Cleavable linkers in antibody drug conjugates

Literature seminar
2020/01/20
M1 Natsuki Konoue

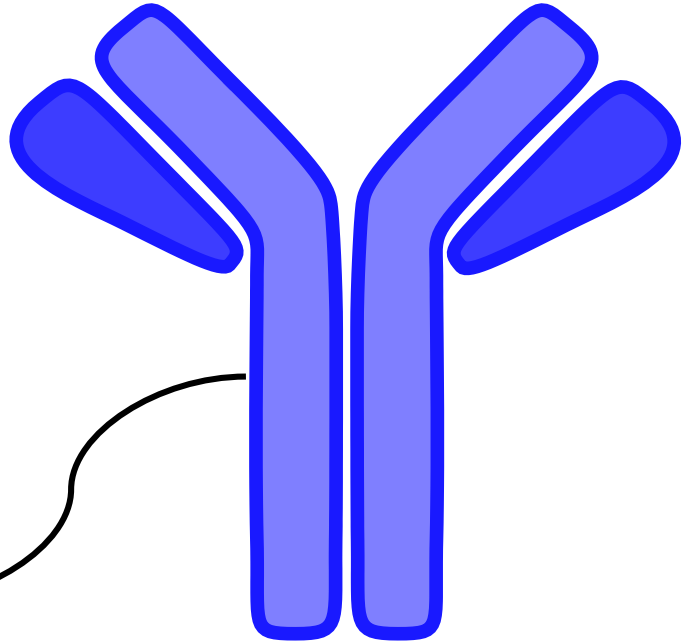
contents

Introduction

Antibody-drug conjugate(ADC)

antibody

the selective delivery system developed against an antigen associated with a specific cancer cell type



chemical linker

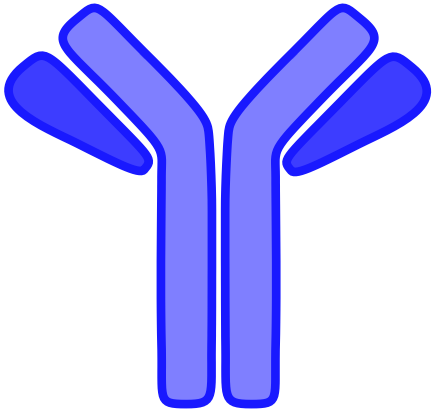
The molecular chain through which the payload is attached to the antibody

payload

the potent anticancer drug

Drug

- ○ high cytotoxicity
- ○ low production cost
- × low selectivity to cancer cell
- × rapid plasma clearance

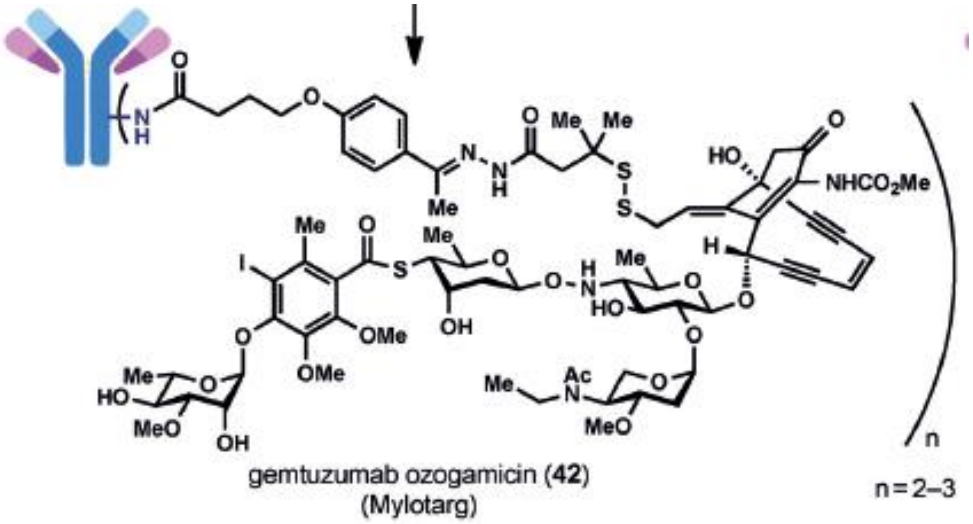


- ○ high selectivity to cancer cell
- ○ long plasma half-lives
- × limited cytotoxicity

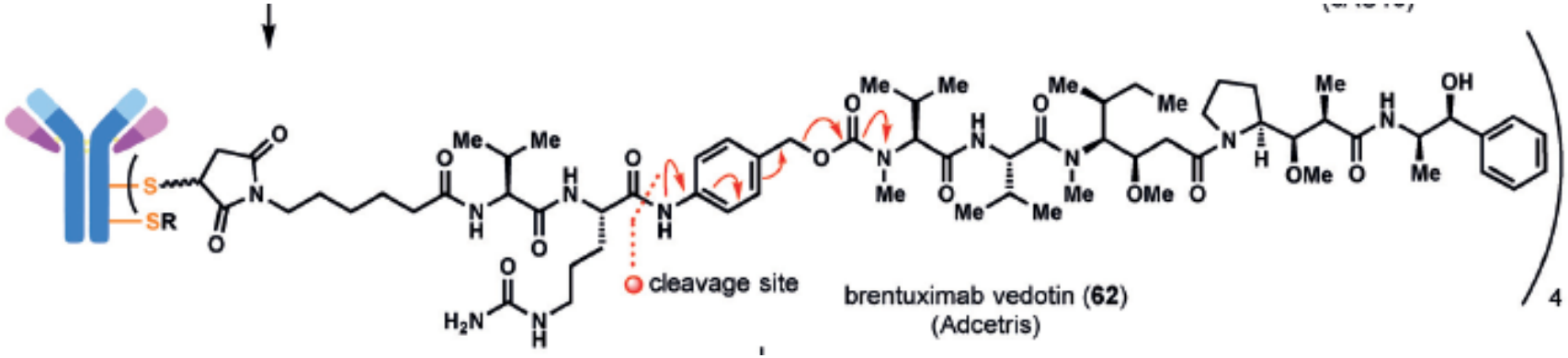
combination

□ High cytotoxic and selective therapeutics with long plasma half-lives

Clinically approved antibody drug conjugates [5]

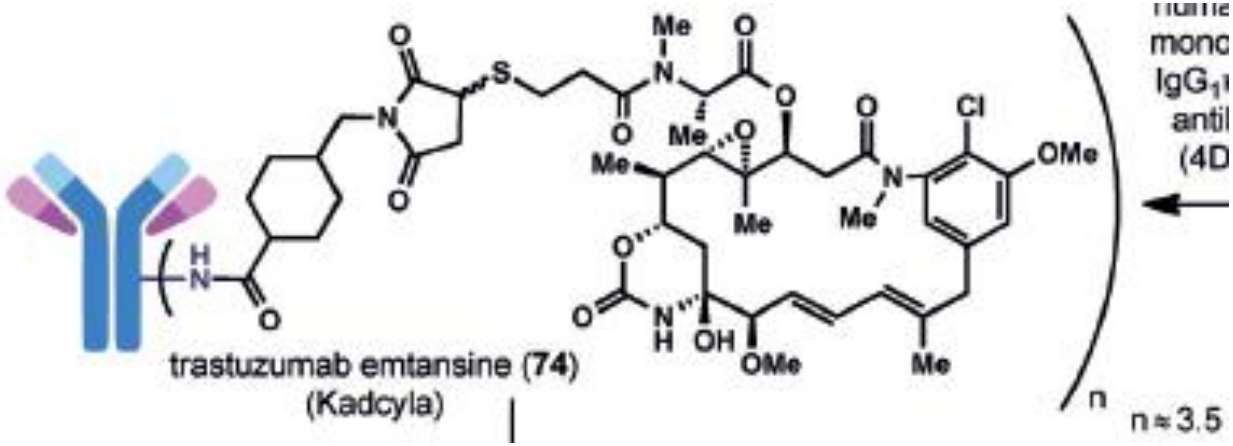


Mylotarg (2000, withdrawn in 2010 and reapproved in 2017)
For acute myeloid leukemia(AML)
急性骨髄性白血病

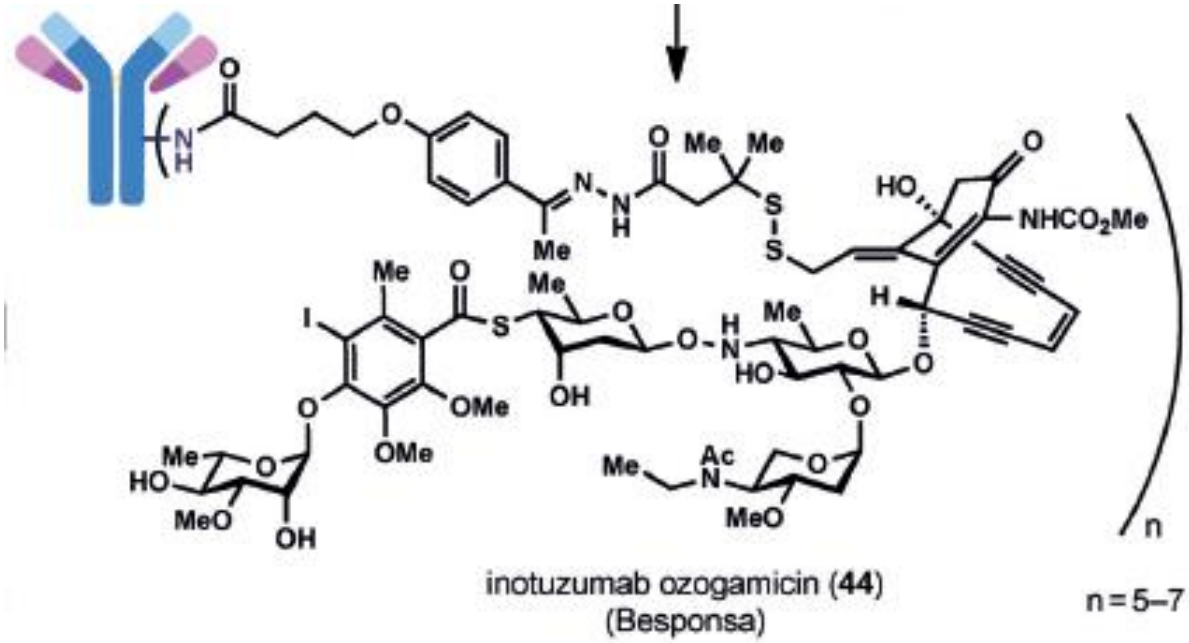


Adcetris(2011)
For relapsed or refractory Hodgkin lymphoma and systemic anaplastic large cell lymphoma (ALCL)
ホジキンリンパ腫、ALK陽性未分化大細胞リンパ腫

