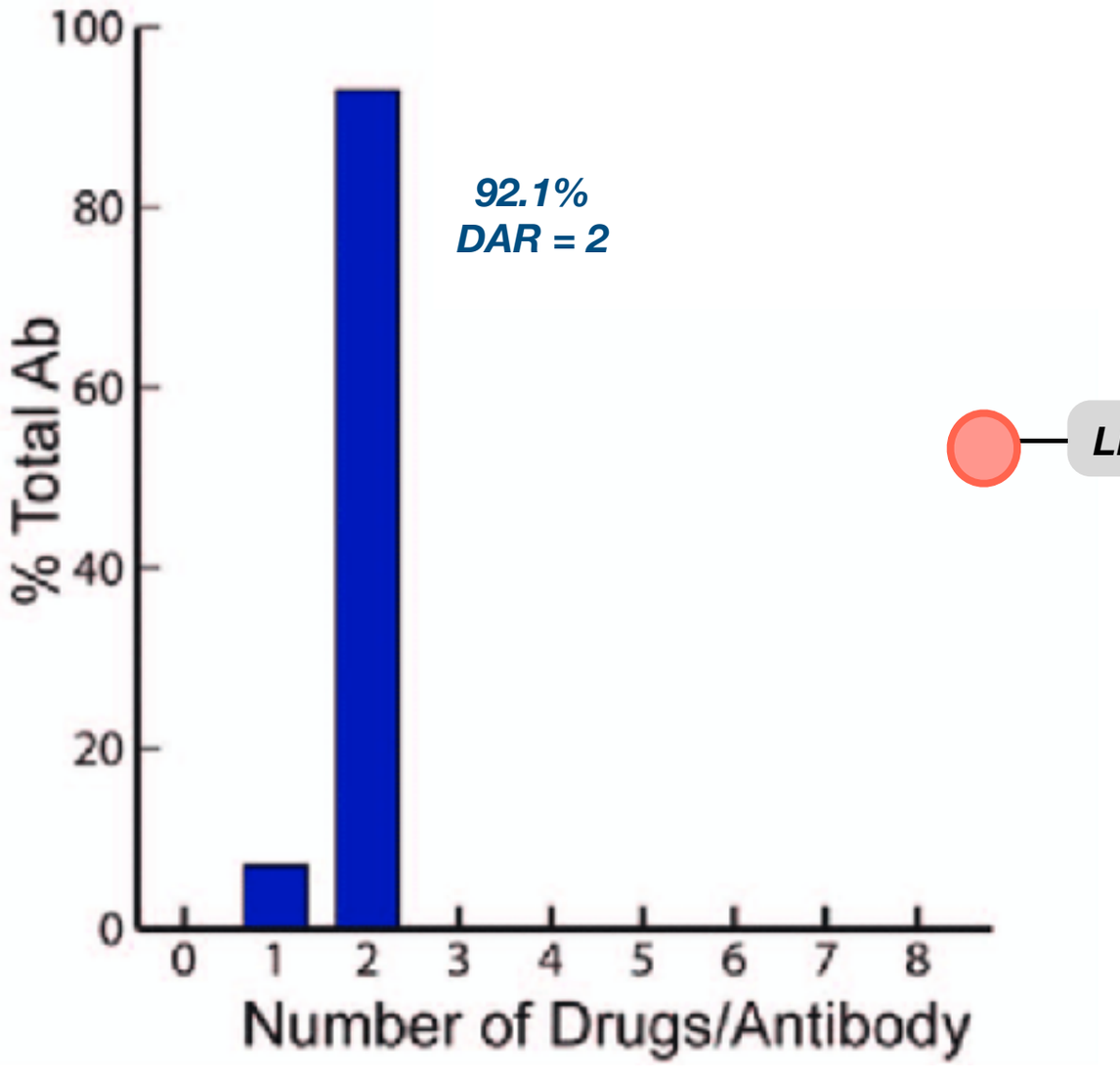


THIOMAB™ antibody

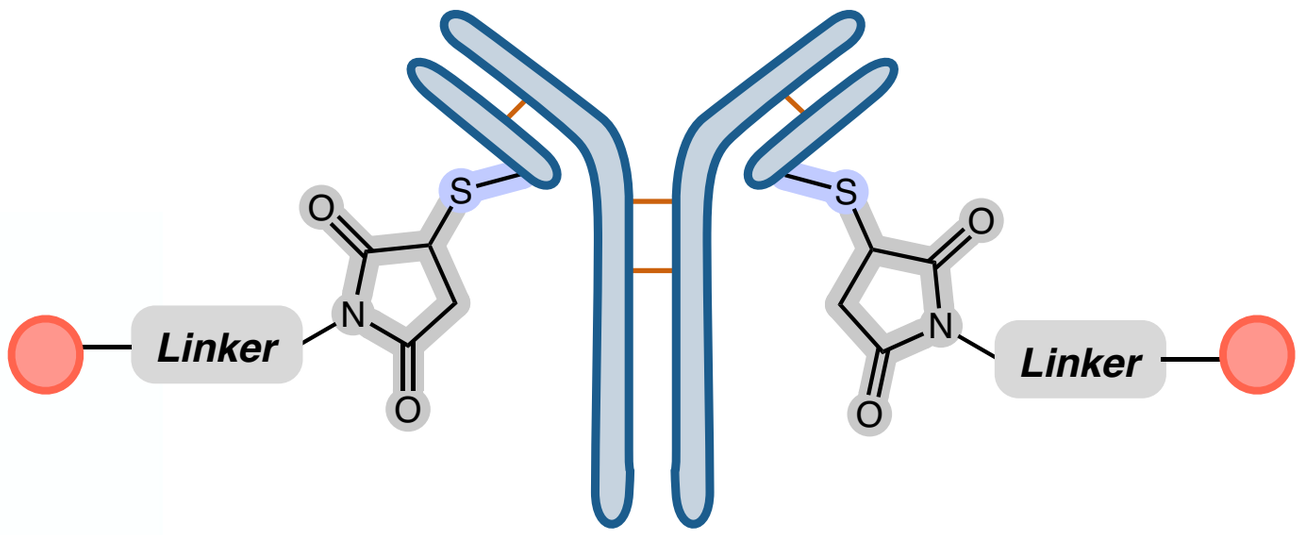
Conjugation through antibody engineering

THIOMAB Technology

Highly homogenous DAR



92.1%
DAR = 2



THIOMAB™ antibody

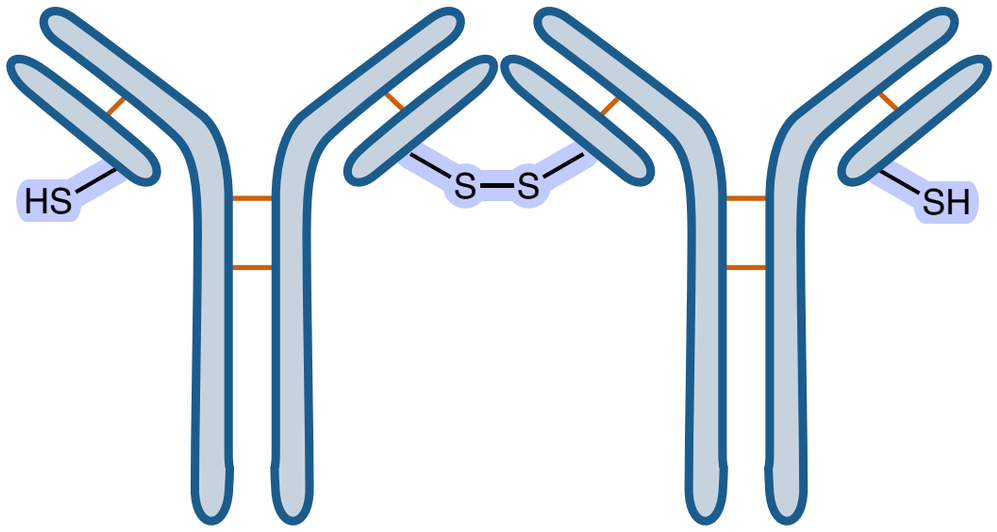
Fu, Z.; Li, S.; Han, S.; Shi, C.; Zhang, Y. *Signal Transduct. Target. Ther.* **2022**, *7* (1), 93.

Conjugation through antibody engineering

THIOMAB Technology

Limitations

- ***Antibody scrambling***



Wrong disulfide bond formation between the two Fabs in the antibody

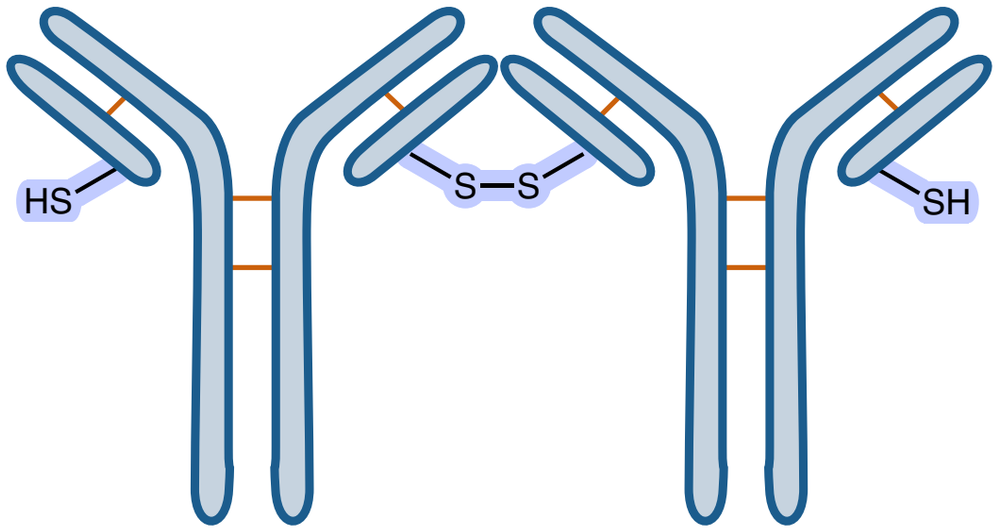
Fu, Z.; Li, S.; Han, S.; Shi, C.; Zhang, Y. *Signal Transduct. Target. Ther.* **2022**, *7* (1), 93.

Conjugation through antibody engineering

THIOMAB Technology

Limitations

- ***Antibody scrambling***



Wrong disulfide bond formation between the two Fabs in the antibody

- ***DAR limited to 2 and does not solve the inherent issues of maleimide chemistry***

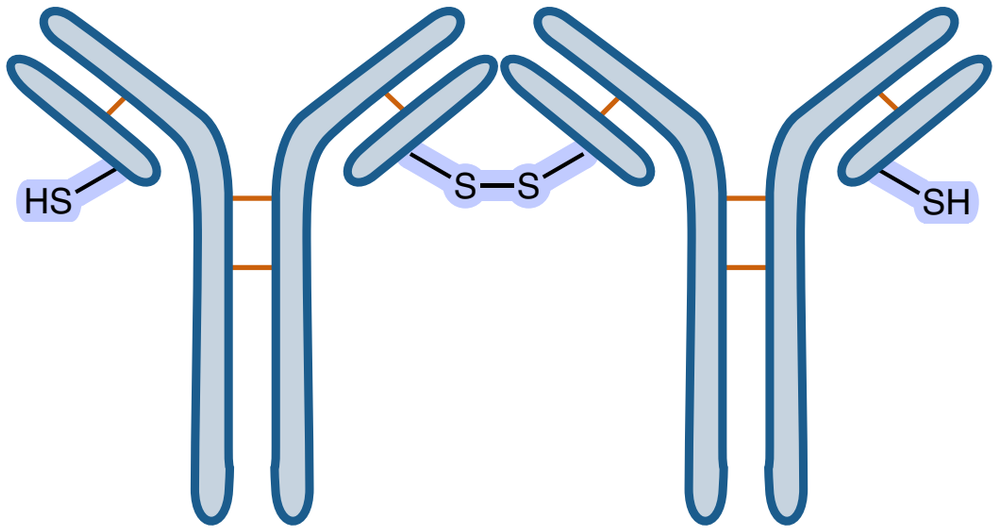
Fu, Z.; Li, S.; Han, S.; Shi, C.; Zhang, Y. *Signal Transduct. Target. Ther.* **2022**, *7* (1), 93.

Conjugation through antibody engineering

THIOMAB Technology

Limitations

- ***Antibody scrambling***



Wrong disulfide bond formation between the two Fabs in the antibody

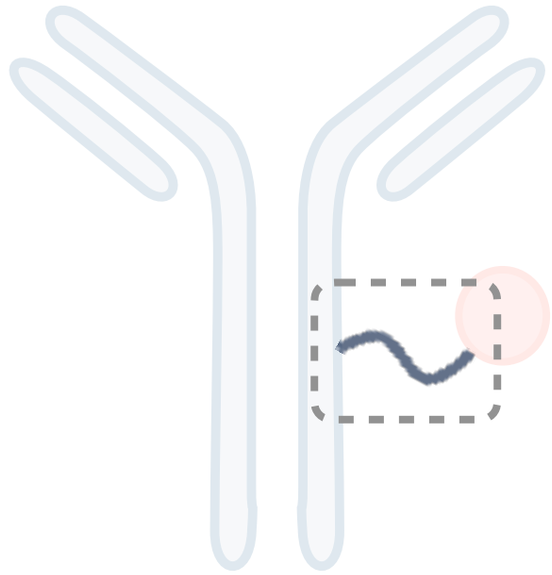
- ***DAR limited to 2 and does not solve the inherent issues of maleimide chemistry***

- ***Engineered antibody: increased risk of immunogenicity***

Fu, Z.; Li, S.; Han, S.; Shi, C.; Zhang, Y. *Signal Transduct. Target. Ther.* **2022**, *7* (1), 93.

Antibody-drug conjugate

Linker



- *Bioconjugation*

- ***Non-cleavable or cleavable***

'C' in ADC

Cleavable or non-cleavable

"Non-cleavable"

"Cleavable"

Mylotarg™

Besponsa™

Adcetris™

Polivy™

Padcev™

Tivdak™

Kadcyla™

Enhertu™

Trodelvy™

Zynlota™

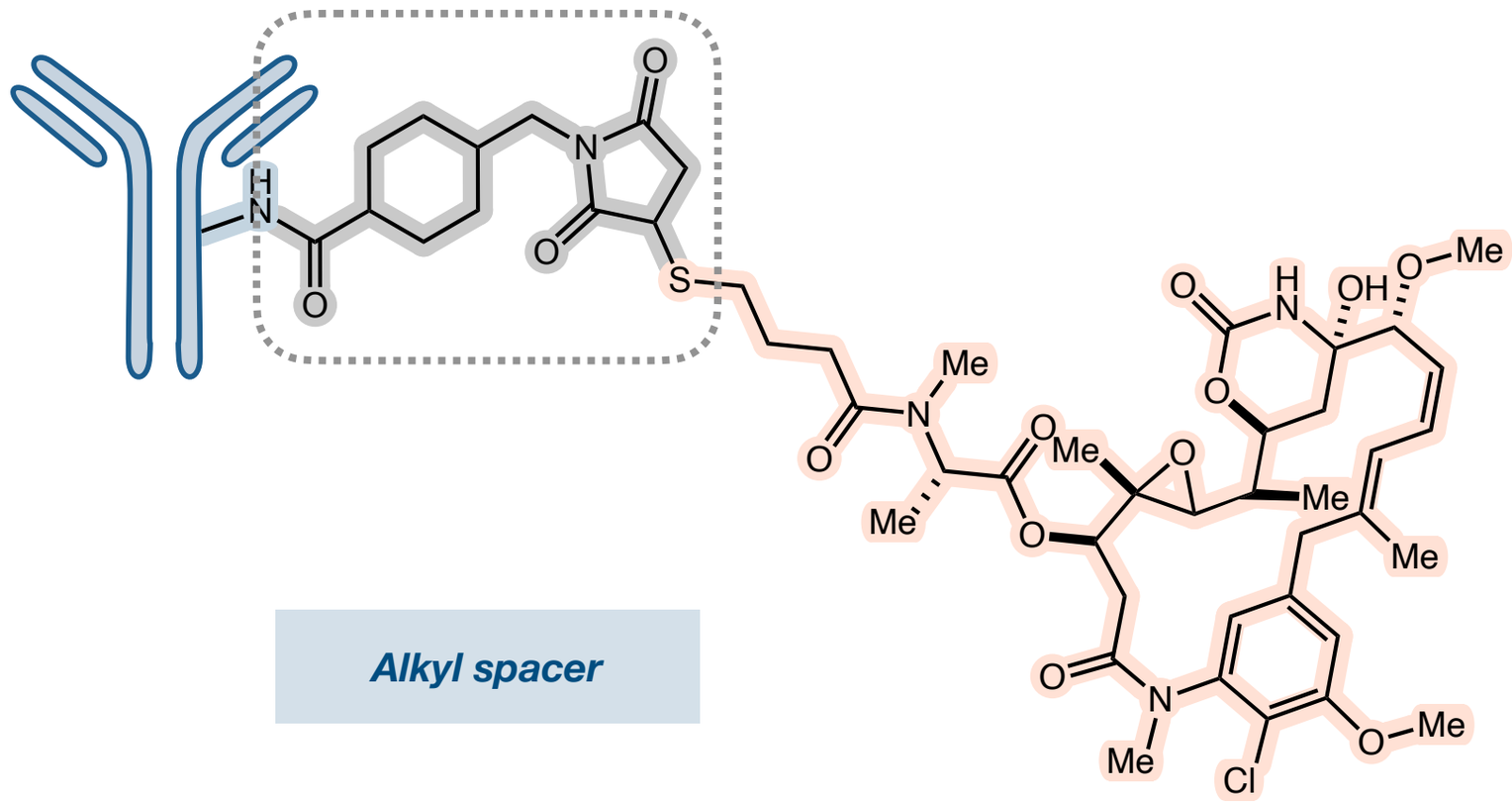
Blenrep™

Elahere™

'C' in ADC

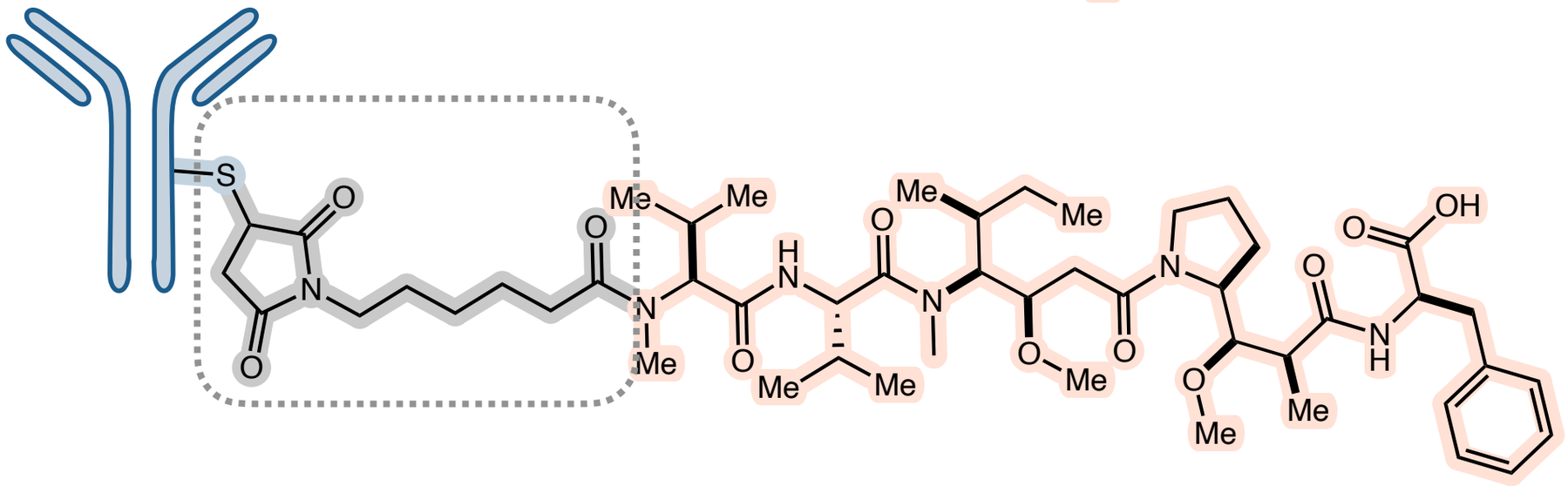
Non-cleavable

Kadcyla™
(Trastuzumab emtansine)



Alkyl spacer

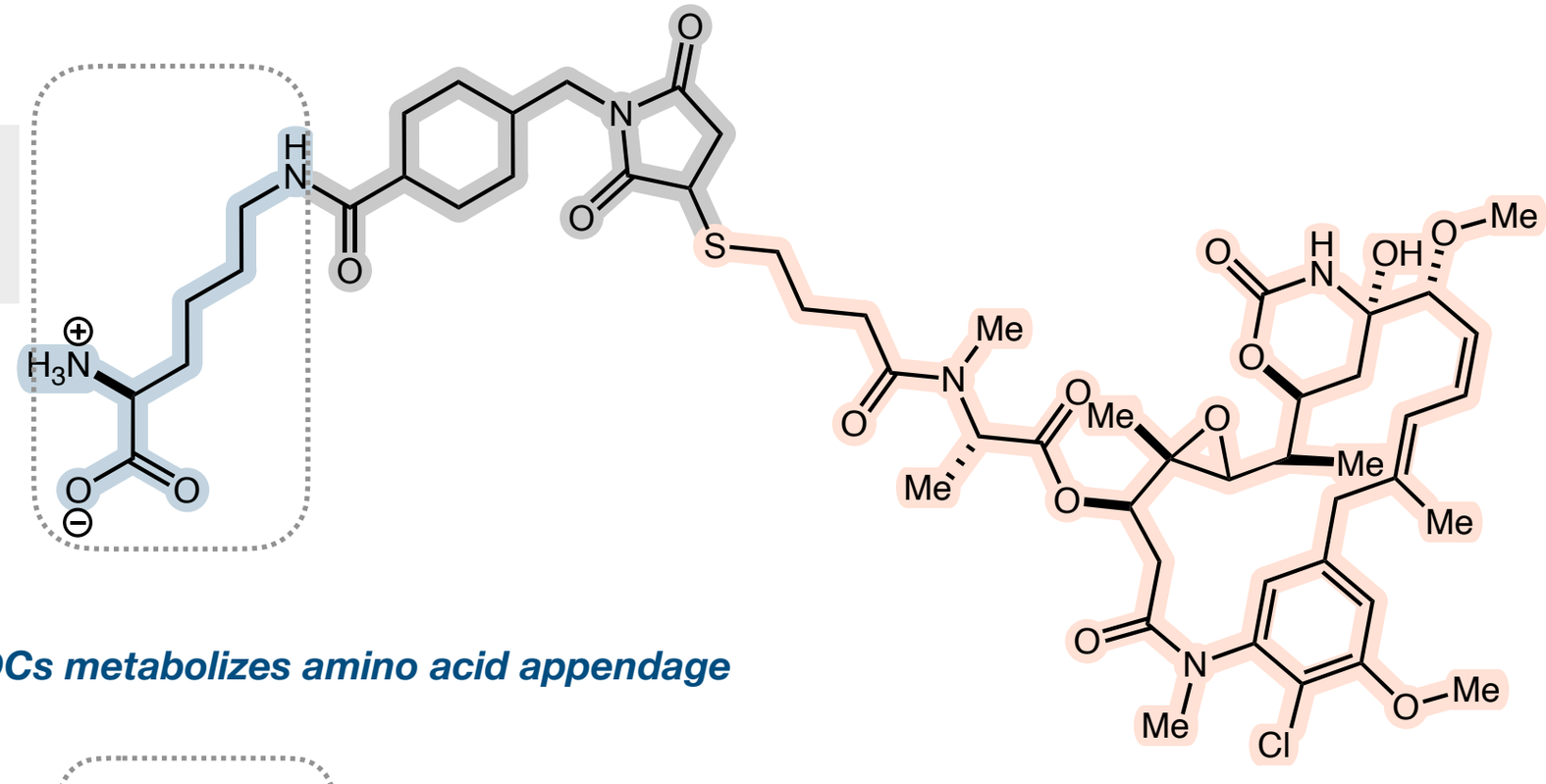
Blenrep™
(Belantamab mafodotin)



Non-cleavable linker

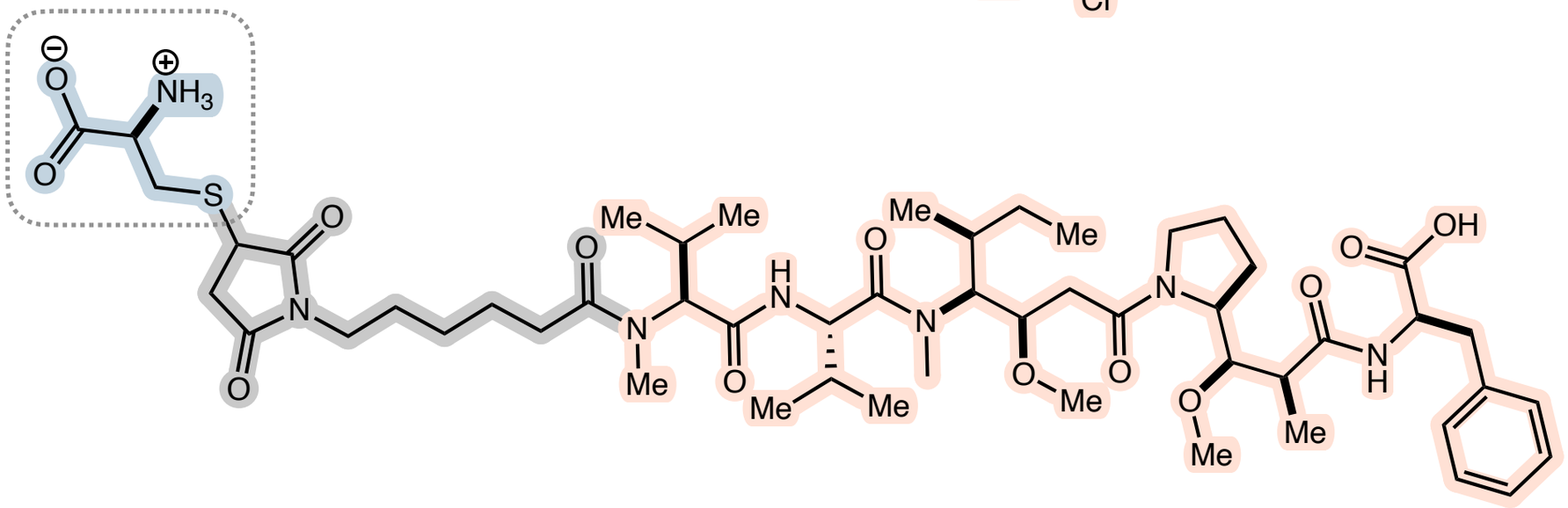
Lysosomal degradation with amino acid appendage

Kadcyla™
(Trastuzumab emtansine)



ADCs metabolizes amino acid appendage

Blenrep™
(Belantamab mafodotin)



'C' in ADC

Cleavable linker

Cleavable linkers make use of the hallmarks of cancer

- ***Acidic microenvironment (~ pH 6.7)***
- ***Overexpressed glutathiones (GSH)***
- ***Overexpressed lysosomal protease (e.g. Cathepsin B)***

Hanahan, D.; Weinberg, R. A. *Cell* **2011**, *144* (5), 646–674.

Cleavable linker

Acidic tumour microenvironment and overexpressed glutathiones

Mylotarg™

Besponsa™

Adcetris™

Polivy™

Padcev™

Tivdak™

Kadcyla™

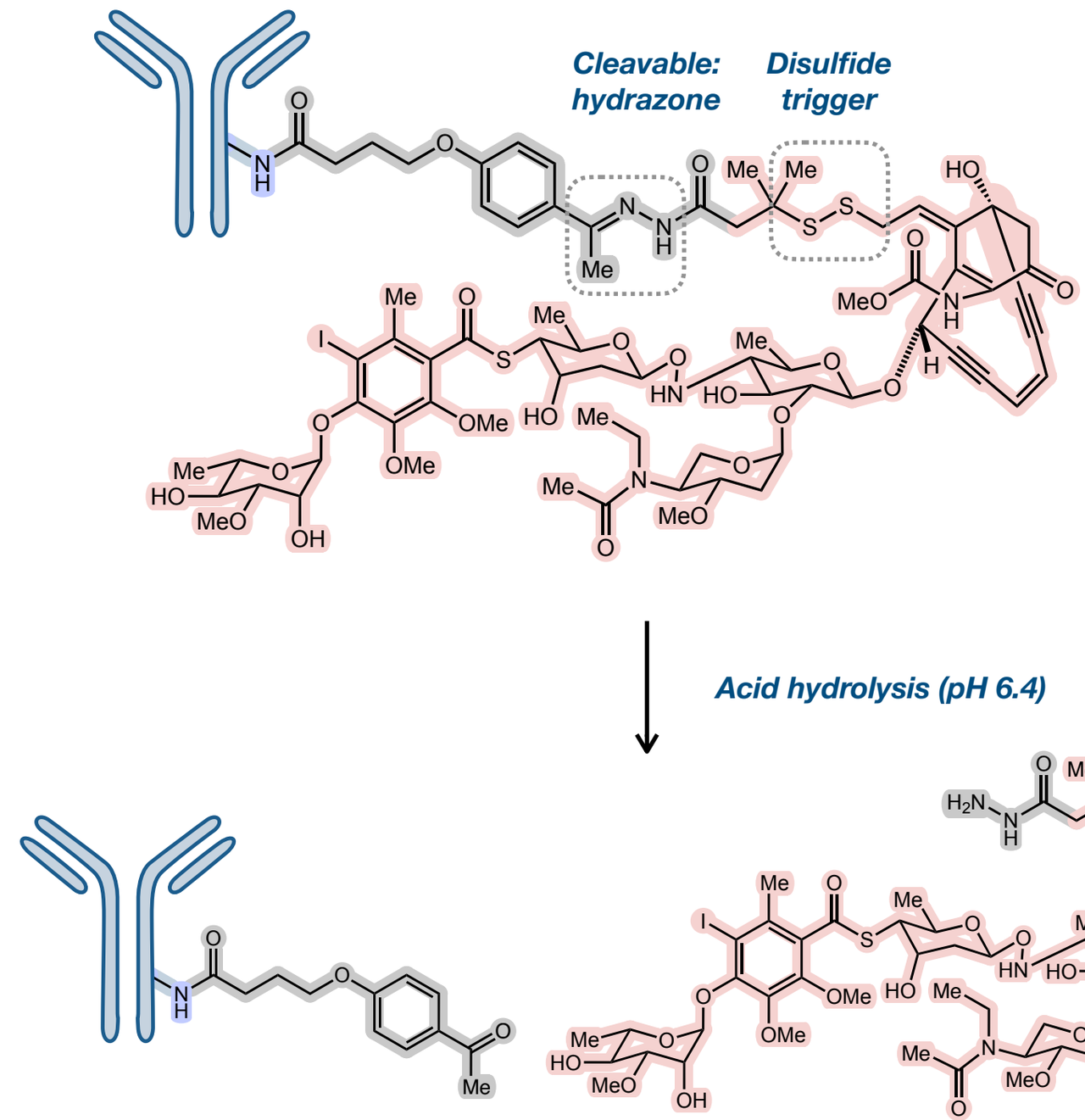
Enhertu™

Trodelvy™

Zynlota™

Blenrep™

Elahere™



Jain, N.; Smith, S. W.; Ghone, S.; Tomczuk, B. *Pharm. Res.* **2015**, *32* (11), 3526–3540

Cleavable linker

Acidic tumour microenvironment and over expressed glutathiones

Mylotarg™

Besponsa™

Adcetris™

Polivy™

Padcev™

Tivdak™

Kadcyla™

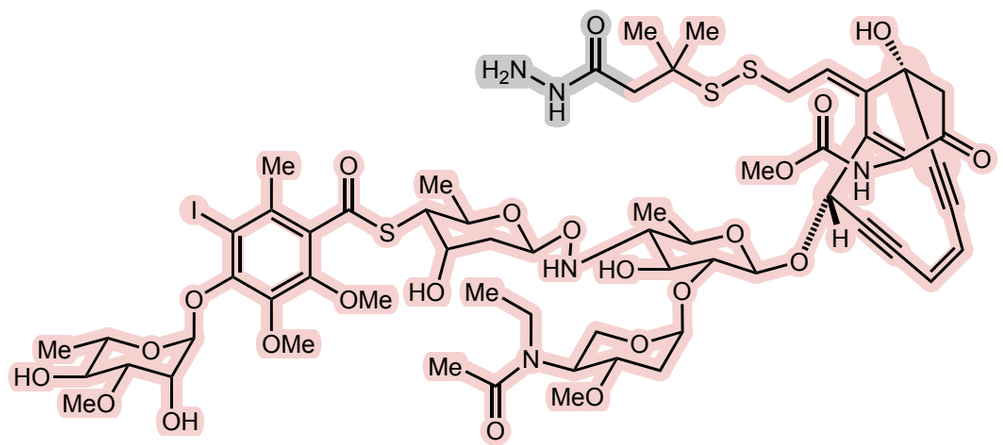
Enhertu™

Trodelvy™

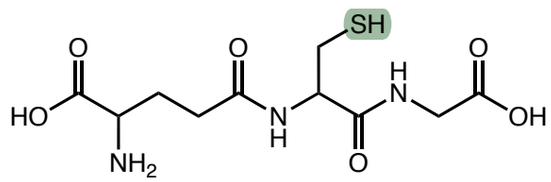
Zynlota™

Blenrep™

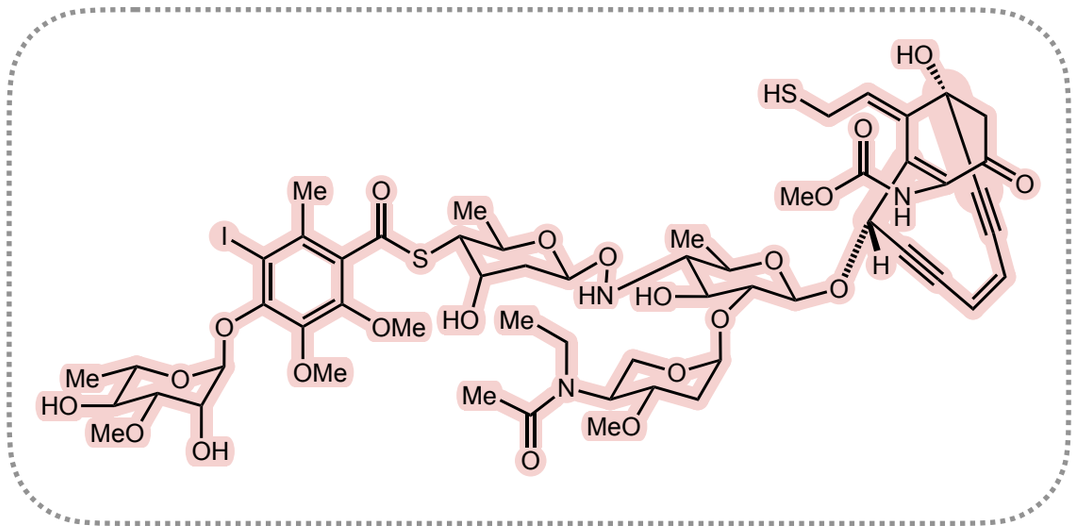
Elahere™



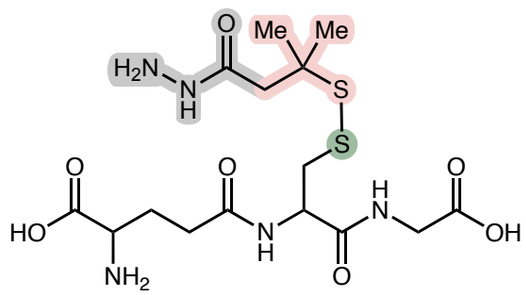
GSH (overexpressed)



Thiol-disulfide exchange



Active drug (N-Acetyl calicheamicin) released



Jain, N.; Smith, S. W.; Ghone, S.; Tomczuk, B. *Pharm. Res.* **2015**, *32* (11), 3526–3540