Angew. Chem. Int. Ed. **2019**, *58*, 11206 – 11241



Kadcyla(2013)
For HER2 positive metastatic breast cancer
HER2陽性転移性乳がん
*HER2: human epidermal growth factor receptor 2



Besponsa(2017)
For relapsed or refractory B-cell precursor acute lymphoblastic leukemia(ALL)
前駆B細胞急性リンパ性白血病

●There are almost 100 ADCs in clinical trials

Angew. Chem. Int. Ed. **2019**, *58*, 11206 – 11241

Mechanism of action

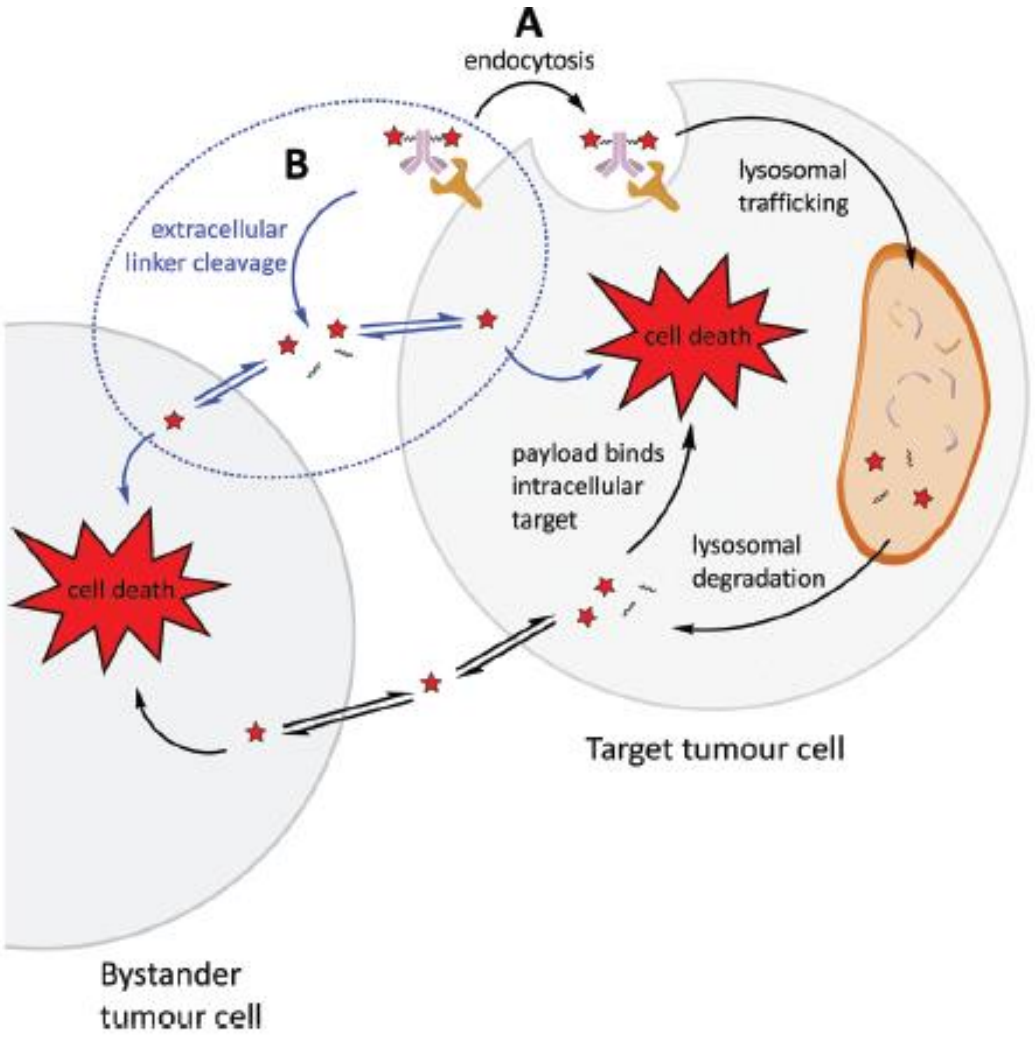


Fig. 2 (A) The traditional mechanism of action, involving endocytosis and intracellular payload release. (B) The non-internalising, extracellular mechanism of action.

Chem. Soc. Rev., **2019**, *48*, 4361-4374

Chemically cleavable linkers

ADC linkers can be classified as 'cleavable' or 'non-cleavable'

Non-cleavable linkers are mainly effective for the treatment of haematological cancers or tumors with high antigen expression

Cleavable linkers are generally preferred to non-cleavable linkers due to their range of applicability

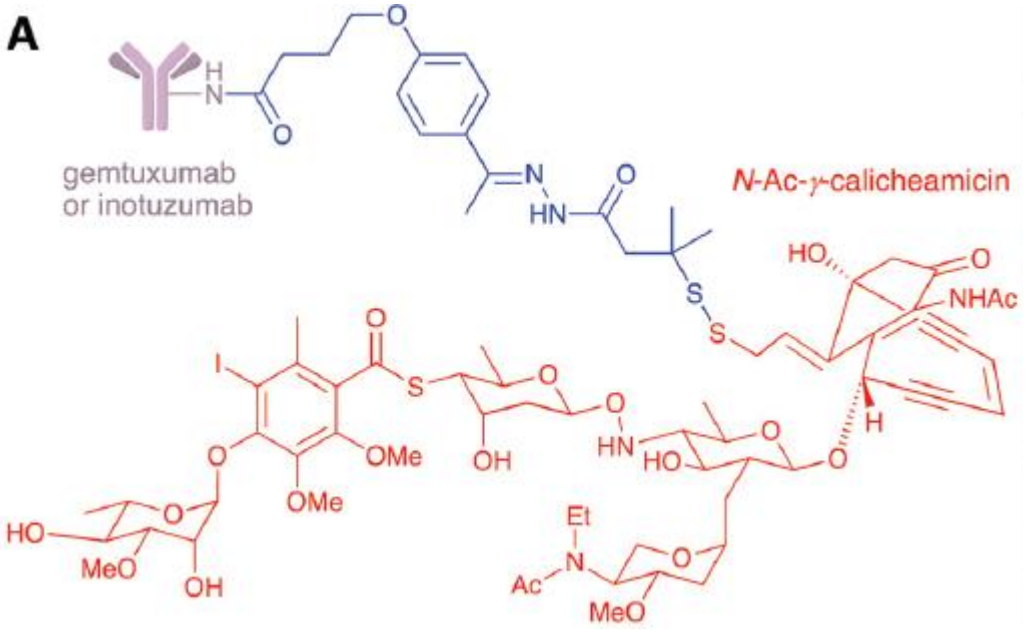
There are 4 types of chemically cleavable linkers

- **Acid cleavable**
- **Reducible disulfides**
- **Cleavable by exogenous stimuli**
- **Enzyme cleavable**

Acid cleavable linkers

Acid cleavable linkers aim to exploit the acidity of the Endosomes(pH 5.5-6.2) and lysosomes(pH 4.5-5.0), whilst maintaining stability in circulation at pH 7.4

This strategy resulted in clinical success in gemtuzumab ozogamicin(*Mylotarg*), inotuzumab ozogamicin(*Besponsa*)



The linker contains an acid-sensitive *N*-acyl hydrazine linkage

Upon acid catalysis, hydrolyses to a ketone and a hydrazide-payload

Besponsa linker showed that hydrazone hydrolysis occurred in circulation at a rate of 1.5-2.0%/day

Bioconjugate Chem.., **2002**, *13*, 47-58

Other hyrazone-containing linker

Phenylketone-derived hydrazine linker

Hydrolysed with $t_{1/2}$ = 2 days in isolated human and mouse plasma despite much higher stability in pH 7.4 buffer

The specific cause is unclear

The highly variable stability of hydrazones has prevented their utility

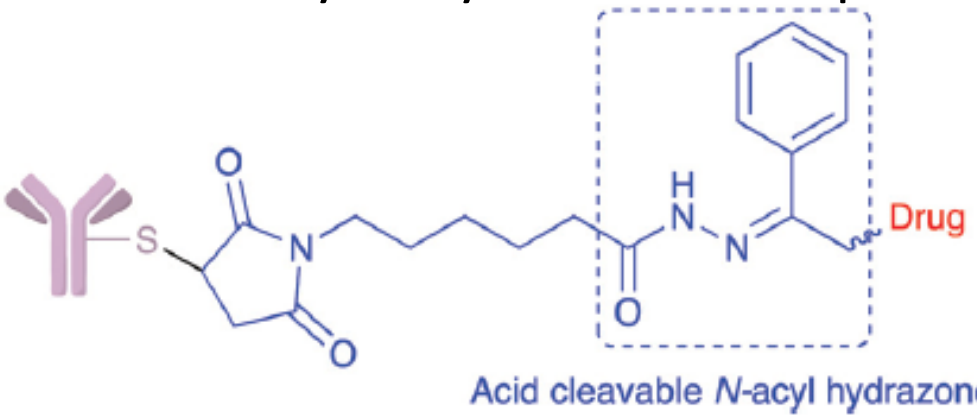


Fig. 3 The structure of a phenylketone-derived hydrazone linker.