Cleavable linker

Overexpressed glutathiones

Mylotarg™

Besponsa™

Adcetris™

Polivy™

Padcev™

Tivdak™

Kadcyla™

Enhertu™

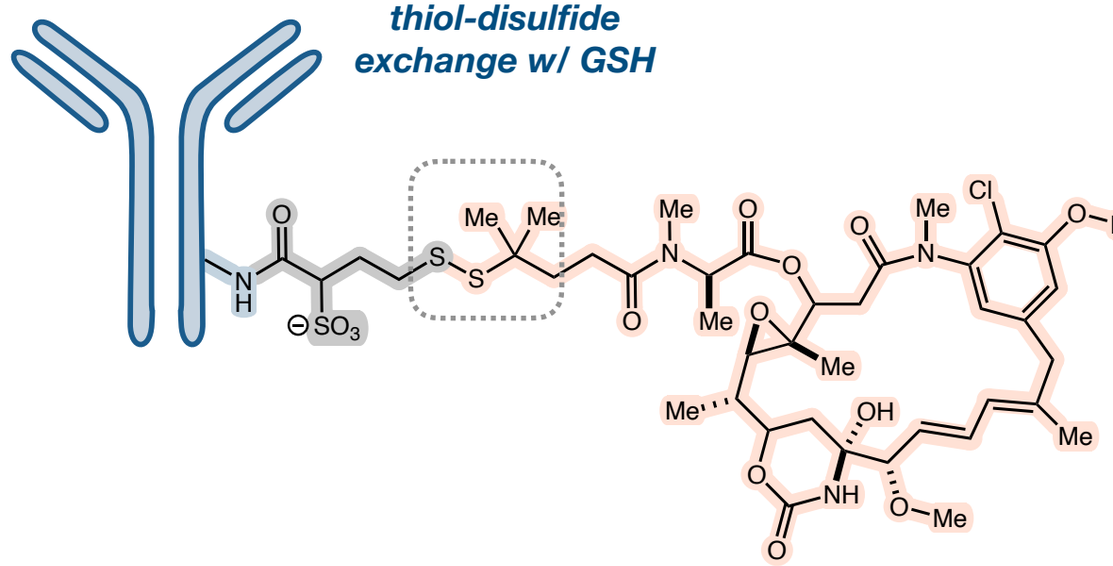
Trodelvy™

Zynlota™

Blenrep™

Elahere™

Disulfide trigger: thiol-disulfide exchange w/ GSH



N. Engl. J. Med. **2023**, *389* (23), 2162–2174

Cleavable linker

Overexpressed cathepsin B

Mylotarg™

Besponsa™

Adcetris™

Polivy™

Padcev™

Tivdak™

Kadcyla™

Enhertu™

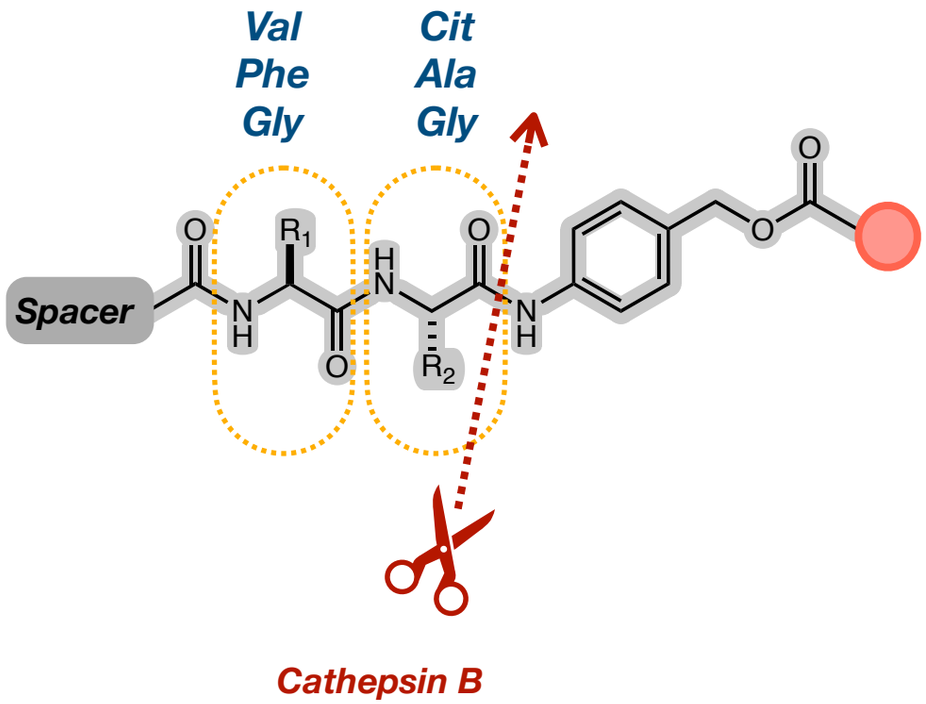
Trodelvy™

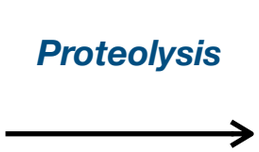
Zynlota™

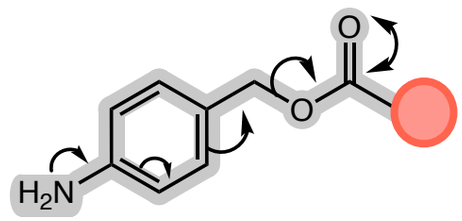
Blenrep™

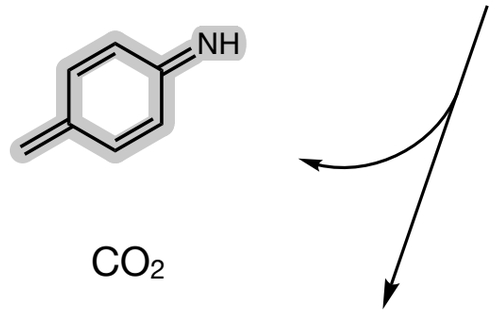
Elahere™

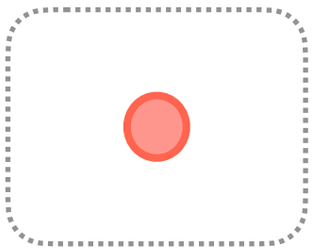
Tetra/Dipeptide









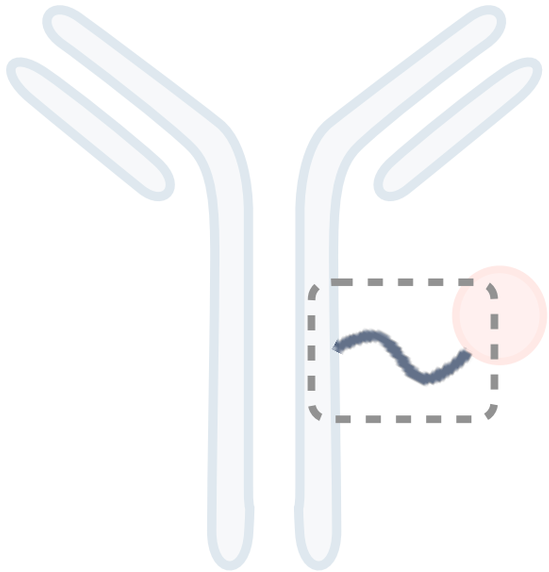


Active drug released

Jain, N.; Smith, S. W.; Ghone, S.; Tomczuk, B. *Pharm. Res.* **2015**, *32* (11), 3526–3540

‘C’ in ADC

What makes a good ‘C’ for ADC

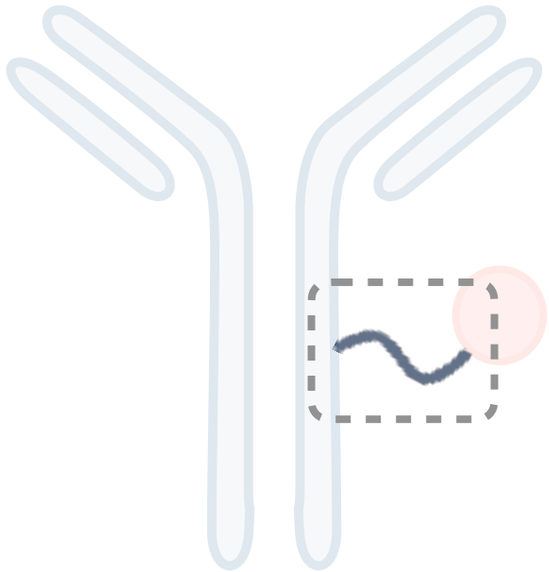


Bioconjugation

- ✓ *Aqueous*
- ✓ *pH neutral*
- ✓ *Below 37 °C*
- ✓ *Low concentration*
- ✓ *Atom-economic*
- ✓ *Rapid*

‘C’ in ADC

What makes a good ‘C’ for ADC



Bioconjugation

- ✓ *Aqueous*
- ✓ *pH neutral*
- ✓ *Below 37 °C*
- ✓ *Low concentration*
- ✓ *Atom-economic*
- ✓ *Rapid*

- ✓ *No side-reactivity with ‘D’*

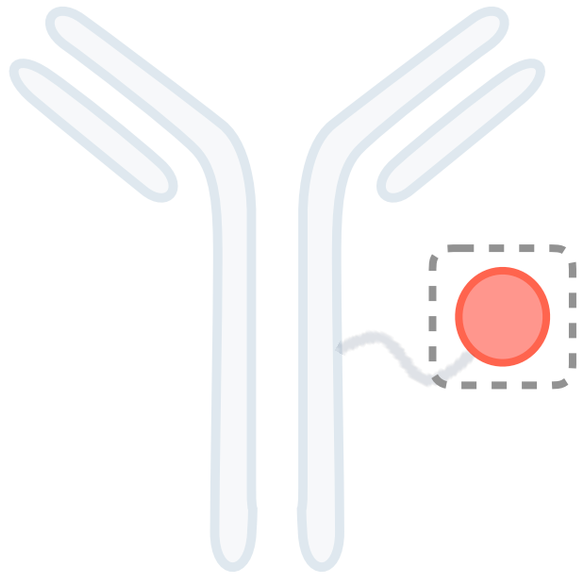
- ✓ *Selective (chemo-, regio-, stereo-) for **DAR control***

Cleavable/Non-cleavable

- ✓ *Cleavable linkers should be able to reliably differentiate between normal and cancer cells*

‘D’ in ADC

Drug



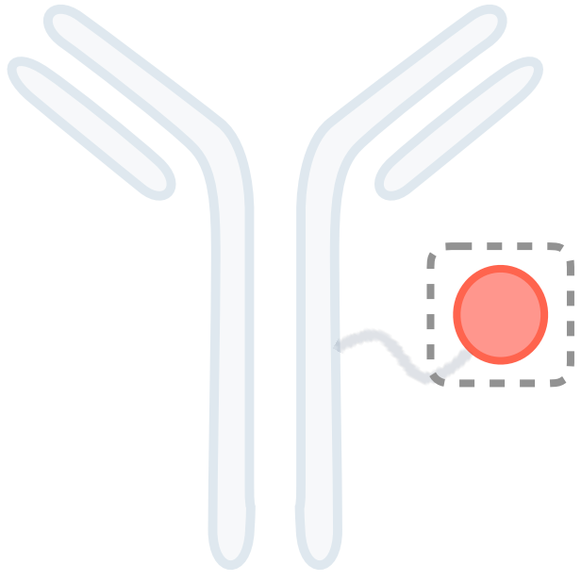
- ***Types of drugs***

- ***Bystander effect***

- ***Other types of ‘D’***

‘D’ in ADC

Drug



- ***Types of drugs***

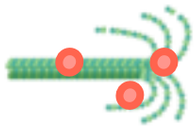
- *Bystander effect*

- *Other types of ‘D’*

'D' in ADC

Types of drugs

Microtubule inhibitor



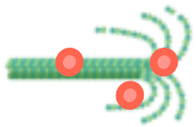
	ADC	***IC_{50}***
MMAE	*Adcteris™* *Polivy™* *Padcev™* *Tivdak™*	*1.1 nM*
MMAF	*Blenrep™*	*137 nM*
DM1	*Kadcyla™*	*30 nM*
DM4	*Elahere™*	*3.3 nM*

Wang, Z.; Li, H.; Gou, L.; Li, W.; Wang, Y. *Acta Pharm. Sin. B* **2023**, *13* (10), 4025–4059

'D' in ADC

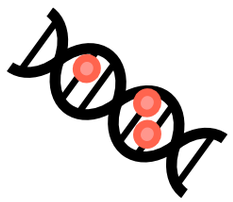
Types of drugs

Microtubule inhibitor



	ADC	IC_{50}
MMAE	*Adcteris™* *Polivy™* *Padcev™* *Tivdak™*	*1.1 nM*
MMAF	*Blenrep™*	*137 nM*
DM1	*Kadcyla™*	*30 nM*
DM4	*Elahere™*	*3.3 nM*

DNA binder



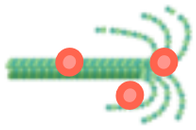
	ADC	IC_{50}
Calicheamicin	*Besponsa™* *Mylotarg™*	*1 nM*
PBD dimer	*Zynlota™*	*1 pM*

Wang, Z.; Li, H.; Gou, L.; Li, W.; Wang, Y. *Acta Pharm. Sin. B* **2023**, *13* (10), 4025–4059

'D' in ADC

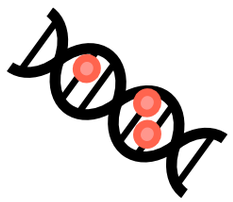
Types of drugs

Microtubule inhibitor



	ADC	IC_{50}
MMAE	*Adcteris™* *Polivy™* *Padcev™* *Tivdak™*	*1.1 nM*
MMAF	*Blenrep™*	*137 nM*
DM1	*Kadcyla™*	*30 nM*
DM4	*Elahere™*	*3.3 nM*

DNA binder



	ADC	IC_{50}
Calicheamicin	*Besponsa™* *Mylotarg™*	*1 nM*
PBD dimer	*Zynlota™*	*1 pM*

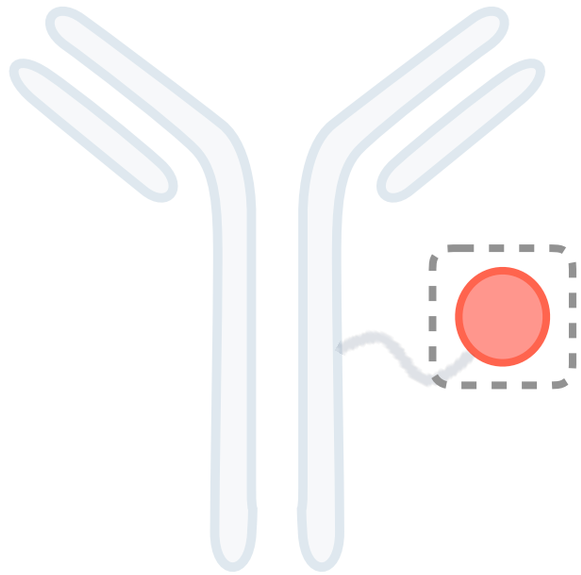
Topoisomerase inhibitor

	ADC	IC_{50}
Exatecan	*Enhertu™*	*0.31 μM*
SN-38	*Trodelvy™*	*0.17 μM*

Wang, Z.; Li, H.; Gou, L.; Li, W.; Wang, Y. *Acta Pharm. Sin. B* **2023**, *13* (10), 4025–4059

‘D’ in ADC

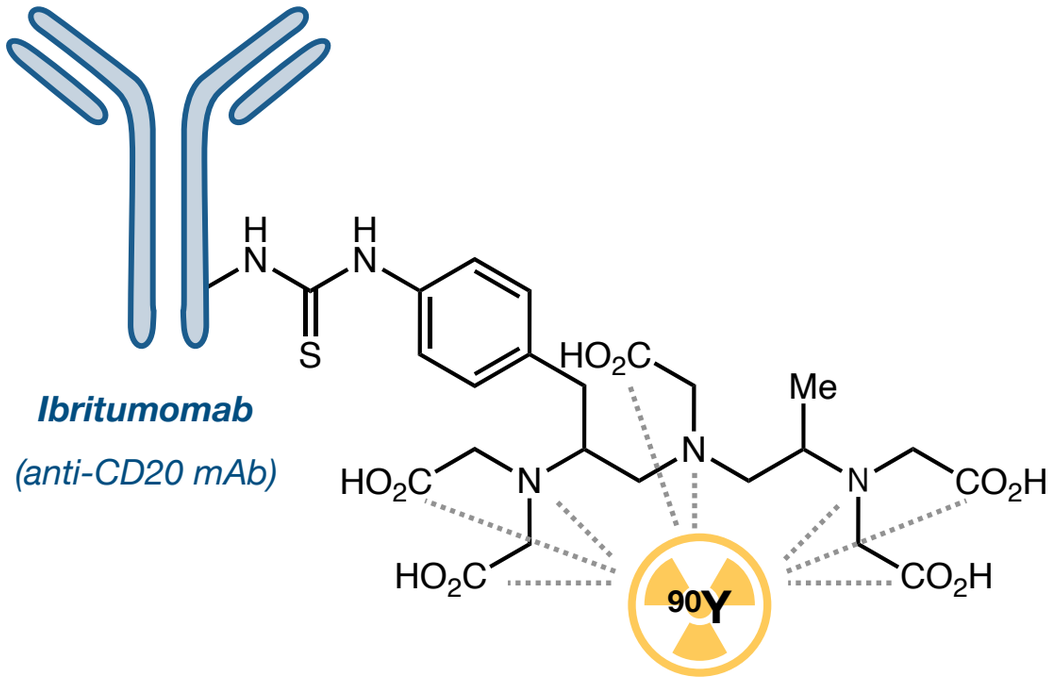
Drug



- *Types of drugs*
- ***Other types of ‘D’***
- *Bystander effect*

Other types of 'D'

Antibody-Radionuclide Conjugate (ARC)



Ibritumomab

(anti-CD20 mAb)

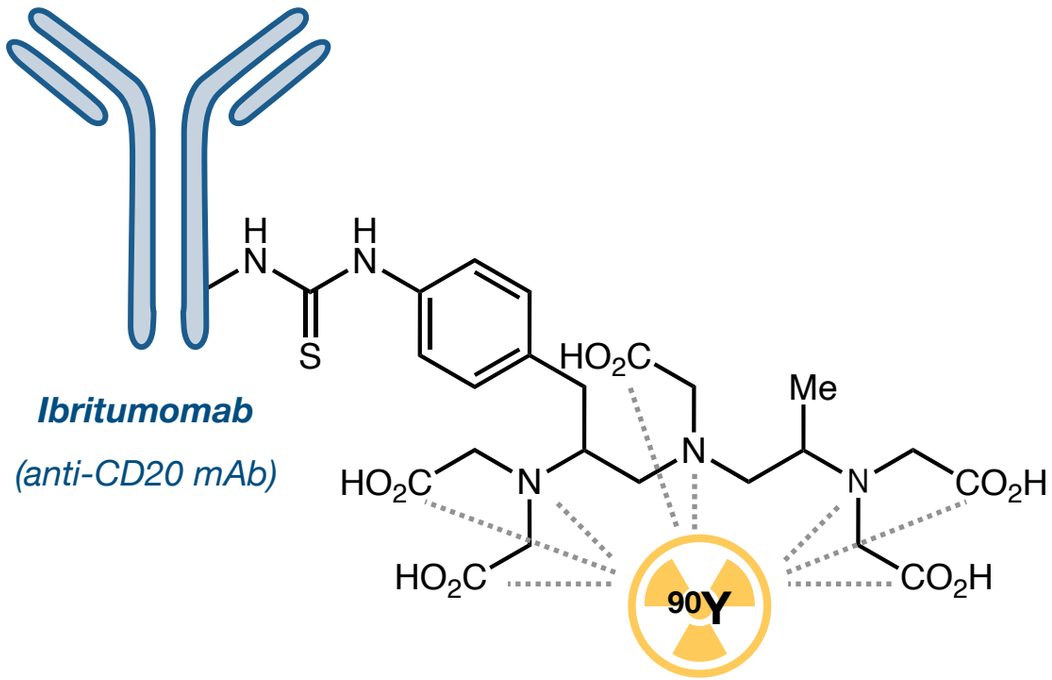
90 Yttrium

β-particle-emitting radionuclide

Sgouros, G.; Bodei, L.; McDevitt, M. R.; Nedrow, J. R. *Nat. Rev. Drug Discov.* **2020**, *19* (9), 589–608

Other types of 'D'

Antibody-Radionuclide Conjugate (ARC)



Ibritumomab

(anti-CD20 mAb)

90 Yttrium

β-particle-emitting radionuclide

***Limited number of approvals & low clinical adoptions**, due to the requirement of a multidisciplinary team of medical oncologists, radiation oncologists, and physicists to develop and deploy the molecules in the clinic*

Sgouros, G.; Bodei, L.; McDevitt, M. R.; Nedrow, J. R. *Nat. Rev. Drug Discov.* **2020**, *19* (9), 589–608

Other types of ‘D’

Degrader-Antibody Conjugate (DAC) and Antibody-Oligonucleotide Conjugate (AOC)

Dovgan, I.; Koniev, O.; Kolodych, S.; Wagner, A. *Bioconjug. Chem.* **2019**, *30* (10), 2483–2501

Dragovich, P. S. *Chem. Soc. Rev.* **2022**, *51* (10), 3886–3897

Other types of ‘D’

Degrader-Antibody Conjugate (DAC) and Antibody-Oligonucleotide Conjugate (AOC)