Nat. Biotechnol.., **2003**, *21*, 778-784

Acid cleavable linkers containing other functional groups

Carbonate linkers with an alcohol-containing SN-38 payload

Introduction of a p-aminobenzyl(PAB)-spacer boosted the serum stability

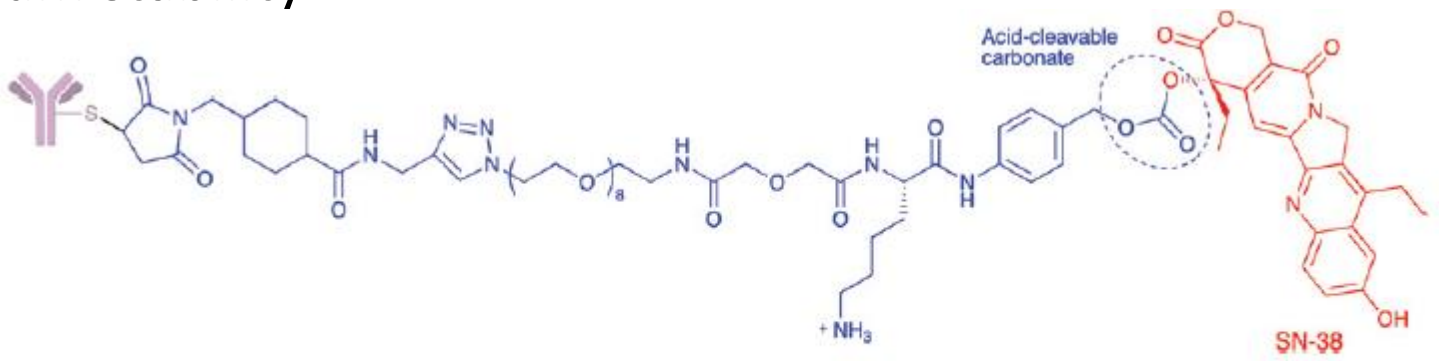


Fig. 4 The structure of SN-38-bearing ADCs with an acid-cleavable PAB-carbonate linker.

Most linker technology has moved away from acid cleavable groups

The requirement for linkers to distinguish between pH 5.0 and pH 7.4 is difficult and development focuses on other approaches

J. Med. Chem.., **2008**, *51*, 6916-6926

Reducible disulfides

Disulfides are the most prominent class of chemically cleavable motifs found in ADC linkers

Disulfides are stable at physiological pH but are sensitive to nucleophilic attack from thiols

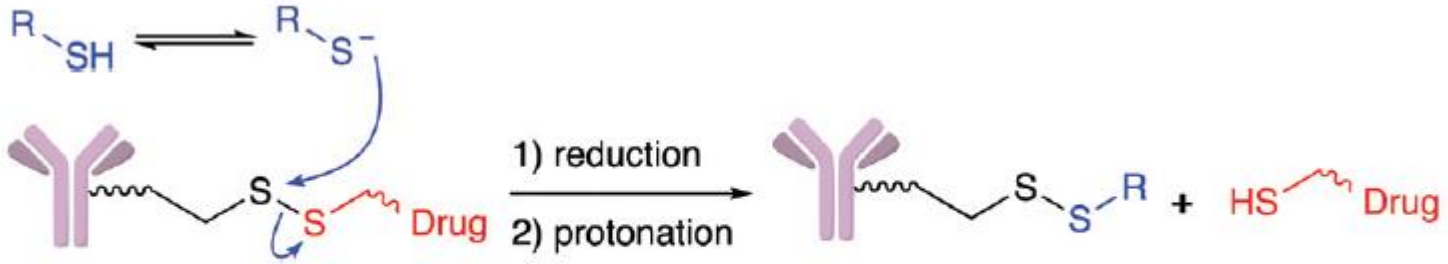


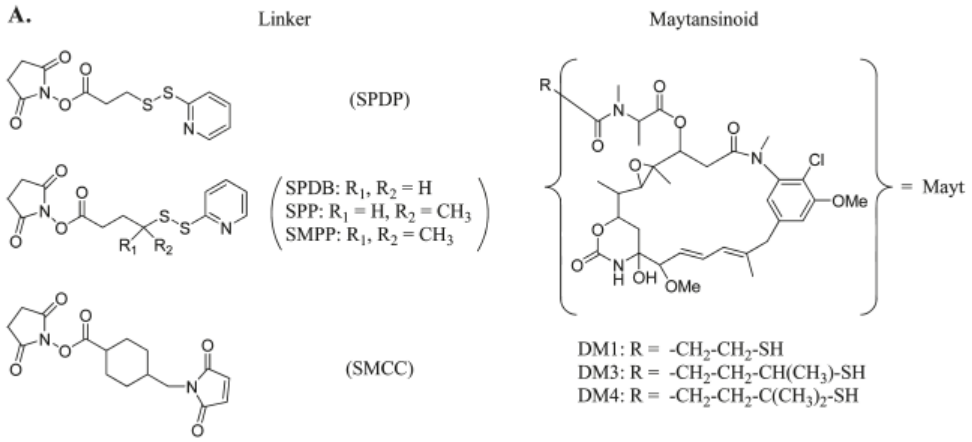
Fig. 5 The reduction of disulfide linkers, mediated by the deprotonated thiolate.

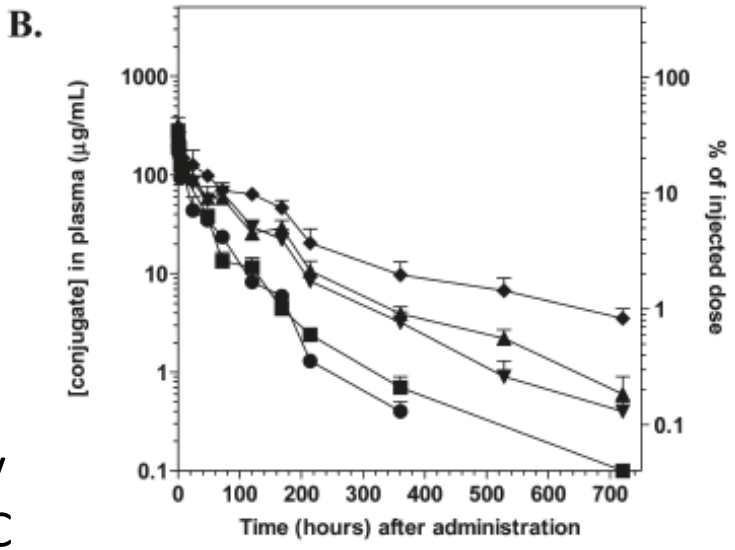
In contrast to the limited reductive power of blood plasma, the cytosol contains high levels of glutathione(GSH) (1-10 mM)

The oxidative stress associated with tumors leads to elevated GSH levels

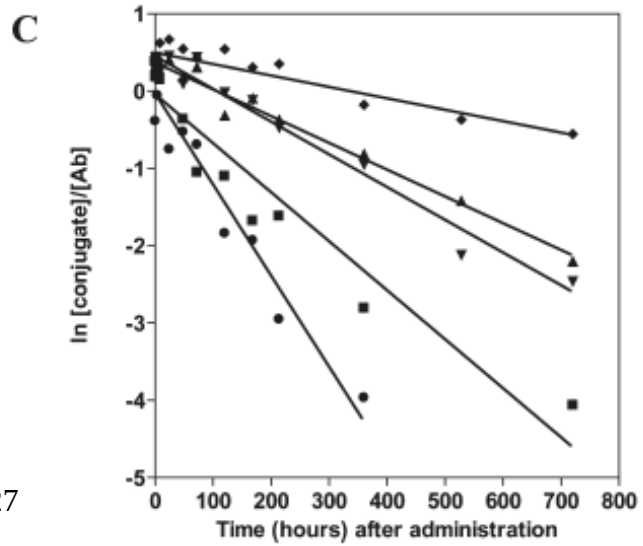
Sterically hindered disulfides

structures of linkers in antibody-maytasinoid conjugates(AMCs)





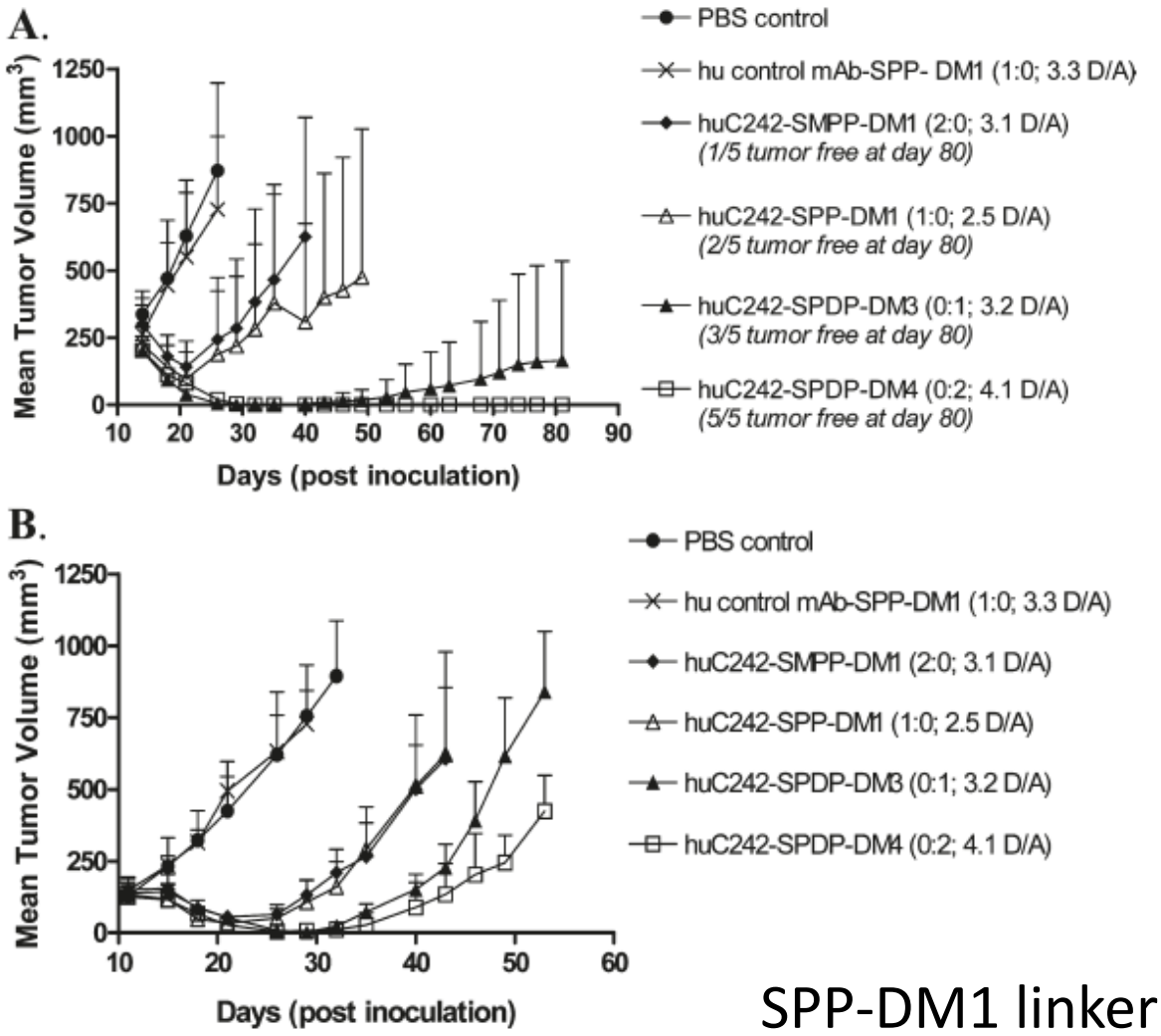
The rate of reduction of disulfide-linked AMCs by DTT was measured at 37 C, pH 6.5, using an HPLC assay to follow the release of maytansinoid



huC242-SMCC-DM1 : ◆
huC242-SPDB-DM4 : ▲
huC242-SPDP-DM4 : ▼
huC242-SPDB-DM3 : ■
huC242-SPPDM1 : ●

Bioconjugate Chem. **2011**, *22*, 717–727

in vivo efficacy of AMCs



A: COLO205 model

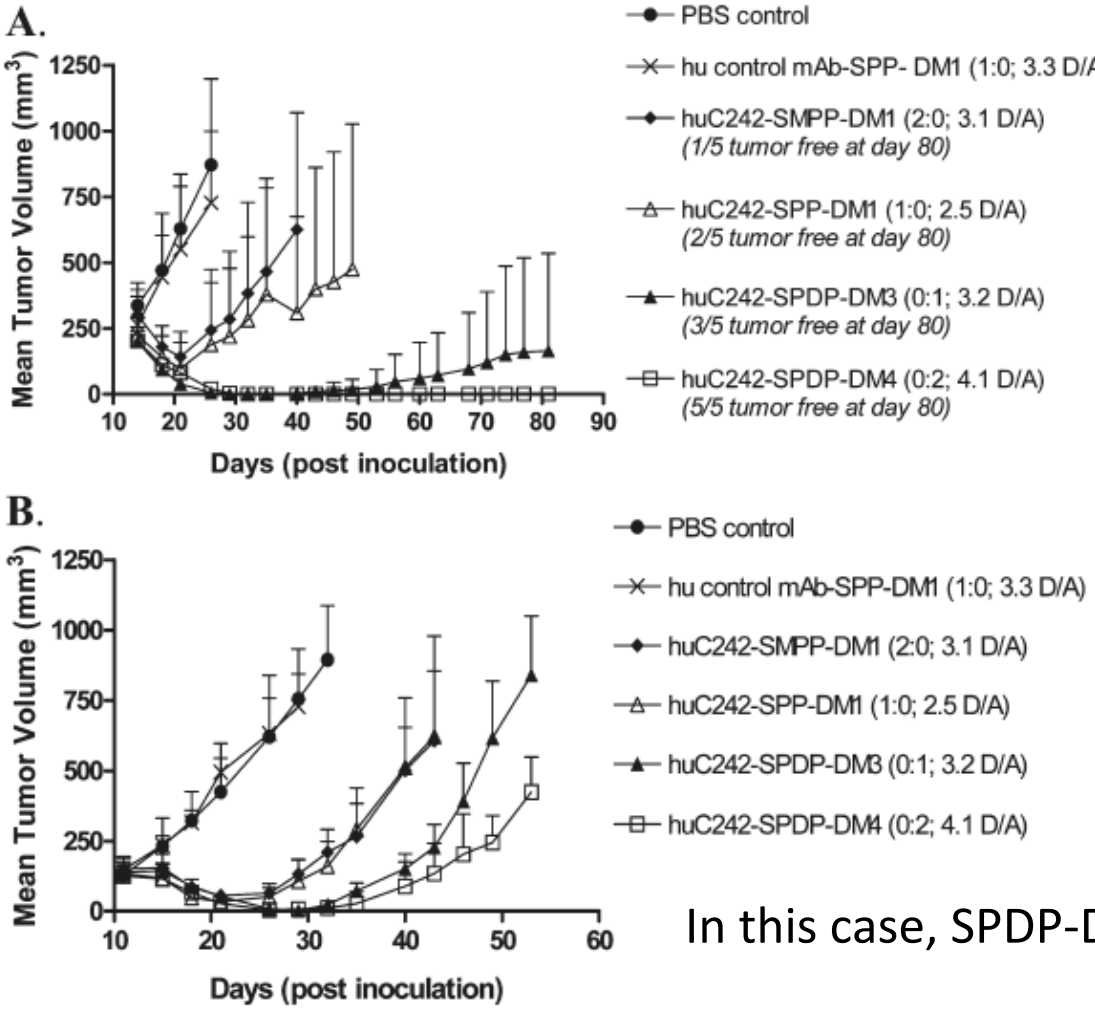
B: HT29 model

Using ADC linked with huC242 (anti-CanAg) antibody

SPP-DM1 linker was most active

Bioconjugate Chem. **2011**, *22*, 717–727

a second set of efficacy studies



A: COLO205 model

B: HT29 model

Using ADC linked with huC242 (anti-CanAg) antibody

In this case, SPDP-DM4 was most active

This result arises not from the stability profile but the increased activity of the DM4 metabolite