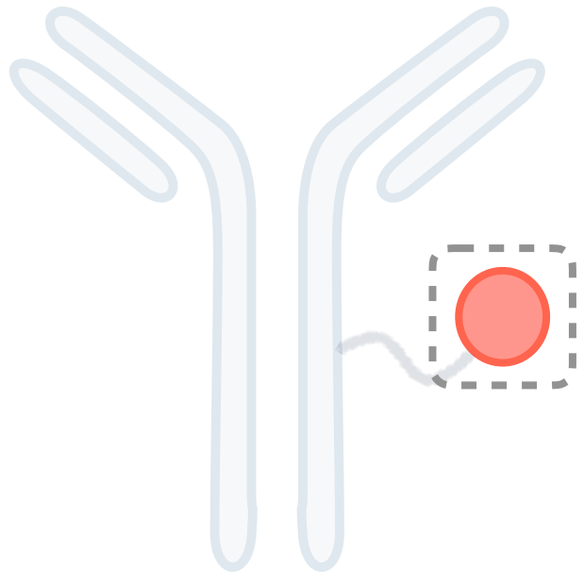
Expansion of modalities beyond ADCs

Dovgan, I.; Koniev, O.; Kolodych, S.; Wagner, A. *Bioconjug. Chem.* **2019**, *30* (10), 2483–2501

Dragovich, P. S. *Chem. Soc. Rev.* **2022**, *51* (10), 3886–3897

‘D’ in ADC

Drug



- *Types of drugs*

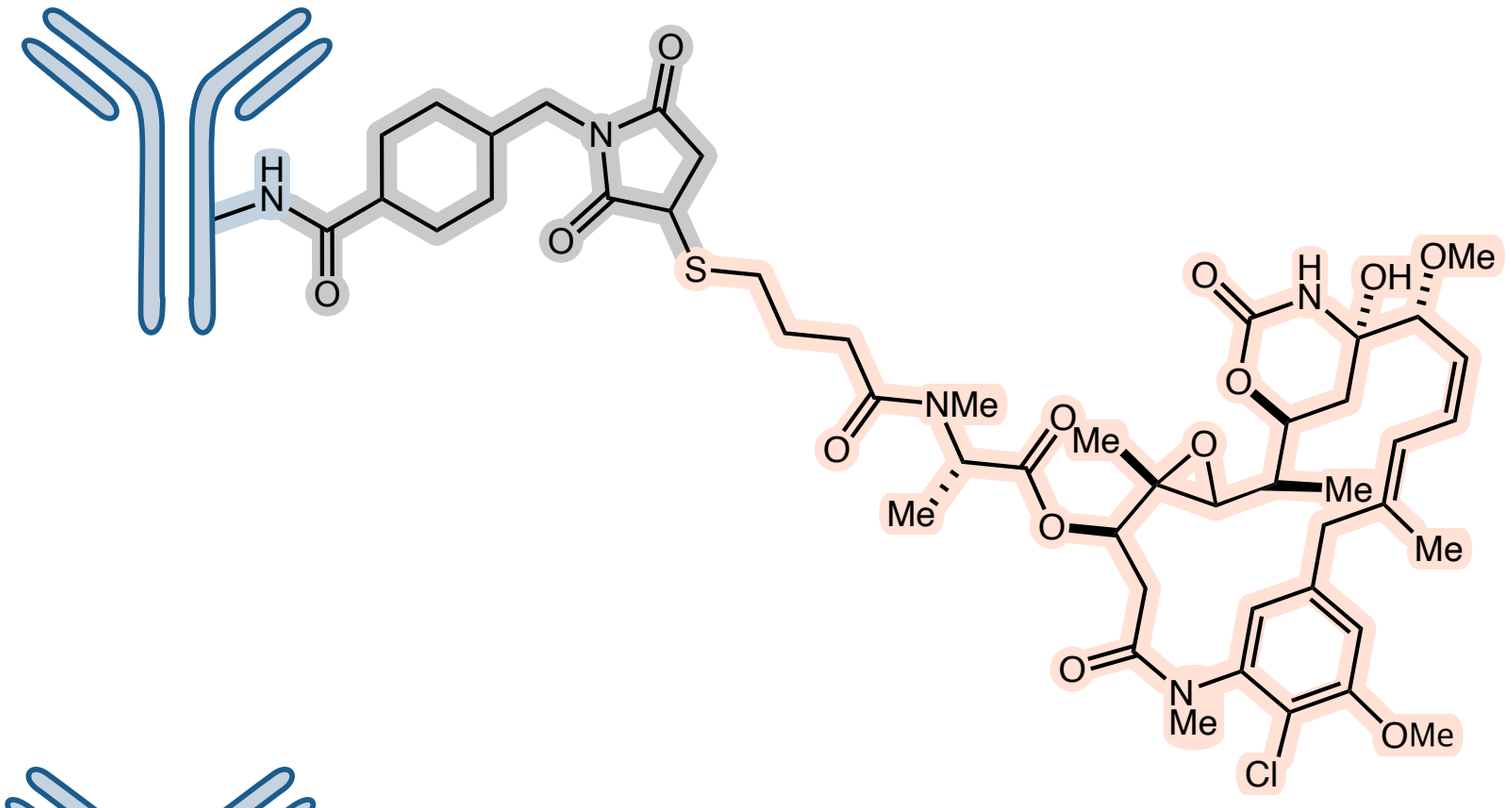
- *Other types of ‘D’*

- ***Bystander effect***

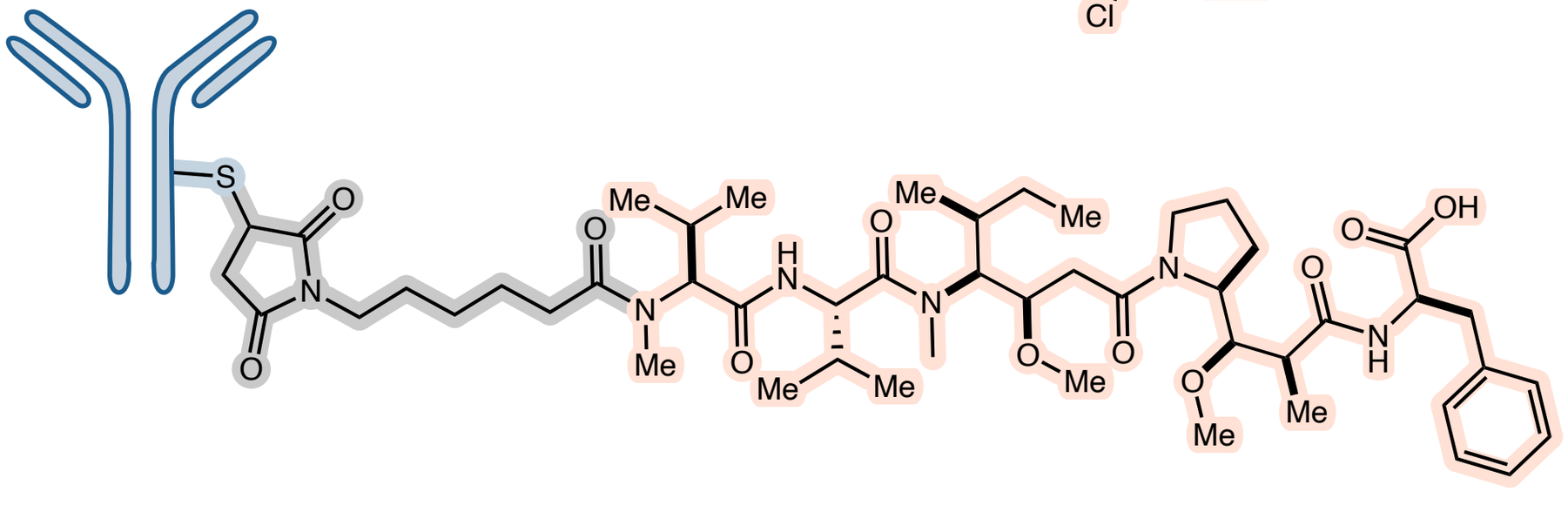
Bystander effect

Drugs tethered to non-cleavable linkers

Kadcyla™
(Trastuzumab emtansine)



Blenrep™
(Belantamab mafodotin)

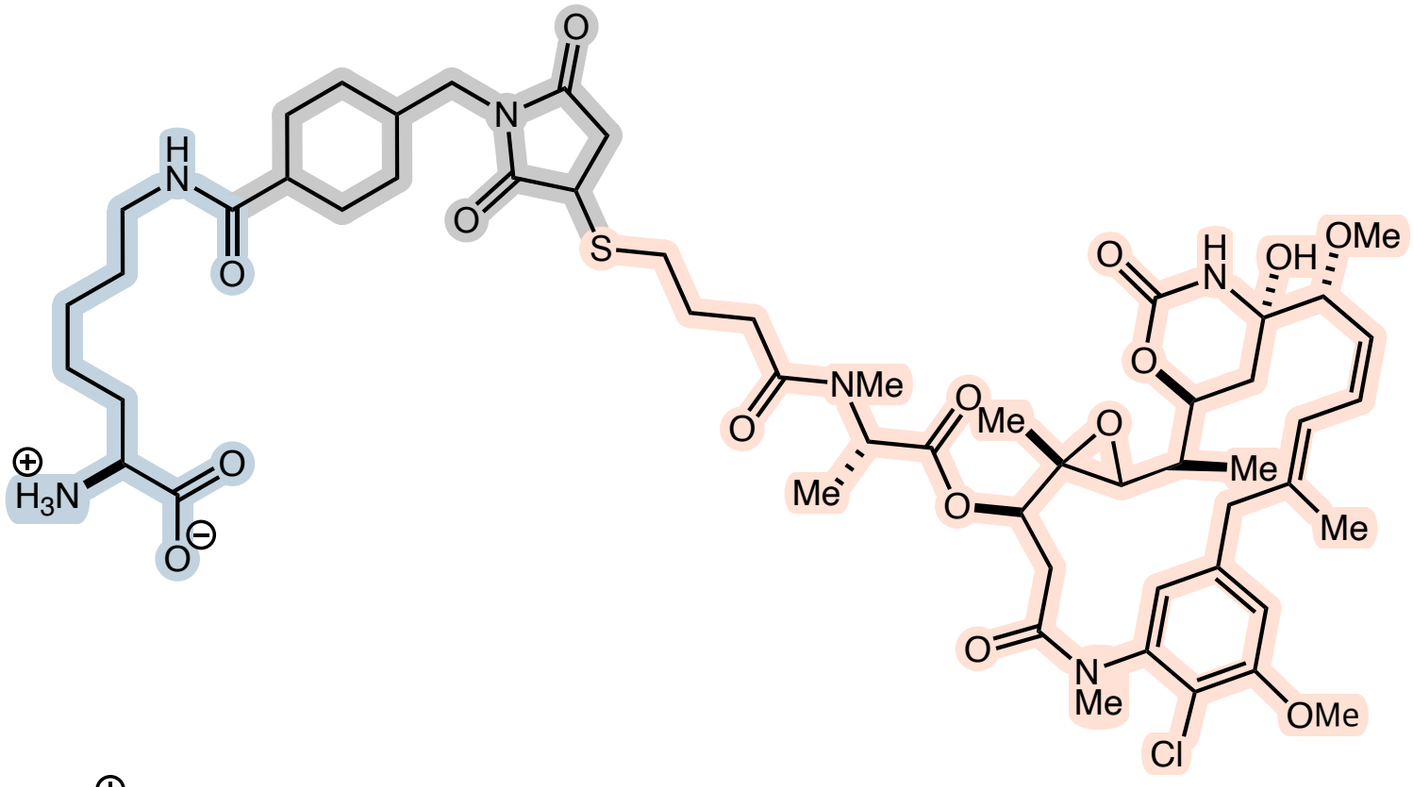


Bystander effect

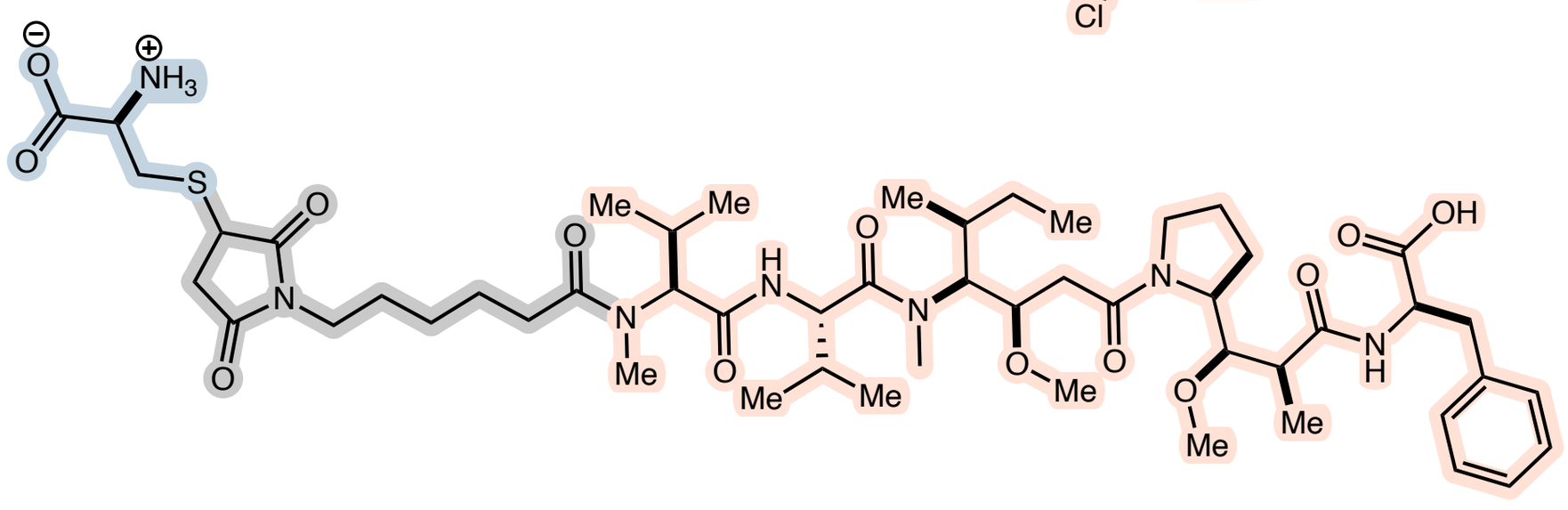
Drugs tethered to non-cleavable linkers

After lysosomal degradation to release drugs

Kadcyla™
(Trastuzumab emtansine)



Blenrep™
(Belantamab mafodotin)

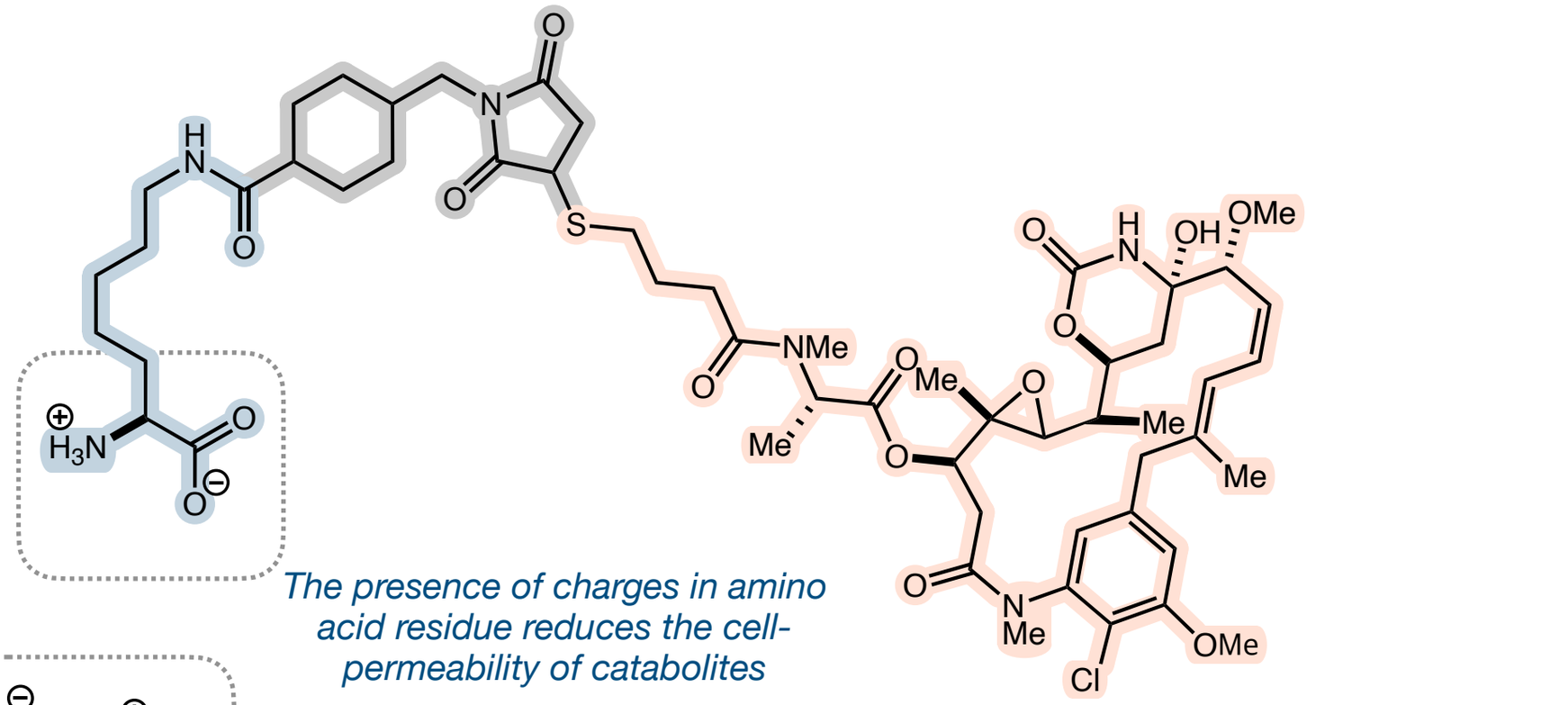


Bystander effect

Drugs tethered to non-cleavable linkers

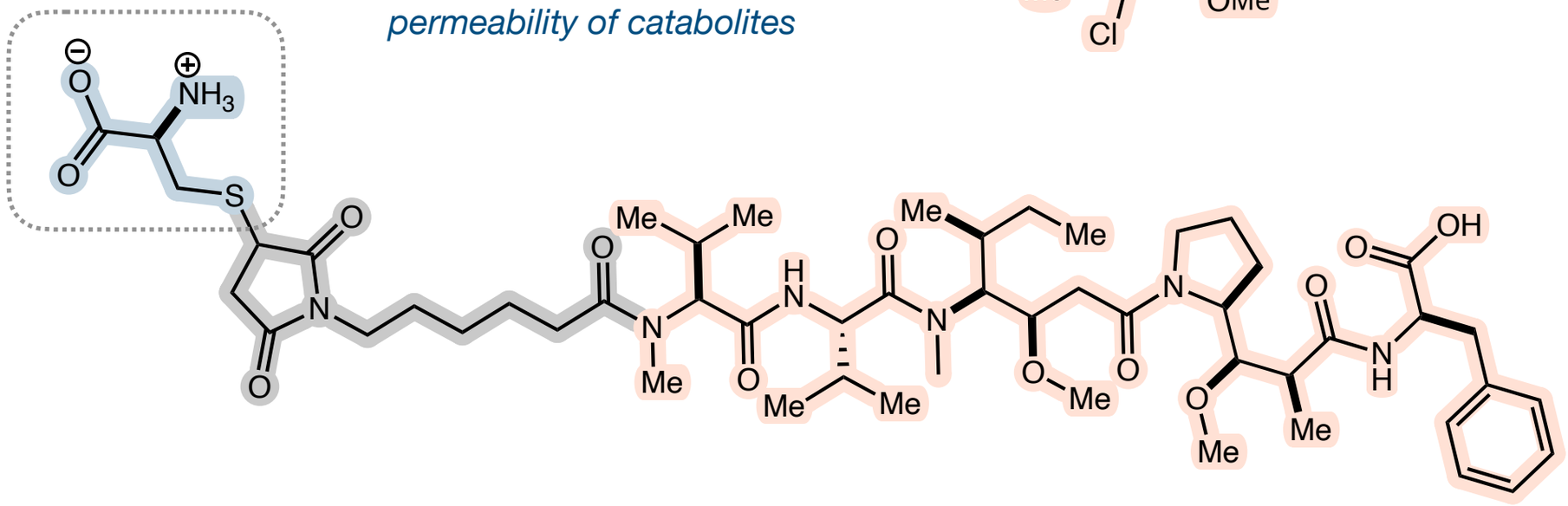
After lysosomal degradation to release drugs