Bioconjugate Chem. **2011**, *22*, 717–727

Direct disulfide-bonding between engineered cys residues

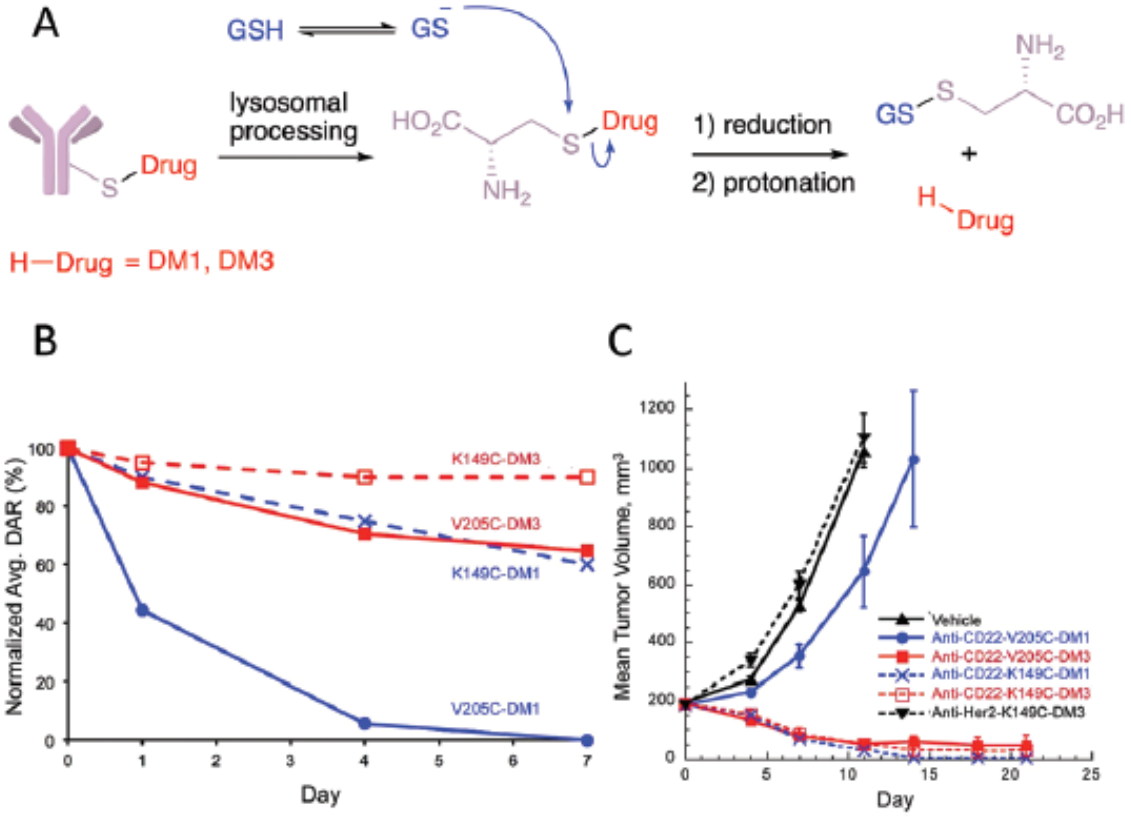


Fig. 9 (A) Metabolism of a linkerless, cysteine-linked maytansinoid ADC (B) *in vivo* stability of the conjugates in healthy mice dosed at 3 mg kg^{-1} (C) antitumour activity of the conjugates in a CD22-xenograft model, dosed at 3 mg kg^{-1}. K149C and V205C refer to the hindered and unhindered attachment sites respectively. Reproduced from ref. 17 with permission from the Royal Society of Chemistry.

Without excessive steric protection around the payloads, excellent *in vivo* mouse plasma stability

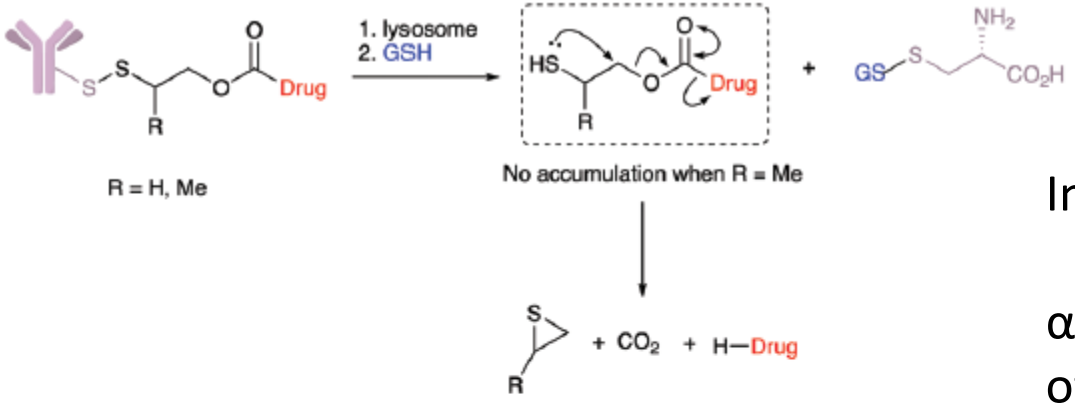
The stability was highly site-dependent

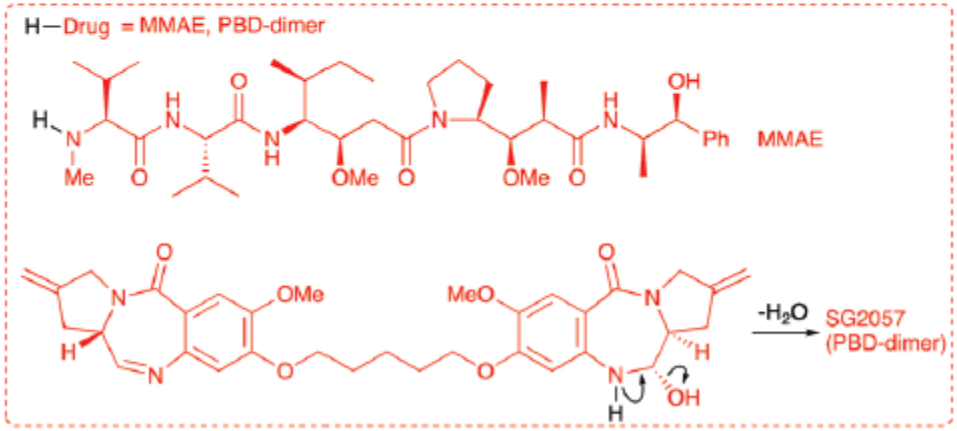
The sterically protected DM3 payload gave the predicted rise in stability over DM1

Chem. Sci., **2017**, *8*, 366–370

Traceless disulfide-carbamate technology

- Novel self-immolating carbamate linker
- This technology allows the benefits of disulfide likers to be applied to a wider range of potential payloads





Increasing stability to reduction

α-methyl group increased the rate of self-immolation with increasing stability to reduction

Fig. 10 Disulfide reduction and immolation of a disulfide-carbamate, releasing amine-linked drugs.

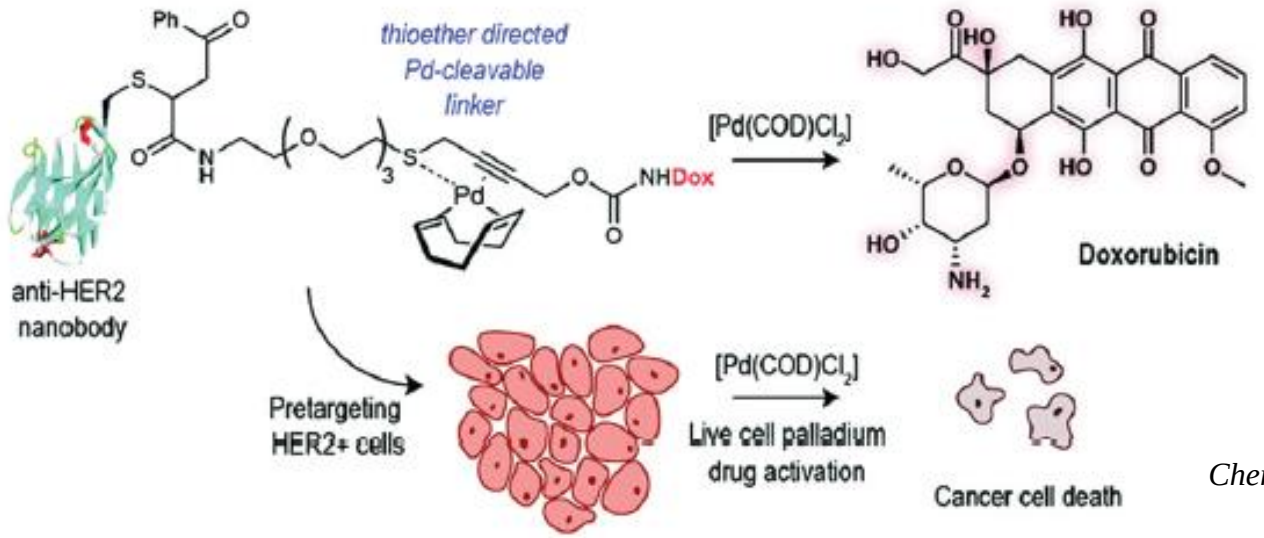
Mol. Cancer Ther., **2017**; *16*, 871-878

Cleavage by exogenous stimuli

Advantages of extracellular drug release

- Avoidance of any disparity in linker cleavage rates caused by variable biology across patients
- The ADCs can be effective when extracellular concentrations of endogenous triggers are insufficient for payload release

Thioether-containing linker



Chem. Sci., **2018**, *9*, 4185-4189

Fig. 11 Mechanism of action of a Pd^0-labile ADC. Reproduced with permission from ref. 26 with permission from the Royal Society of Chemistry.

Under exposure to Pd^0, this liker releases an amine-linked payload

In the presence of the $[Pd(COD)Cl_2]$, the ADC was potent

The concentration of Pd required fro efficient decaging was non-toxic in kidney cells, but it is unlikely to be tolerated *in vivo*

Trans-cyclooctene linker

Diels-Alder reaction with an externally administered tetrazine
Releasing an amine-containing payload *in vivo*