Kadcyla™
(Trastuzumab emtansine)



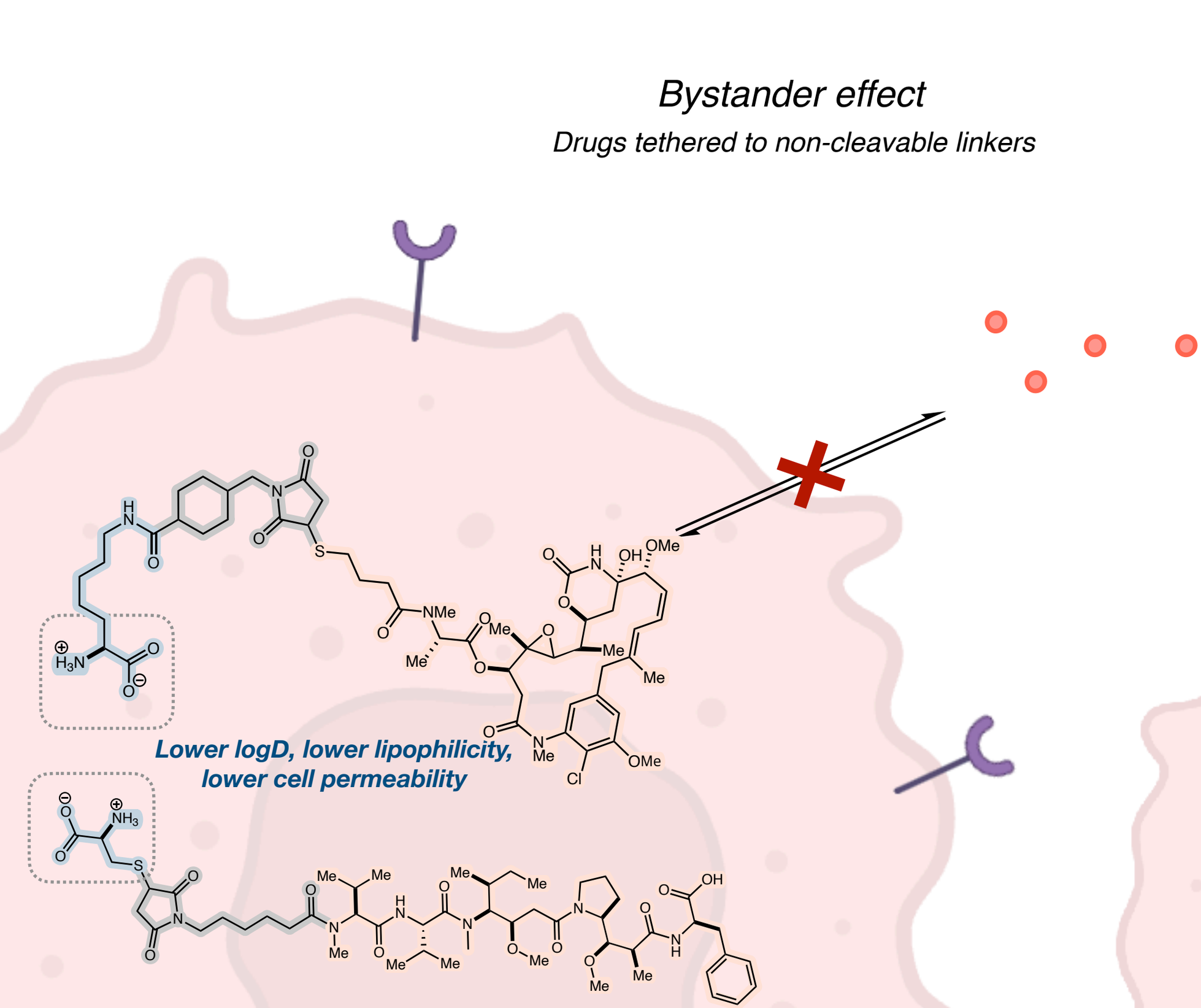
The presence of charges in amino acid residue reduces the cell-permeability of catabolites

Blenrep™
(Belantamab mafodotin)



Bystander effect

Drugs tethered to non-cleavable linkers



Neighbouring antigen-negative tumor cell

Drugs tethered to non-cleavable linkers are NOT able to exert bystander effects

Bystander effect

Drugs tethered to cleavable linkers

Mylotarg™

Besponsa™

Adcetris™

Polivy™

Padcev™

Tivdak™

Kadcyla™

Enhertu™

Trodelvy™

Zynlota™

Blenrep™

Elahere™

Bystander effect

Ozogamicin (Calicheamicin)-containing ADC

Mylotarg™

Besponsa™

Adcetris™

Polivy™

Padcev™

Tivdak™

Kadcyla™

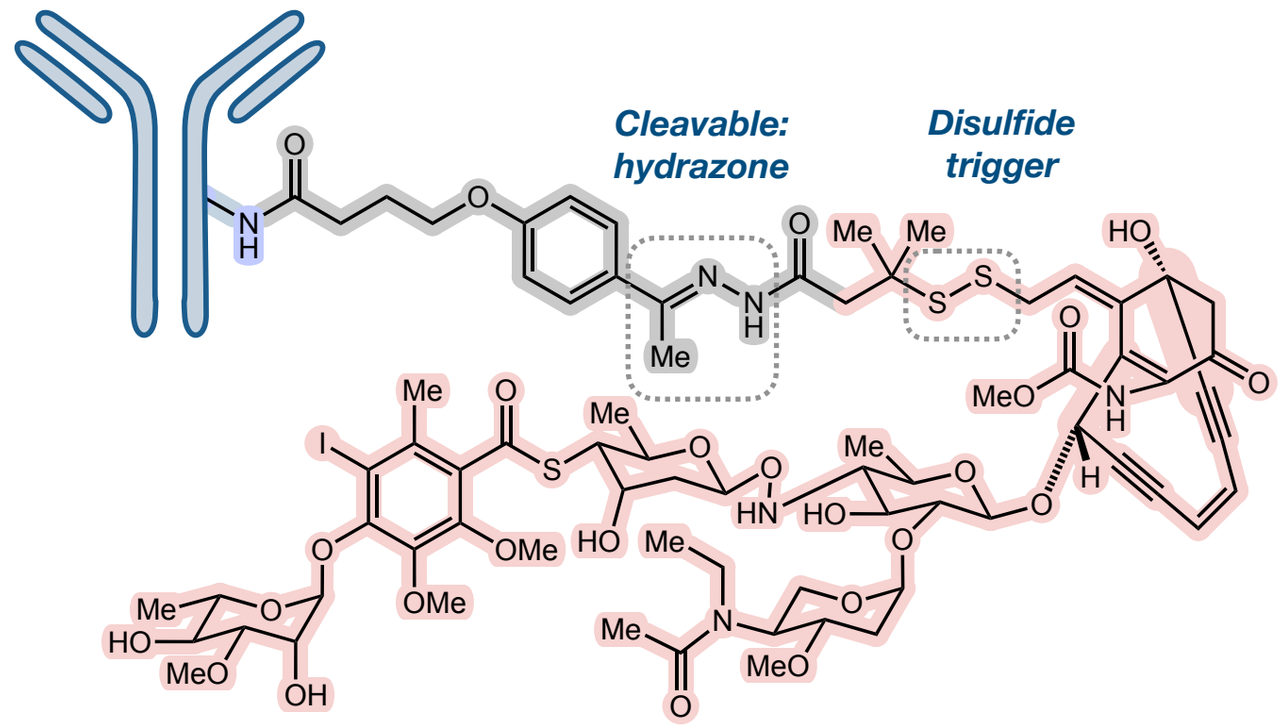
Enhertu™

Trodelvy™

Zynlota™

Blenrep™

Elahere™



Bystander effect

Ozogamicin (Calicheamicin)-containing ADC

Mylotarg™

Besponsa™

Adcetris™

Polivy™

Padcev™

Tivdak™

Kadcyla™

Enhertu™

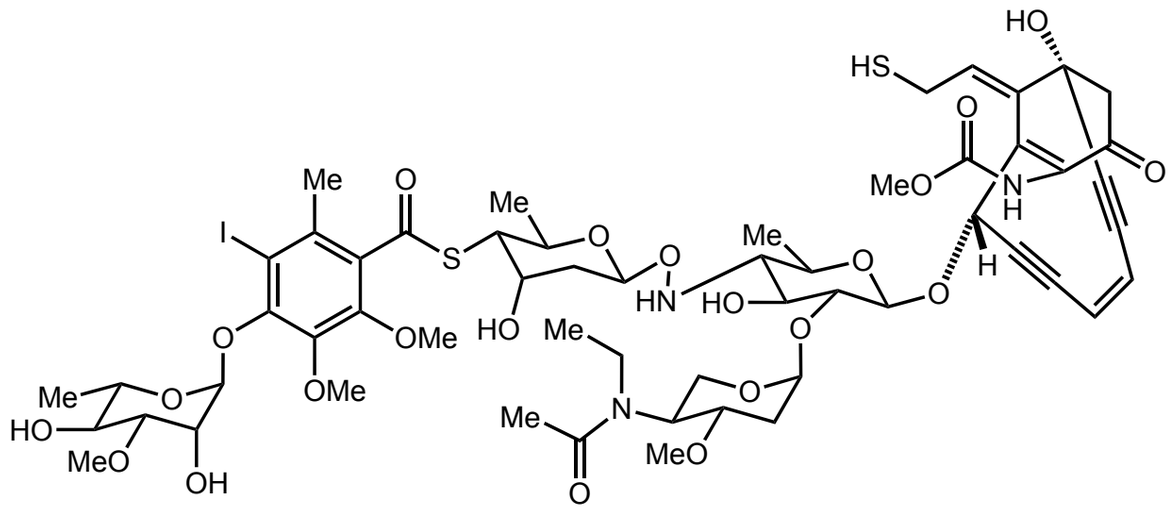
Trodelvy™

Zynlota™

Blenrep™

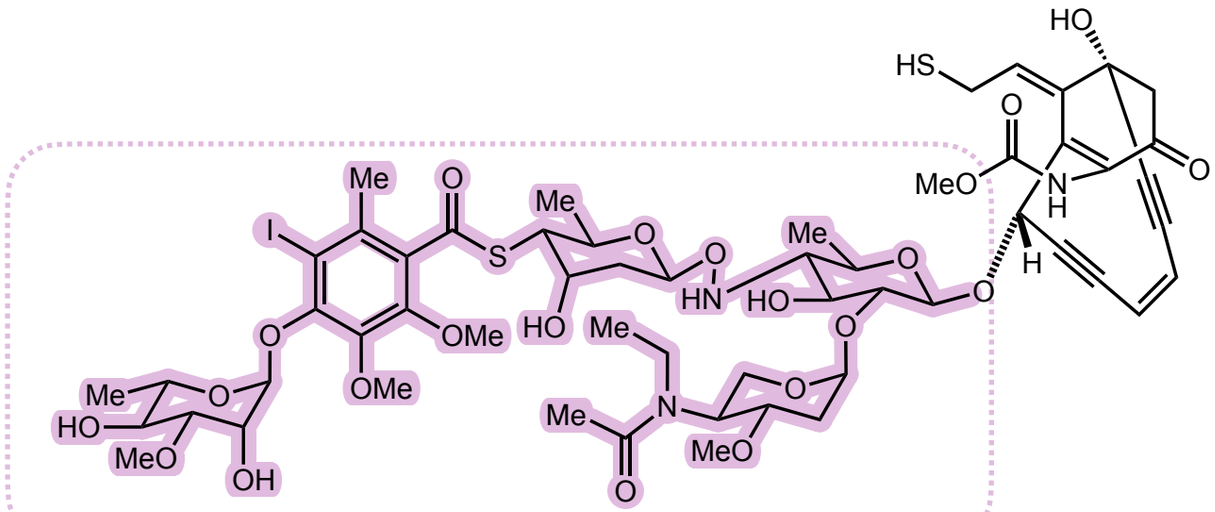
Elahere™

After linker cleavage to release drugs



Bystander effect

Ozogamicin (Calicheamicin)-containing ADC

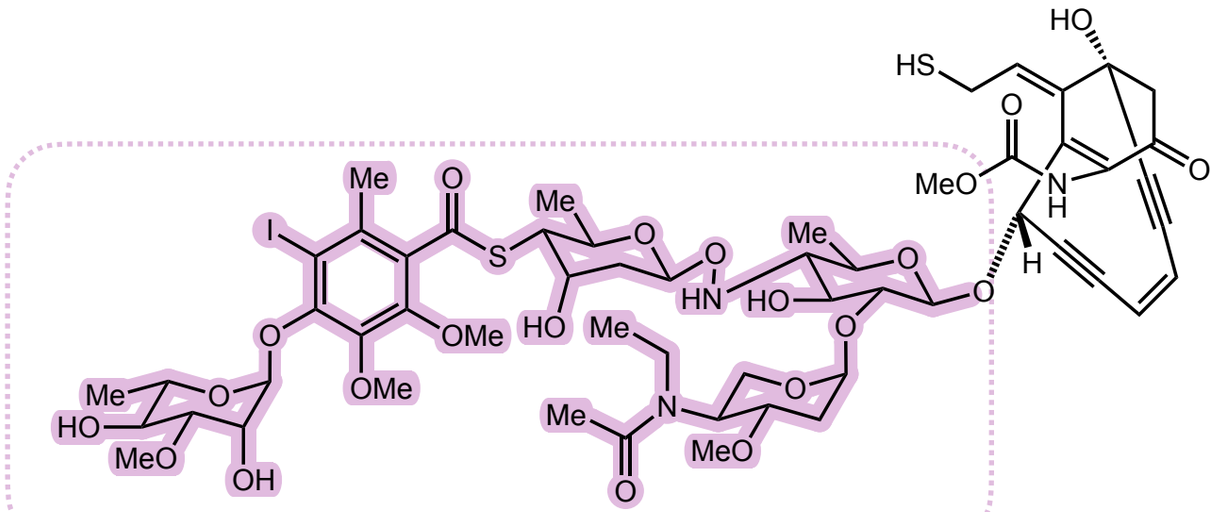


Trisaccharide directs the enediyne moiety to bind specifically to the minor groove of DNA

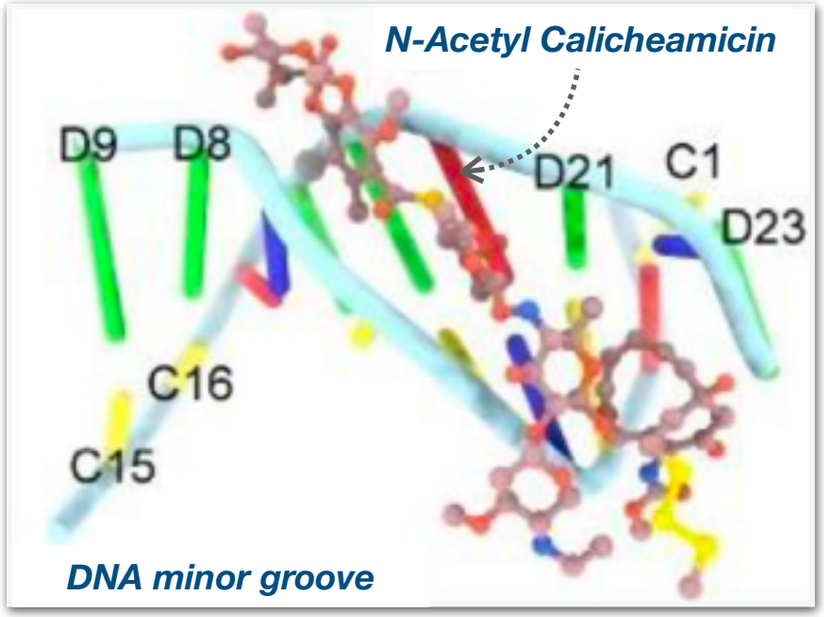
Wang, Z.; Li, H.; Gou, L.; Li, W.; Wang, Y. *Acta Pharm. Sin. B* **2023**, *13* (10), 4025–4059

Bystander effect

Ozogamicin (Calicheamicin)-containing ADC



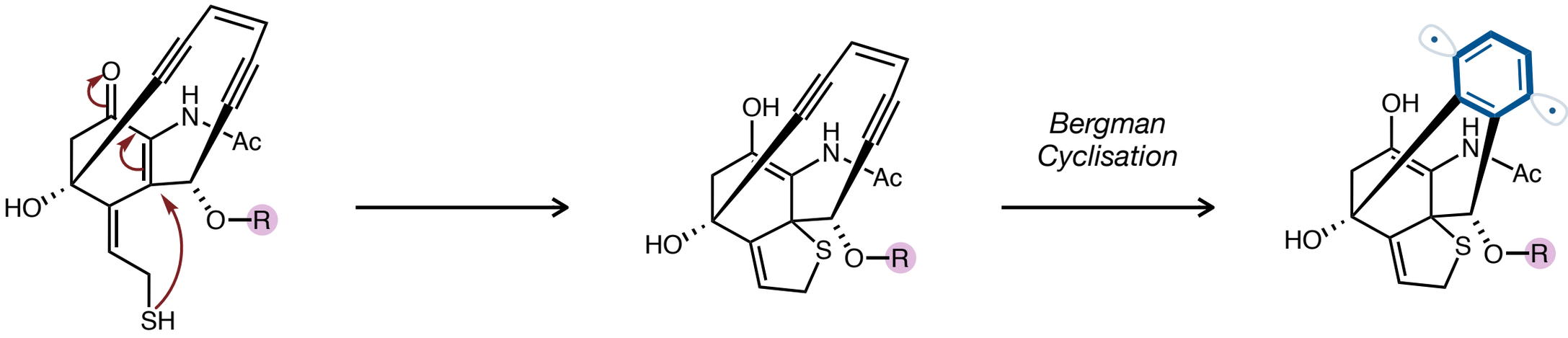
Trisaccharide directs the enediyne moiety to bind specifically to the minor groove of DNA



Wang, Z.; Li, H.; Gou, L.; Li, W.; Wang, Y. *Acta Pharm. Sin. B* **2023**, *13* (10), 4025–4059