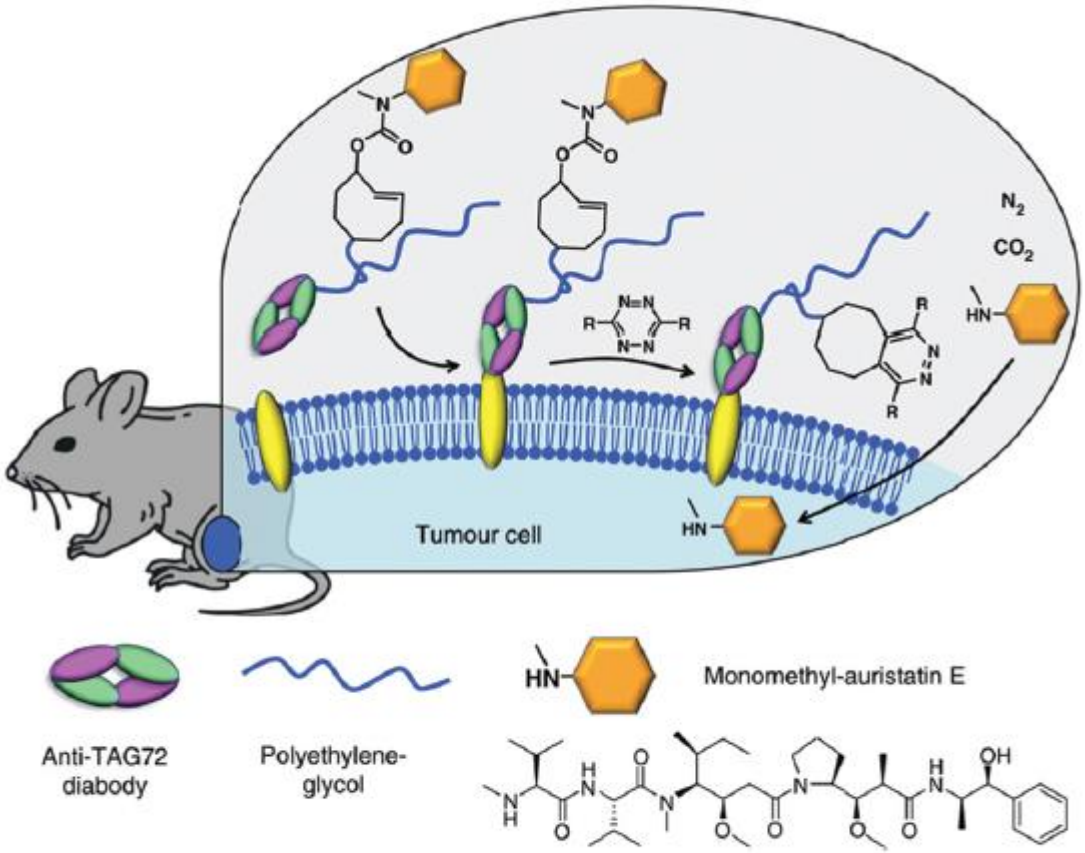


Fig. 12 The mechanism of action of a *trans*-cyclooctene-containing ADC, cleaved by a IEDDA reaction. Reproduced from ref. 28, used under license CC BY.

Nat. Commun.., **2018**, *9*, 1484

The tetrazines with fast kinetic profiles were unsuitable for use on their own, due to their rapid plasma clearance rate($t_{1/2}$≈1 min.)

partially proved this problem by employing a pegylated 3-methyl-6-trimethylenetetrazine linked to a chelated lutetium(III) species

Instability of the trans-cycloocten-Dox conjugate to alkene isomerism was observed, but this adverse effect on the therapeutic window was not severe

Enzyme cleavable linkers

- Dipeptide-containing linkers
- Glycosidase-cleavable linkers
- Phosphatase-cleavable linkers

Dipeptide-containing linkers

Dipeptide-containing linkers are present in the majority of ADCs

Previously known lysosome cleavable tetrapeptides **Gly-Phe-Leu-Gly** and **Ala-Leu-Ala-Leu** were unsuitable for prodrug or ADC applications due to their slow release kinetics, hydrophobicity and complexity

The carboxydipeptidase activity of cathepsin B enables to cleave dipeptide linkers

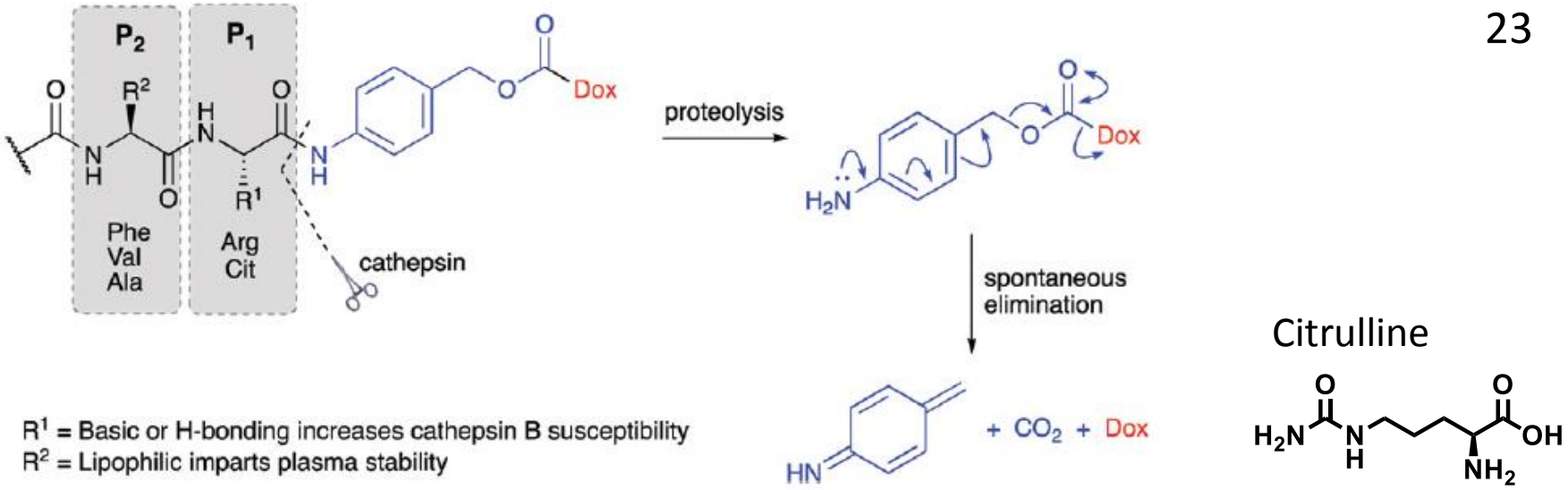


Fig. 13 The structure and cleavage mechanism of the Aaa-Aaa-PABC-Dox motif.

General trend

- A hydrophilic residue at P_1 is required and hydrolysis rates increase with basicity
- Citrulline(Cit) is isoelectronic with Arg, and was preferred to Arg due to synthetic ease
- The hydrophobic residues Phe, Val and Ala at P_2 enable cleavage by cathepsin B with plasma stability

Due to the steric bulk of the Dox payload, a spacer unit was required for enzymatic activity

A *p*-aminobenzyl carbamate(PABC) linkage was used as a self-immolative spacer

Dipeptide-containing ADCs

More selective and potent than acid-cleavable hydrazone-containing ADC

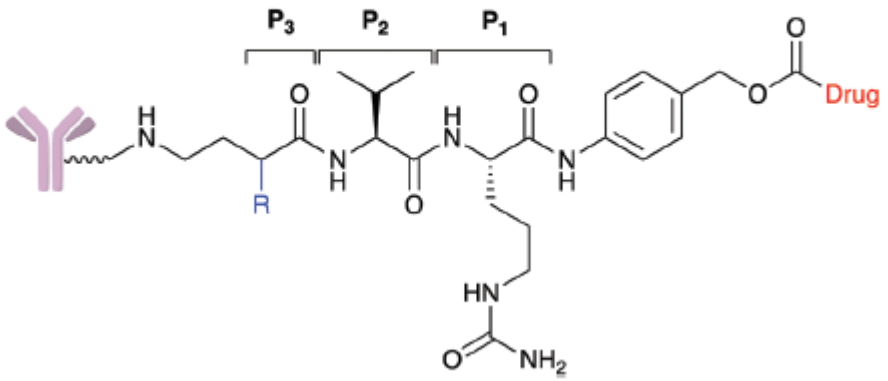
Phe-Lys-PABC and **Val-Cit-PABC** containing linkers were successfully applied to ADCs

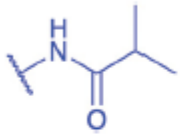
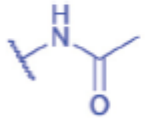
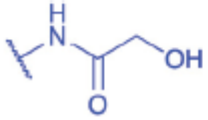
When incubated in isolated human plasma, they exhibited long half-lives, but **in mouse plasma the conjugates were less stable**

The enzyme responsible for the instability **was not cathepsin but carboxyl esterase 1C(Ces1C)**

Improving the mouse plasma stability of Val-Cit

Table 1 The stability of modified Val-Cit-PABC-containing ADCs in rodent plasma. The shaded entry signifies the linker used in an ADC that outperformed the unmodified variant in a mouse xenograft model



R group	% Stability after 4.5 days	
	Mouse plasma	Rat plasma
	0	75
	5	94
	65	96
	84	97

The addition of hydrophilic P_3 residues at the N-terminus increased the mouse plasma stability of the ADC, without negatively affecting cathepsin B-cleavage

Tripeptidic linkers

Tripeptidic linkers with more hydrophilicity at P_3 to eliminate cleavage by Ces1C and provide improved aqueous solubility

P_3 = Ser gave only slightly increased stability and P_3 = Lys reduced stability over the native Val-Cit, the use of acidic Glu and Asp residues dramatically increased linker stability, with Glu slightly outperforming Asp