Bystander effect

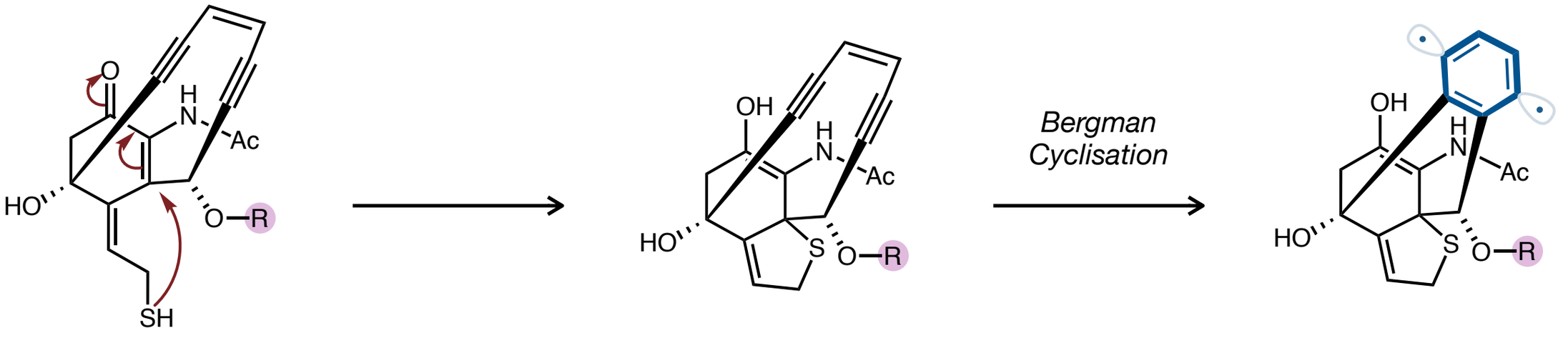
Ozogamicin (Calicheamicin)-containing ADC

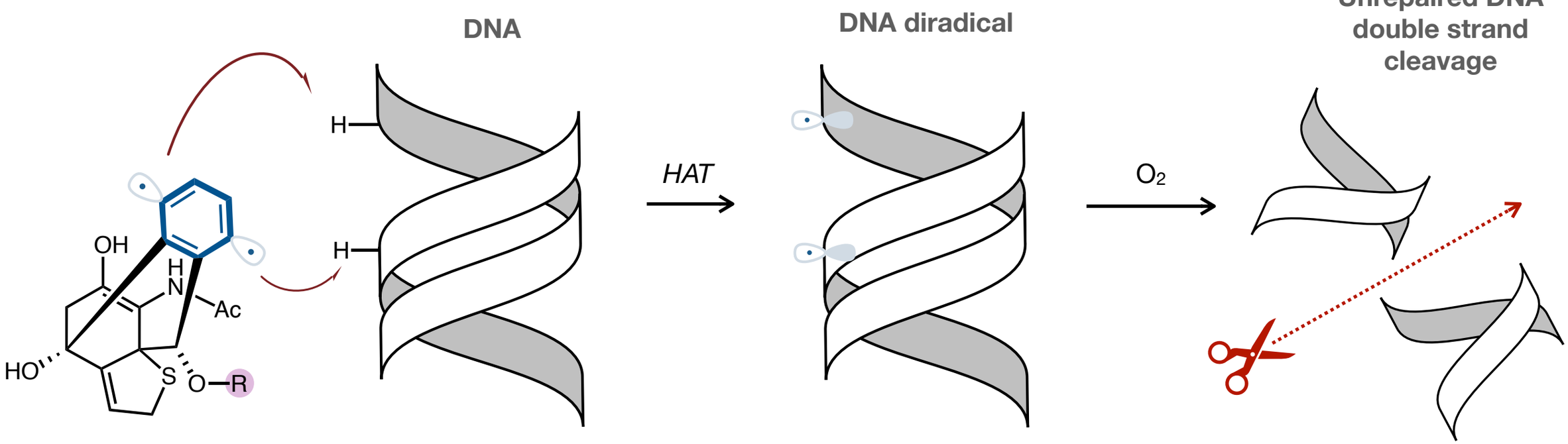


R = Sugar

Bystander effect

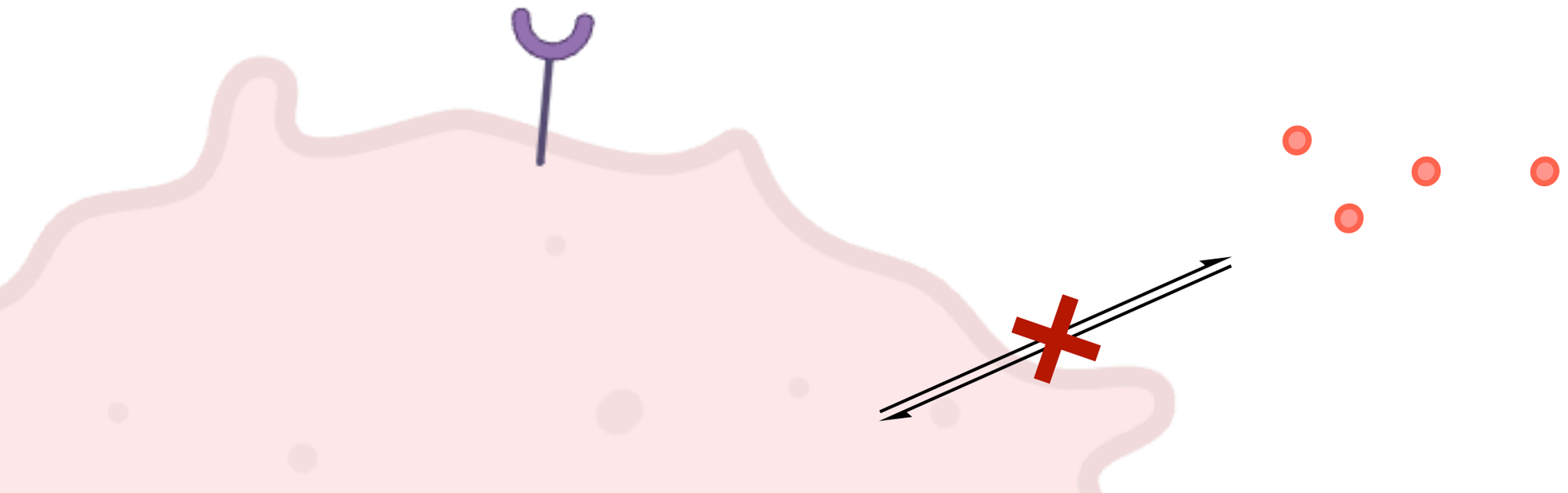
Ozogamicin (Calicheamicin)-containing ADC

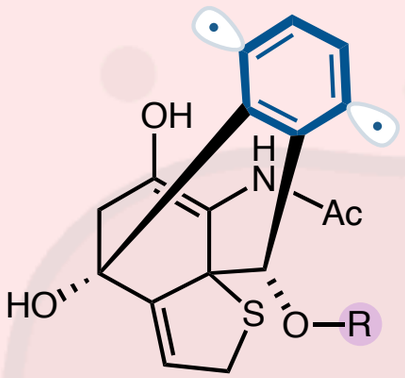




Bystander effect

Ozogamicin (Calicheamicin)-containing ADC





Short-lived nature of activated diradical

Neighbouring antigen-negative tumor cell

Calicheamicin-containing ADCs (Mylotarg™ and Besponsa™) do not exert bystander effects

Bystander effect

ADCs which do not exhibit bystander effect

Mylotarg™

Besponsa™

Calicheamicin-containing ADC

Adcetris™

Polivy™

Padcev™

Tivdak™

Kadcyla™

Enhertu™

Trodelvy™

Non-cleavable linker

Zynlota™

Blenrep™

Elahere™

Bystander effect

ADCs which exhibit bystander effect

Mylotarg™

Besponsa™

Adcetris™

Polivy™

Padcev™

Tivdak™

Kadcyla™

Enhertu™

Trodelvy™

Zynlota™

Blenrep™

Elahere™

How powerful is ‘bystander effect’ in the efficacy profile of ADCs?

Kadcyla™ versus Enhertu™

DESTINY Breast03 trial

The NEW ENGLAND JOURNAL of MEDICINE

RESEARCH SUMMARY

Trastuzumab Deruxtecan versus Trastuzumab Emtansine for Breast Cancer

Cortés J et al. DOI: 10.1056/NEJMoa2115022

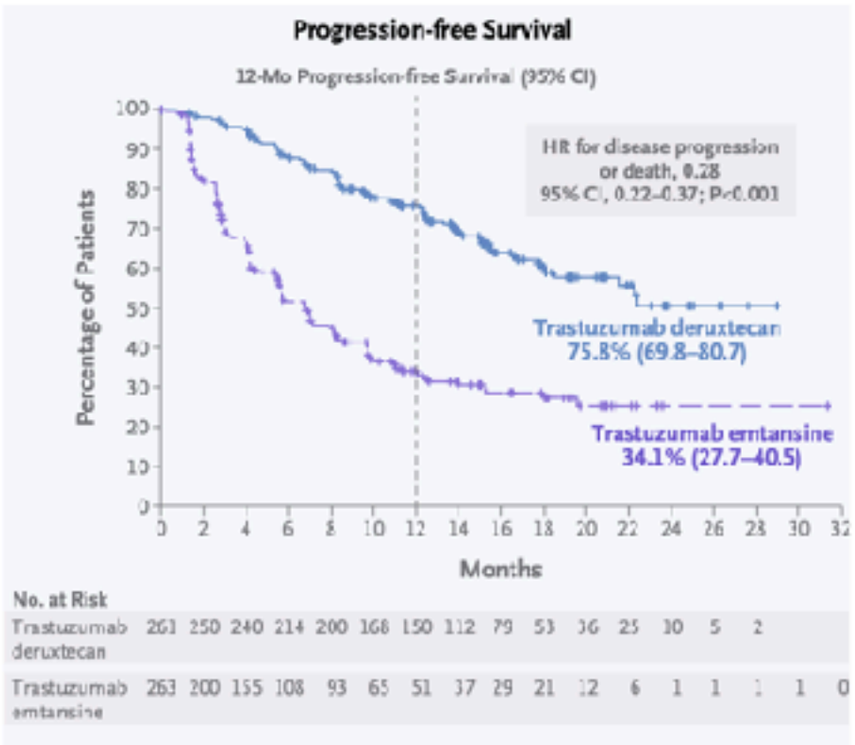
CLINICAL PROBLEM

The antibody–drug conjugate trastuzumab deruxtecan is approved in the United States to treat patients with human epidermal growth factor receptor 2 (HER2)–positive metastatic breast cancer who have received at least two previous anti-HER2 regimens in the context of metastatic disease. The benefits of trastuzumab deruxtecan as second-line therapy are unknown.

CLINICAL TRIAL

Design: A phase 3, multicenter, open-label, randomized, controlled trial compared trastuzumab deruxtecan with standard second-line treatment, trastuzumab emtansine, in patients with HER2-positive metastatic breast cancer.

Intervention: 524 patients with metastatic cancer that had progressed during or after treatment with trastuzumab and a taxane or that had progressed within 6 months after neoadjuvant or adjuvant treatment with trastuzumab or a taxane were assigned to receive either trastuzumab deruxtecan or trastuzumab emtansine intravenously every 3 weeks. The primary end point was progression-free survival.



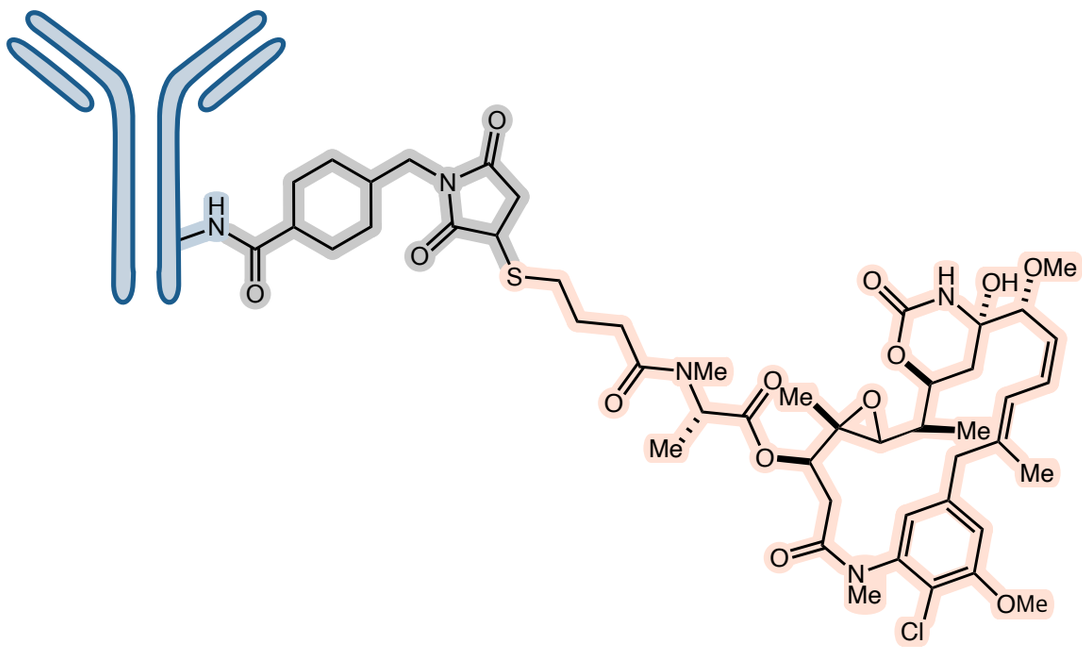
N. Engl. J. Med. **2022**, *386* (12), 1143–1154

Kadcyla™ versus Enhertu™

DESTINY Breast03 trial

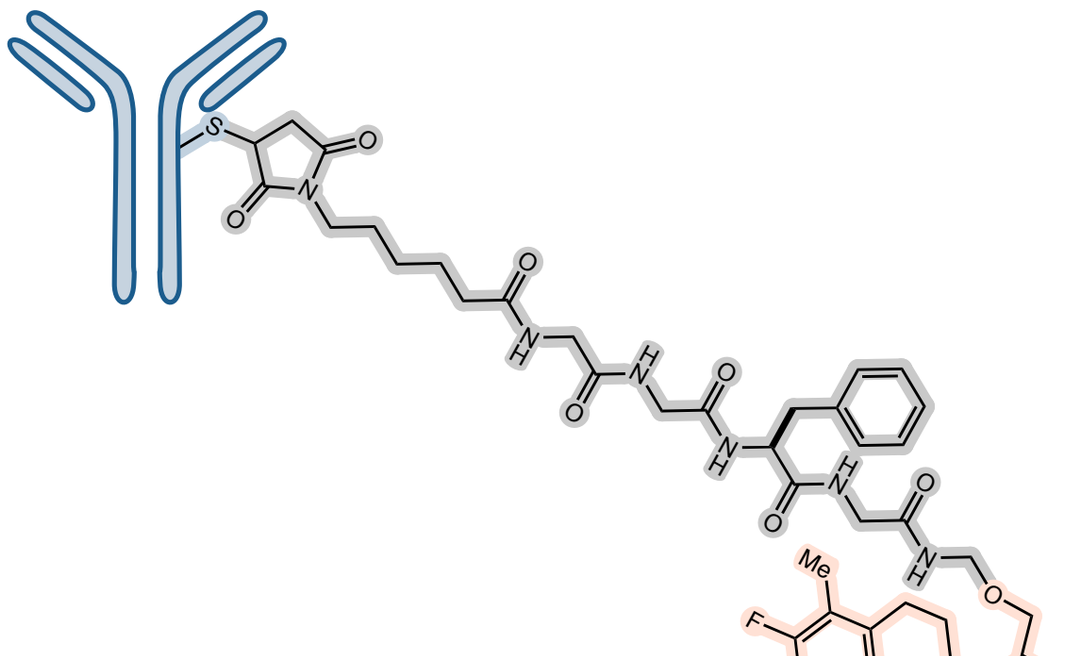
Kadcyla™
(Trastuzumab emtasine)

Genentech



Enhertu™
(Trastuzumab deruxtecan)

Daiichi-Sankyo



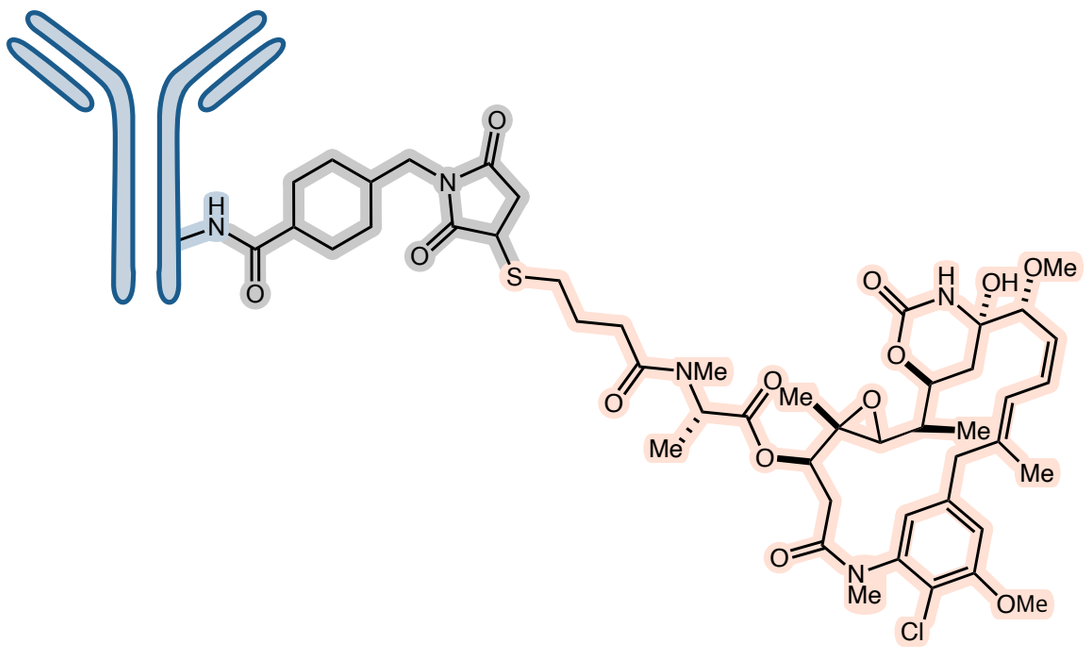
N. Engl. J. Med. **2022**, *386* (12), 1143–1154

Kadcyla™ versus Enhertu™

Emtansine versus Deruxtecan

Kadcyla™
(Trastuzumab emtasine)