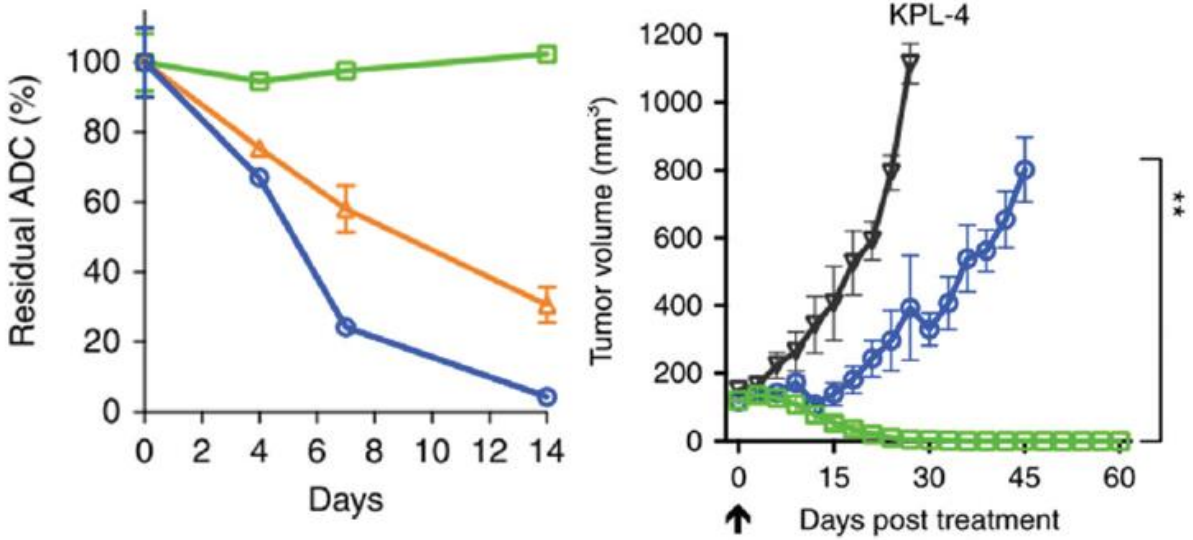


Fig. 14 The *in vitro* mouse plasma stability and *in vivo* antitumour activities of Glu-Val-Cit (green) and Val-Cit (blue) containing ADCs, stability data for the Ser-Val-Cit ADC (orange) is also included. Reproduced from ref. 33, used under license CC BY.

Nat. Commun.., **2018**, *9*, 2512.

Approach to specifically target cathepsin B

Removal of the P_1–P_2 amide bond, as well as replacing the P_2 residue, eventually settling with the cyclobutane-1,1-dicarboxamide (cBu) moiety

The cBu-Cit moiety is more selective towards cathepsin B than Val-Cit and does not require other proteases in order to effectively release the payload

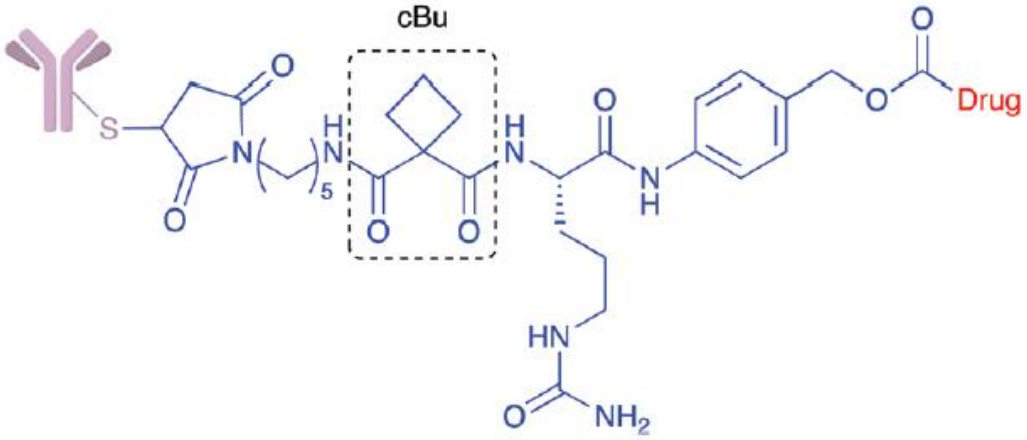


Fig. 15 The structure of the cBu-Cit-PABC-containing ADCs where drug = MMAE or PBD-dimer.

J. Med. Chem.., **2018**, *61*, 989-1000.

The Val-Ala motif

the Val-Ala moiety has also emerged as an effective protease-cleavable group

In the case of Val-Ala, 7.4 drugs per mAb were loaded without significant aggregation(>10%)

Val-Cit-containing linker could not reach a DAR >4 because of excessive aggregation and precipitation

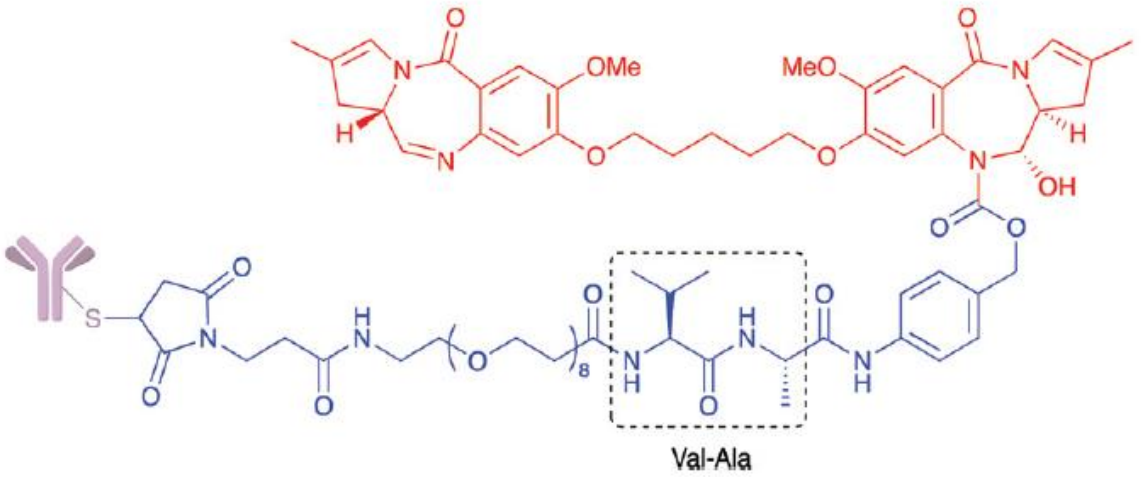


Fig. 16 The structure of rovalpituzumab tesirine.

Med. Chem. Lett.., **2006**, *16*, 358-362.

Glycosidase-cleavable linkers

β-Glucuronidase-cleavable linkers

β-Glucuronidases are hydrolytic lysosomal enzymes in the glycosidase class that catalyse the breakdown of β-glucuronic acid residues in polysaccharides

Attachment to the antibody was achieved by substitution of an amide bond ortho- to the enzyme-cleavable group

Crucially, the conjugates were highly stable in isolated rat plasma, with an extrapolated half-life of 81 days, far superior to the dipeptidic linkers

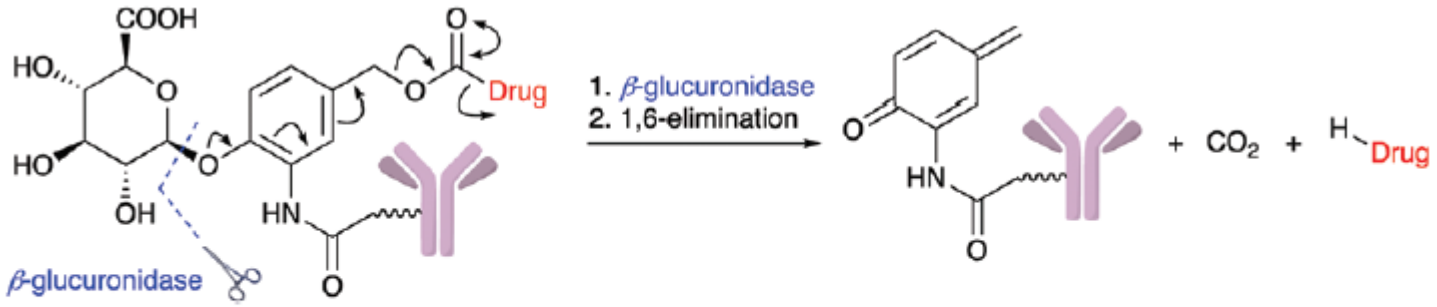


Fig. 18 The structure and release mechanism of β-glucuronic acid-containing ADCs.

Bioconjugate Chem. **2009,** *20,* 1242–1250

Novel linker releasing alkyl alcohol-linked payloads

a methylene-alkoxy group was added to the PAB spacer to allow the release of alkohols

N, *N*-dimethyl ethyl group was stable at pH 7.4 and efficiently eliminated the payload upon enzyme hydrolysis

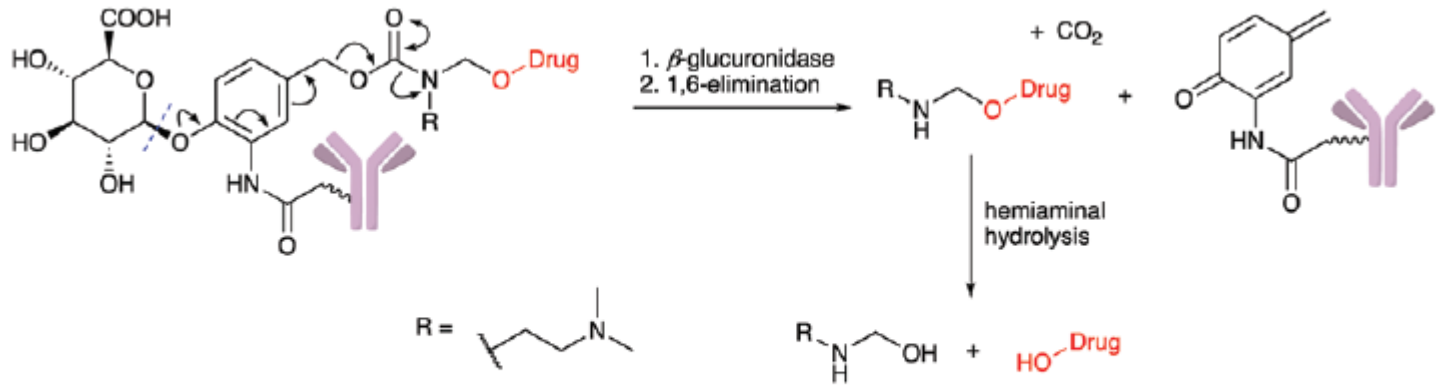


Fig. 19 The structure and release mechanism of a methylene-alkoxy-containing β-glucuronidase-cleavable ADC.