Genentech

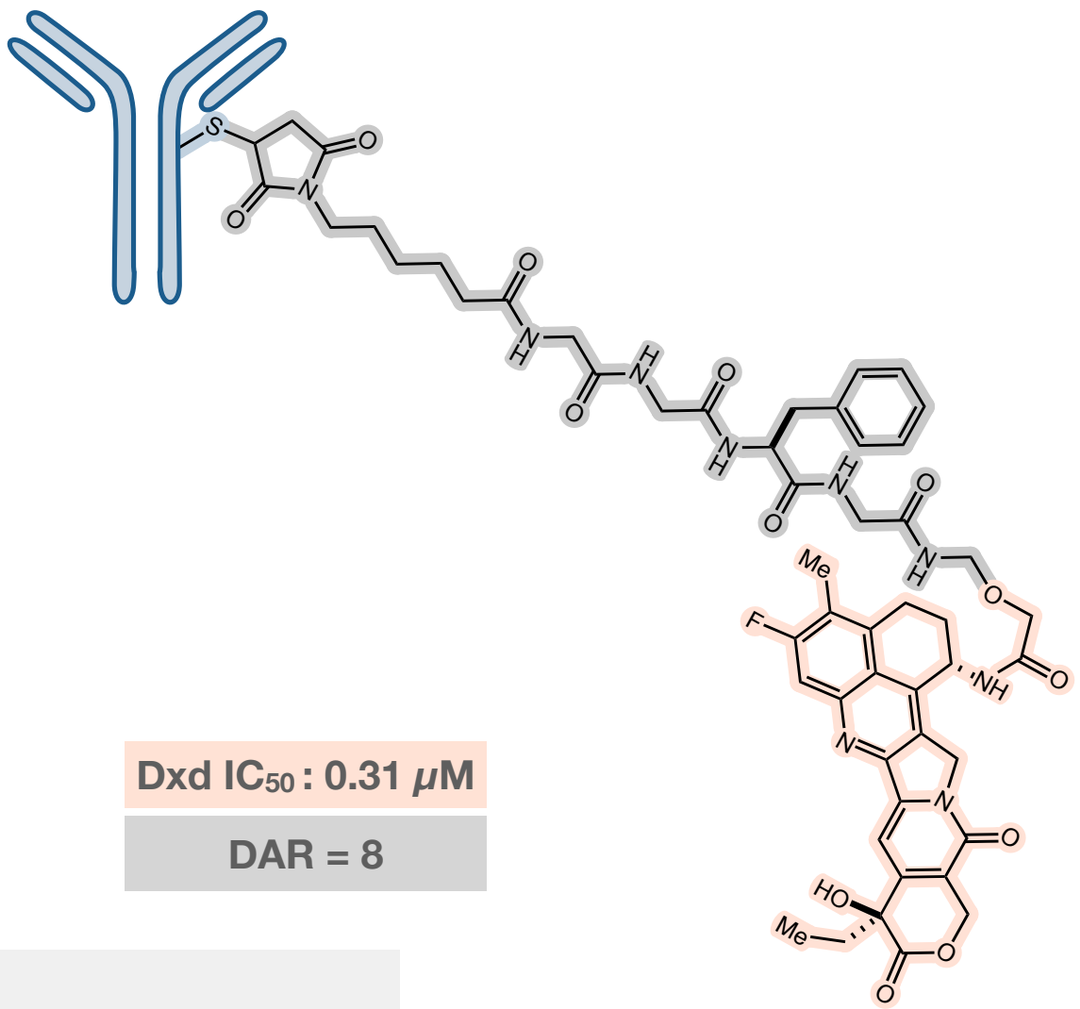


DM1 IC_{50} : 0.03 nM

DAR = 3.5

Enhertu™
(Trastuzumab deruxtecan)

Daiichi-Sankyo



Dxd IC_{50} : 0.31 μM

DAR = 8

Same 'A' (trastuzumab)
Different 'DC'

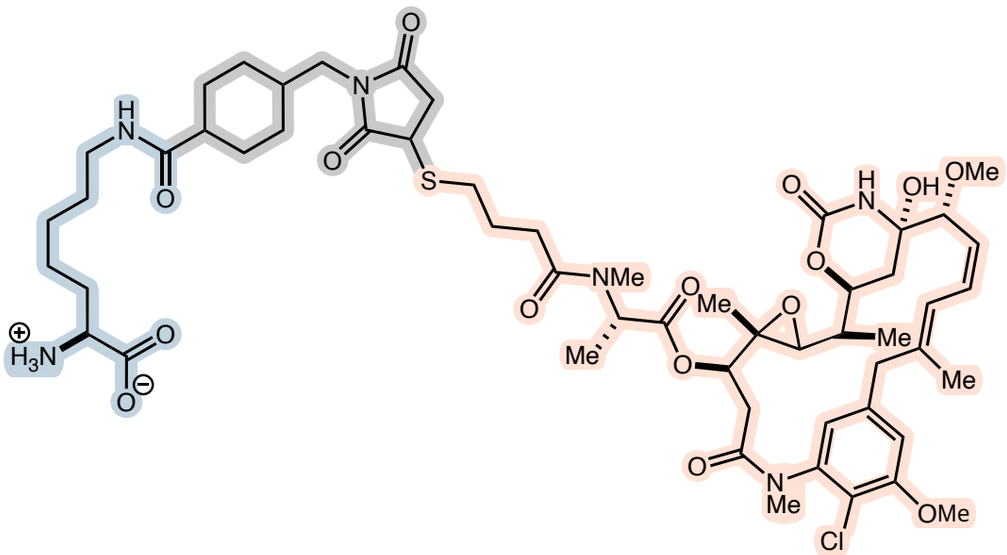
Azar, I.; Alkassis, S.; Fukui, J.; Alsawah, F.; Fedak, K.; Al Hallak, M. N.; Sukari, A.; Nagasaka, M. *Lung Cancer Targets Ther.* **2021**, *Volume 12*, 103–114

Kadcyla™ versus Enhertu™

Emtansine versus Deruxtecan

Kadcyla™
(Trastuzumab emtasine)

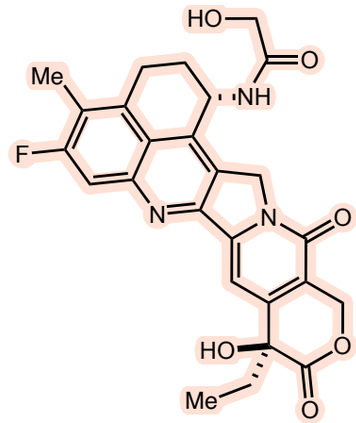
Genentech



logD = 0.2
Low cell-permeability
(No bystander effect)

Enhertu™
(Trastuzumab deruxtecan)

Daiichi-Sankyo



logD = 2.3
High cell-permeability
(Bystander effect)

Chen, R.; Ren, Z.; Bai, L.; Hu, X.; Chen, Y.; Ye, Q.; Hu, Y.; Shi, J. *Bioorganic Chem.* **2024**, *151*, 107697

Kadcyla™ versus Enhertu™

DESTINY Breast03 trial

*Phase 3, open-label, **HER2-positive patients (2L)***
Multi-centre (15 countries)
Jun 2018 - Jun 2020

Kadcyla™ *(Trastuzumab emtasine)*	***Enhertu™*** *(Trastuzumab deruxtecan)*
263 patients	***261 patients***

N. Engl. J. Med. **2022**, *386* (12), 1143–1154

Kadcyla™ versus Enhertu™

DESTINY Breast03 trial

*Phase 3, open-label, **HER2-positive patients (2L)***
Multi-centre (15 countries)
Jun 2018 - Jun 2020

Kadcyla™ *(Trastuzumab emtasine)*	***Enhertu™*** *(Trastuzumab deruxtecan)*
263 patients	***261 patients***

Progression-free survival

34.1% of participants were still alive without their cancer getting worse after 12 months

75.8% of participants were still alive without their cancer getting worse after 12 months

N. Engl. J. Med. **2022**, *386* (12), 1143–1154

Kadcyla™ versus Enhertu™

DESTINY Breast03 trial

Phase 3, open-label, ***HER2-positive patients (2L)***
Multi-centre (15 countries)
Jun 2018 - Jun 2020

Kadcyla™ *(Trastuzumab emtasine)*	***Enhertu™*** *(Trastuzumab deruxtecan)*
263 patients	***261 patients***

Overall response rate

34.2% of participants partially or completely responded to Kadcyla™

79.7% of participants partially or completely responded to Enhertu™

N. Engl. J. Med. **2022**, *386* (12), 1143–1154

Kadcyla™ versus Enhertu™

DESTINY Breast03 trial

*Phase 3, open-label, **HER2-positive patients (2L)***
Multi-centre (15 countries)
Jun 2018 - Jun 2020

Kadcyla™ *(Trastuzumab emtasine)*	***Enhertu™*** *(Trastuzumab deruxtecan)*
263 patients	***261 patients***

Overall response rate

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***79.7%** of participants partially or completely responded to Enhertu™*

N. Engl. J. Med. **2022**, *386* (12), 1143–1154

Enhertu™ in HER2-low advanced patients

DESTINY Breast04 trial

The NEW ENGLAND JOURNAL of MEDICINE

RESEARCH SUMMARY

Trastuzumab Deruxtecan in Previously Treated HER2-Low Advanced Breast Cancer

Modi S et al. DOI: 10.1056/NEJMoa2203690

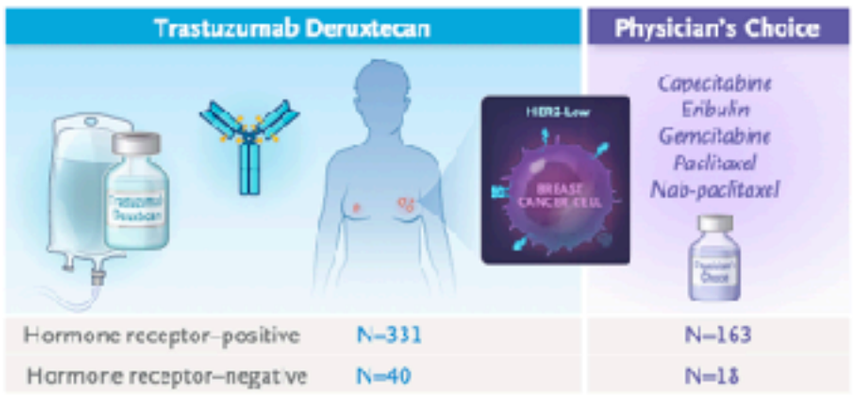
CLINICAL PROBLEM

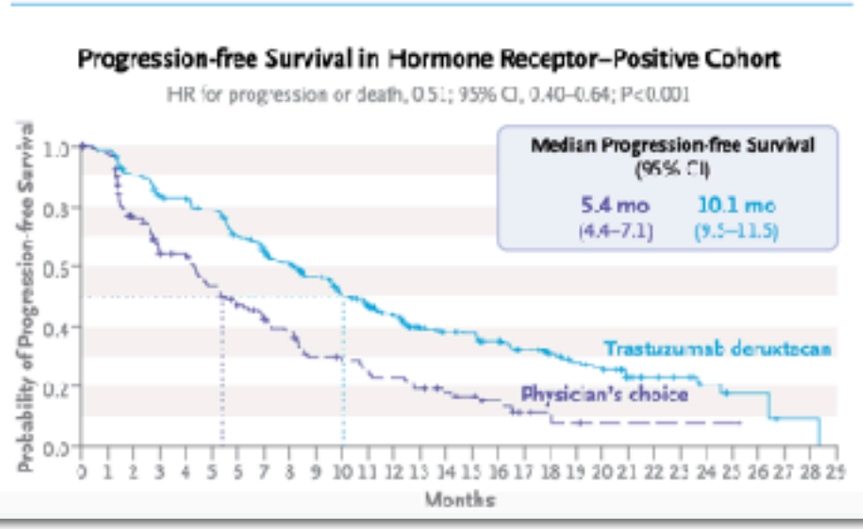
Patients with HER2-low metastatic breast cancer, which lacks overexpression or amplification of HER2, have limited targeted treatment options. The antibody–drug conjugate trastuzumab deruxtecan has shown efficacy in these patients in phase 1 and 2 trials.

CLINICAL TRIAL

Design: A phase 3, open-label, randomized trial examined the efficacy and safety of trastuzumab deruxtecan in patients with previously treated unresectable or metastatic HER2-low breast cancer. Low HER2 was defined by a score of 1+ on immunohistochemical (IHC) analysis or by an IHC score of 2+ and negative results on in situ hybridization.

Intervention: 557 patients with HER2-low metastatic breast cancer were randomly assigned in a 2:1 ratio to receive either trastuzumab deruxtecan intravenously every 3 weeks at a dose of 5.4 mg per kilogram of body weight or the physician's choice of untargeted chemotherapy. The primary end point was progression-free survival among patients with hormone receptor–positive cancer (approximately 89% of all patients).





N. Engl. J. Med. **2022**, *387* (1), 9–20

Enhertu™ in HER2-low advanced patients

DESTINY Breast04 trial

Physician's choice	***Enhertu™*** *(Trastuzumab deruxtecan)*
181 patients	***371 patients***
Paclitaxel, Gemcitabine, Eribulin etc	

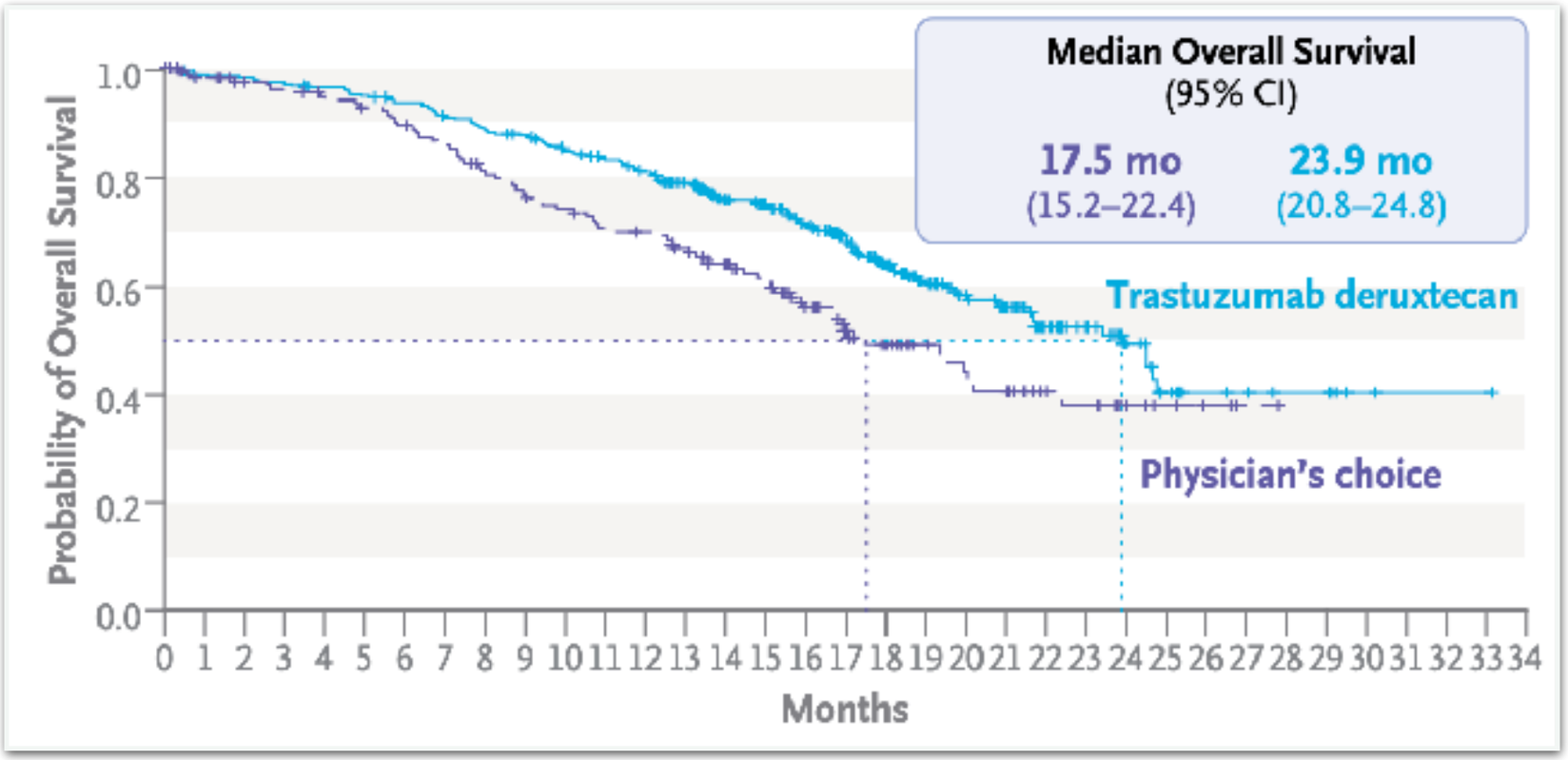
N. Engl. J. Med. **2022**, *387* (1), 9–20

Enhertu™ in HER2-low advanced patients

DESTINY Breast04 trial

Physician's choice	***Enhertu™*** *(Trastuzumab deruxtecan)*
181 patients	***371 patients***
Paclitaxel, Gemcitabine, Eribulin etc	

Overall survival



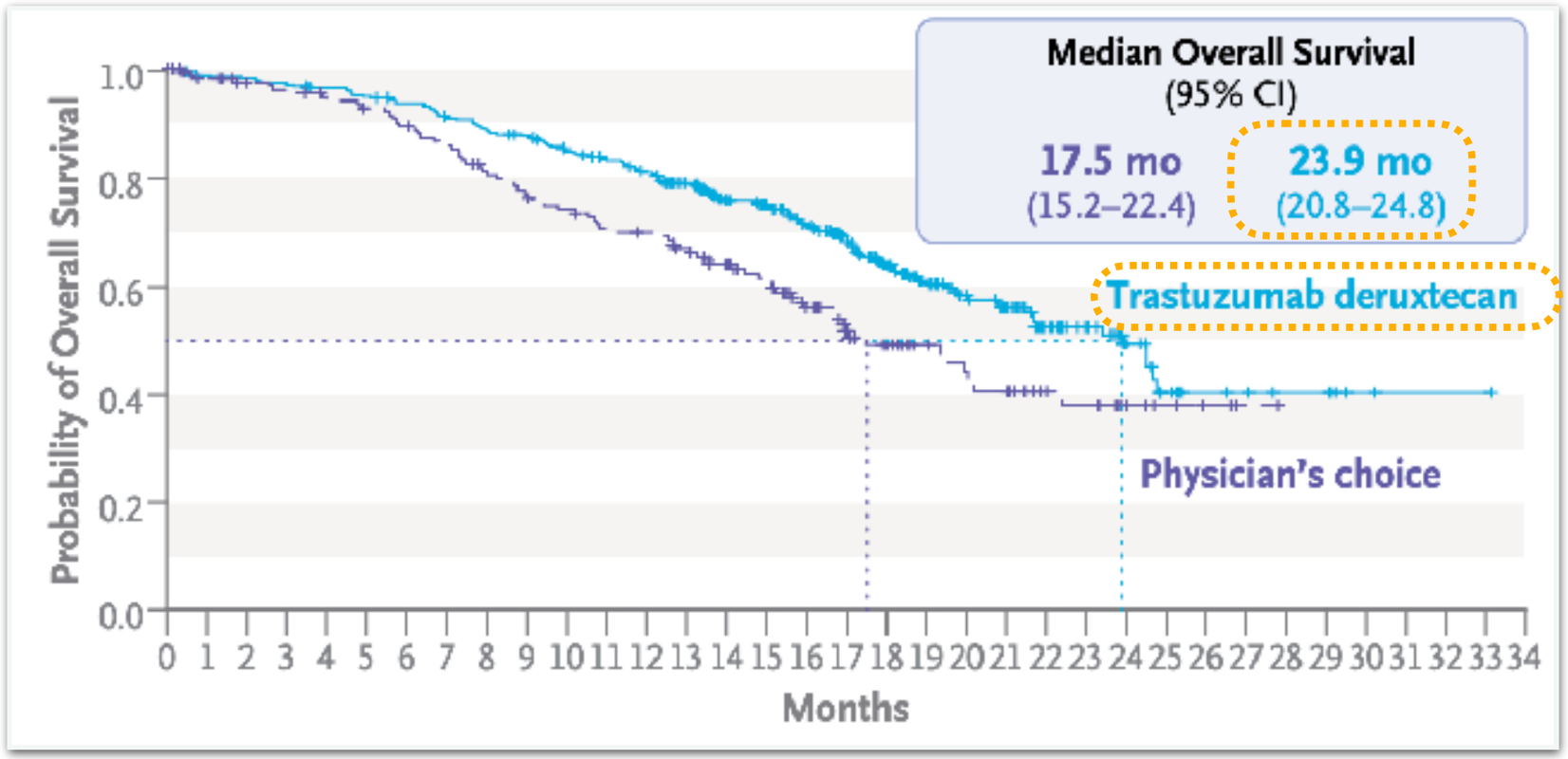
N. Engl. J. Med. **2022**, *387* (1), 9–20

Enhertu™ in HER2-low patients

DESTINY Breast04 trial

Physician's choice	***Enhertu™*** *(Trastuzumab deruxtecan)*
181 patients	***371 patients***
Paclitaxel, Gemcitabine, Eribulin etc	

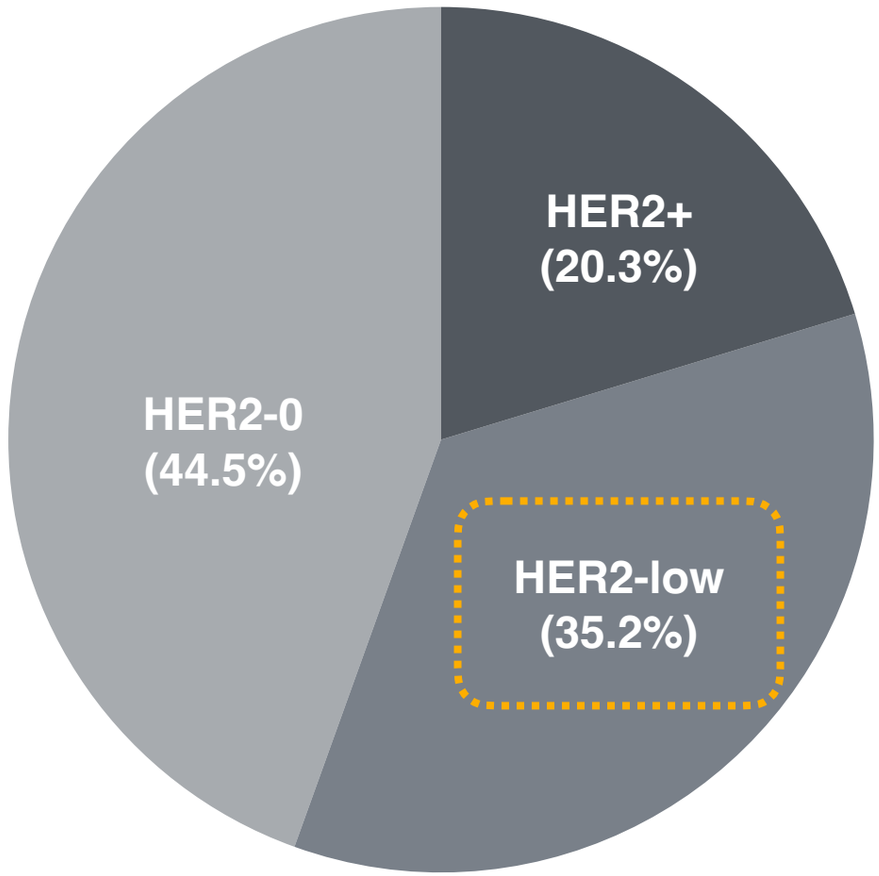
Overall survival



N. Engl. J. Med. **2022**, *387* (1), 9–20

Enhertu™ in HER2-low patients

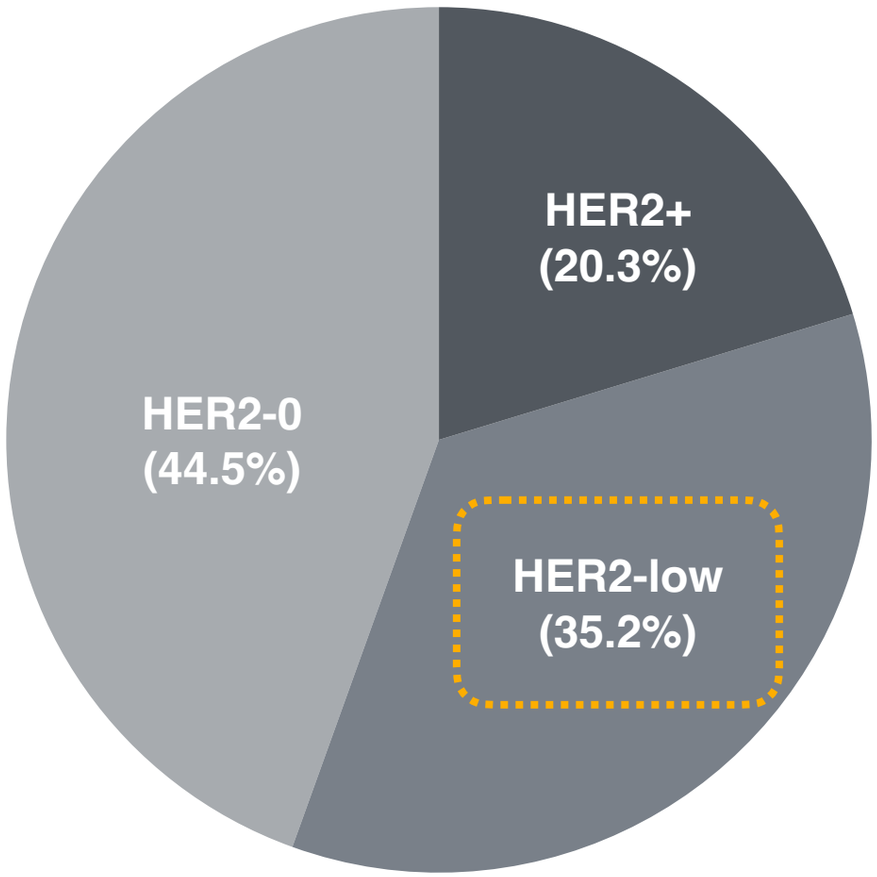
DESTINY Breast04 trial



Gampenrieder, S. P.; Rinnerthaler, G.; Greil, R.*Breast Cancer Res.* **2021**, *23* (1), 112

Enhertu™ in HER2-low patients

DESTINY Breast04 trial

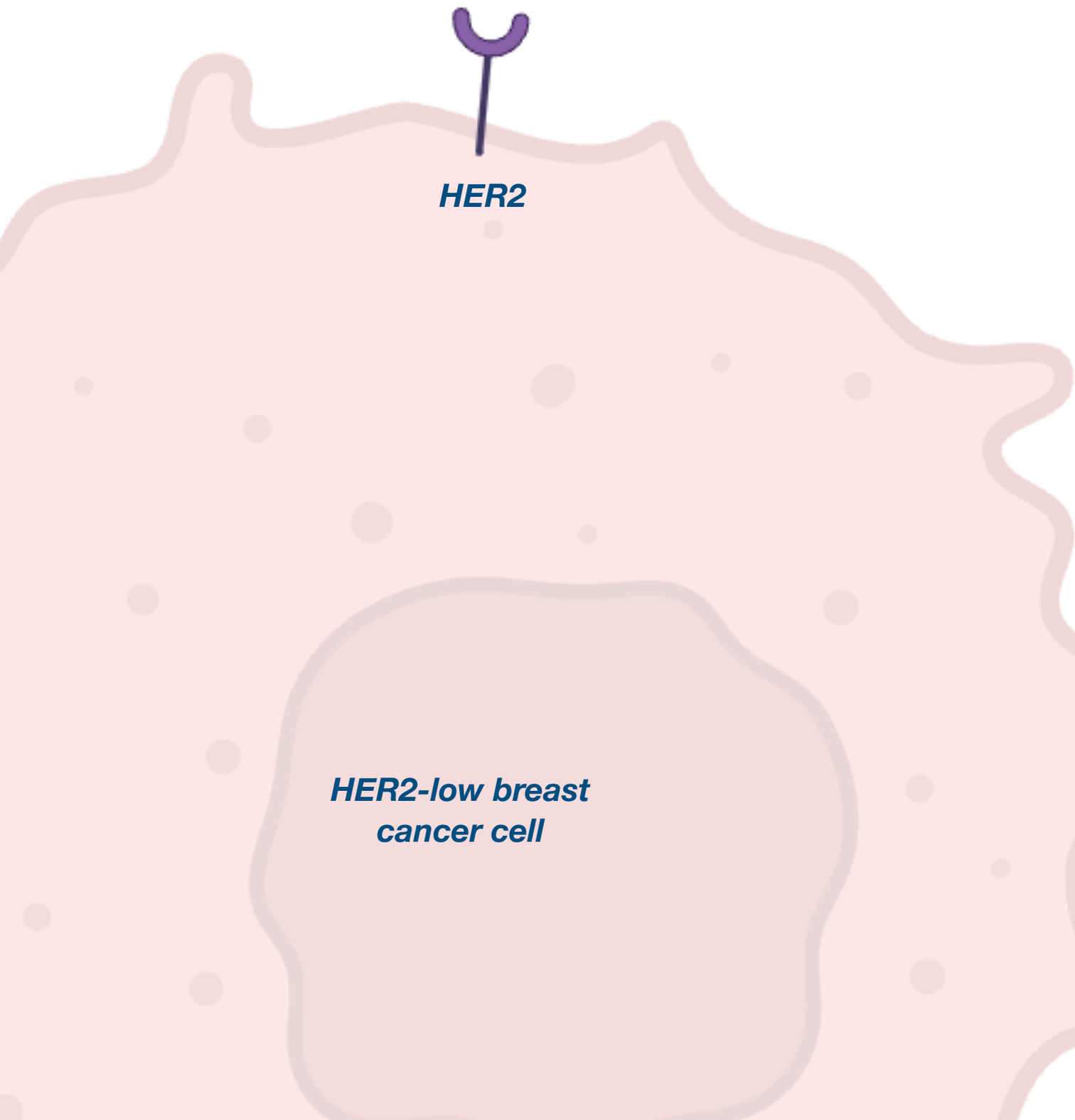


(ASCO 2022)

Breast Cancer Res. **2021**, *23* (1), 112

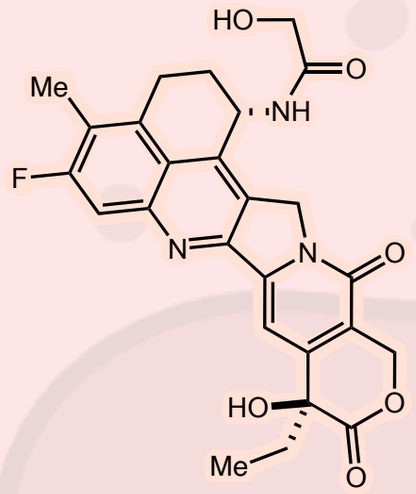
Enhertu™ in HER2-low patients

DESTINY Breast04 trial



Enhertu™ in HER2-low patients

DESTINY Breast04 trial



logD = 2.3
High cell-permeability
(Bystander effect)

“Bystander effect”

HER2-low breast cancer cell

The bystander effect of Enhertu™ has enabled its use in tumors with low/heterogenous HER2 expression

Enhertu™ in HER2-low patients

DESTINY Breast04 trial

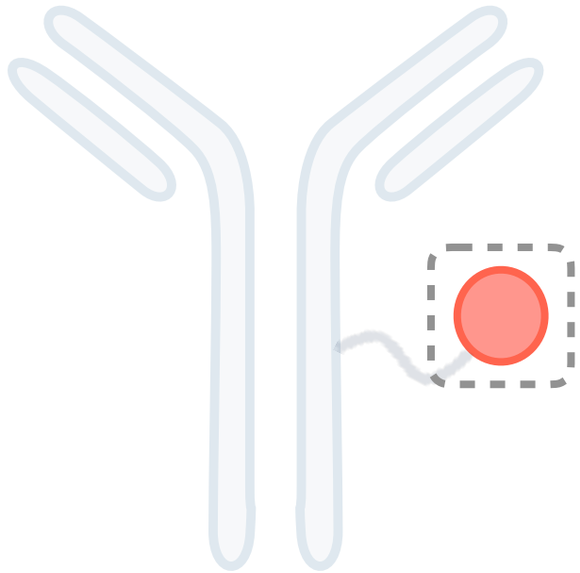
In interview with Breast Cancer Research Foundation, on DESTINY trial (ASCO 2022)

"… The key feature that makes T-DXd different is the linker. T-DXd unlike T-DM1 has a cleavable linker. When the chemo is lopped off from the antibody, it leaves the chemotherapy in a membrane-permeable state. So now when the chemotherapy is released in the cancer cell, it can kill that HER2-positive cancer cell. And that's where most ADCs would stop. That's where T-DM1 would stop. But now this chemotherapy can pass through the cell membrane and can enter the microenvironment and neighboring cells and kill those cells as well. And that can include cells that have variable levels of HER2 expression—even HER2-low cells. ***That's the tremendous advantage of T-DXd, not only over T-DM1, frankly, but over all of our currently available HER2-targeted therapies****"*

Dr Shanu Modi, Study Leader of DESTINY trial (Memorial Sloan Kettering Cancer Center)

‘D’ in ADC

What makes a good ‘D’ for ADC



- ✓ *Sufficiently cytotoxic (micromolar/nanomolar)*
- ✓ *Bystander effect*
- ✓ *New class of ‘D’: Expansion of scope and modality*

Conclusion and outlooks

Final thoughts

- ***ADC: Realization of the ‘Magic Bullet’ concept***

Conclusion and outlooks

Final thoughts

- ***ADC: Realization of the ‘Magic Bullet’ concept***

- ***‘D’ of ADC: The bystander effect plays a crucial role in enhancing ADC efficacy***

Conclusion and outlooks

Final thoughts

- ***ADC: Realization of the 'Magic Bullet' concept***
- ***'D' of ADC: The bystander effect plays a crucial role in enhancing ADC efficacy***
- ***'C' of ADC: ADCs with heterogeneous DARs are not likely to be approved by the US FDA in the future***

Conclusion and outlooks

Final thoughts

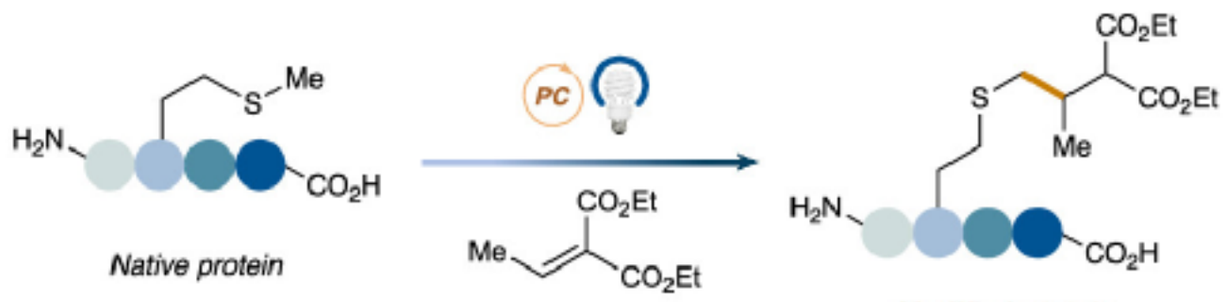
- ***ADC: Realization of the 'Magic Bullet' concept***
- ***'D' of ADC: The bystander effect plays a crucial role in enhancing ADC efficacy***
- ***'C' of ADC: ADCs with heterogeneous DARs are not likely to be approved by the US FDA in the future***
- ***'A' of ADC: Better elucidation of mechanism of action of 'A' (endocytosis to lysosomal degradation) will lead us to a better selection of 'A' for ADC***

Conclusion and outlooks

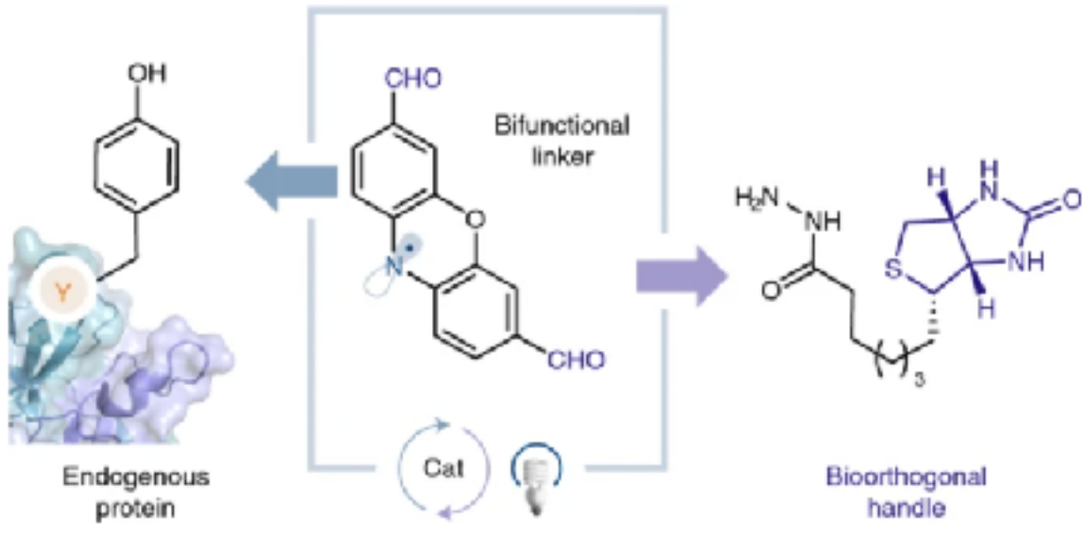
Final thoughts

- ***'C' of ADC: A novel array of bioconjugation methods with photoredox catalysis will enable rapid access to conjugate modalities beyond ADCs***

Methionine conjugation (J. Am. Chem. Soc., 2020)



Tyrosine conjugation (Nat. Chem. 2021)



Our own group's chemical biology tool can be used to further develop this field

Acknowledgements

ADC patent

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
16 May 2024 (16.05.2024)

WIPO | PCT

(10) International Publication Number
WO 2024/102078 A1

(51) International Patent Classification:
C07C 233/49 (2006.01) *A61K 47/54* (2017.01)
C07D 498/18 (2006.01) *A61P 35/00* (2006.01)
A61K 31/537 (2006.01)

(21) International Application Number:
PCT/SG2023/050745

(22) International Filing Date:
09 November 2023 (09.11.2023)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
10202260033S 10 November 2022 (10.11.2022) SG

(71) Applicant: **NANYANG TECHNOLOGICAL UNIVERSITY** [SG/SG]; 50 Nanyang Avenue, Singapore 639798 (SG).

(72) Inventors: **LOH, Teck Peng**, c/o Nanyang Technological University, 50 Nanyang Avenue, Singapore 639798 (SG). **KANG, Min Sun**, c/o Nanyang Technological University, 50 Nanyang Avenue, Singapore 639798 (SG). **KHOO, Yi Xin Joycelyn**; c/o Nanyang Technological University, 50 Nanyang Avenue, Singapore 639798 (SG).

(74) Agent: **KINNAIRD, James Welsh**, Marks & Clerk Singapore LLP, Tanjong Pagar Post Office, P O Box 636, Singapore 910816 (SG).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CV, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, MG, MK, MN, MU, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

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Prof. Teck-Peng Loh

(Nanyang Technological University)

Acknowledgements

Prof. David W. C. MacMillan

The MacMillan Group

PRINCETON UNIVERSITY

Questions?