Angew. Chem. Int. Ed., **2016,** *55,* 7948-7951

β-Galactosidase-cleavable linkers

The enzyme is analogous to β-glucuronidase in its hydrolytic activity, but instead hydrolyses β-galactoside.

The authors found that this type of linker, when used with trastuzumab and MMAE, was more potent than the Val-Cit-PABC analogue

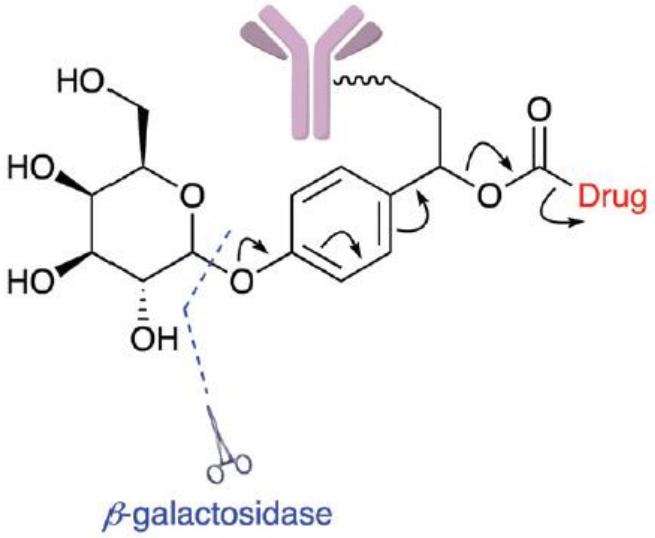


Fig. 20 The structure and release mechanism of an β-galactoside-containing ADC.

Eur. J. Med.. **2017**, *142*, 376-382

Phosphatase-cleavable linkers

Lysosomal acid pyrophosphatase and acid phosphatase are enzymes that hydrolyse pyrophosphates and terminal monophosphates respectively to their parent alcohols in the lysosome

Targeting these enzymes for drug release offers two distinct opportunities

- the substrates are naturally highly hydrophilic

- alkyl alcohol payloads can be released

Dipeptide-phosphate-containing ADC

Upon lysosomal proteolysis of the Val-Cit group, the PAB unit was able to eliminate a terminal monophosphate

The terminal phosphate was hydrolysed by acid phosphatase upon elimination, releasing an alcohol payload

Plasma stability studies were limited to only six hours, insufficient for the lifetimes associated with ADCs

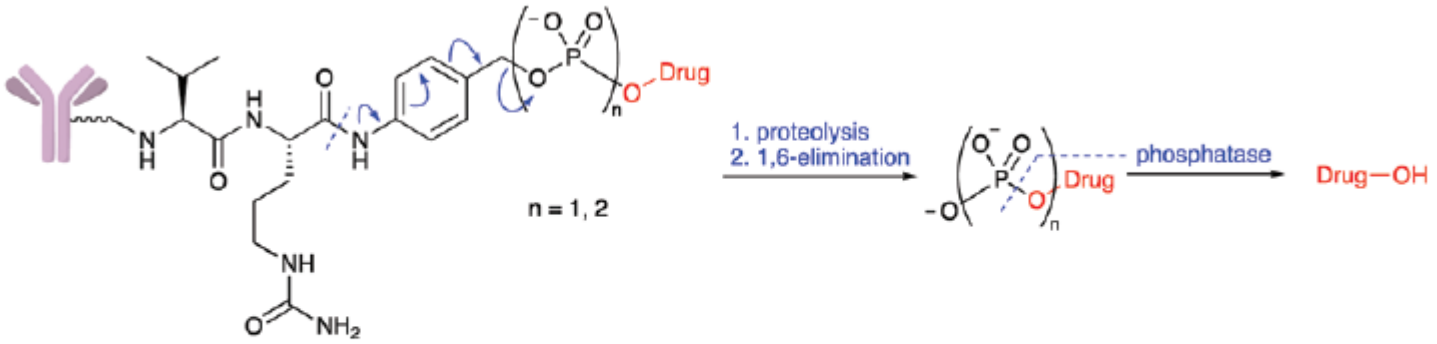


Fig. 21 The structure of cleavage mechanism of a dipeptide-phosphate-containing ADC linker.

Bioconjugate Chem. **2016**, *27*, 2081−2088

Pyrophophate-containing ADCs

The linkers were hydrolysed in lysosomal extract media, first releasing the monophosphateappendaged alcohol payload, then the unfunctionalised glucocorticoid drug

Slow drug release was improved by introducing an acetal spacer

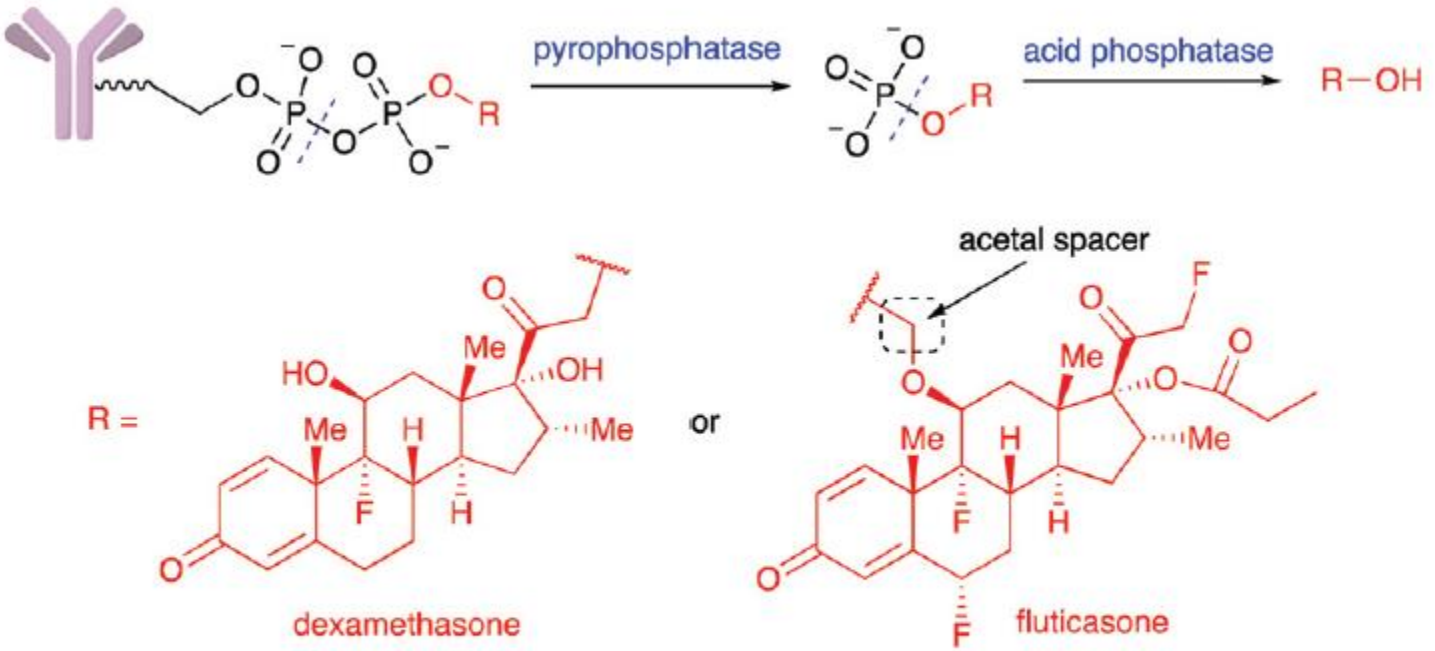


Fig. 22 The structure of cleavage mechanism of pyrophosphate-containing ADCs bearing glucocorticoid payloads.

J. Am. Chem. Soc., **2016**, *138*, 1430-1445

Summary

- Suitable linker technology at the payload attachment site is required for safe and efficacious ADCs
- Cleavable linkers are the preferred choice in the treatment of cancer due to the importance of bystander killing and payload cytotoxicity
- Acid cleavable ADC linkers were initially promising but stability requirements has reduced their value
- The vast majority of current ADCs are disulfide or dipeptide technologies due to their ability to distinguish between plasma and target cell conditions

- Non-dipeptide based enzyme-cleavable linkers have not yet been clinically validated despite their encouraging preclinical results

- Glycosidase- and phosphatase-cleavable linkers have exhibited excellent solubility and stability

Appendix

Ozogamicin
N-acetyl calicheamicin γ_1^I derivative
DNA cleavage activity

Vedotin
monometyl auristatin E ; MMAE
microtubule inhibitor

Emtansine
maytansinoid derivative
Microtubule inhibitor

SN-38
active metabolite of irinotecan
DNA topoisomerase Ⅰ inhibitor

PBD dimer
DNA binding activity

Doxorubicin
anthracycline antibiotics
DNA intercalational compound

Dexamethasone, Fluticasone
Steroidal Anti-Inflammatory Drugs(SAIDs)

Intracellular disulfide metabolism

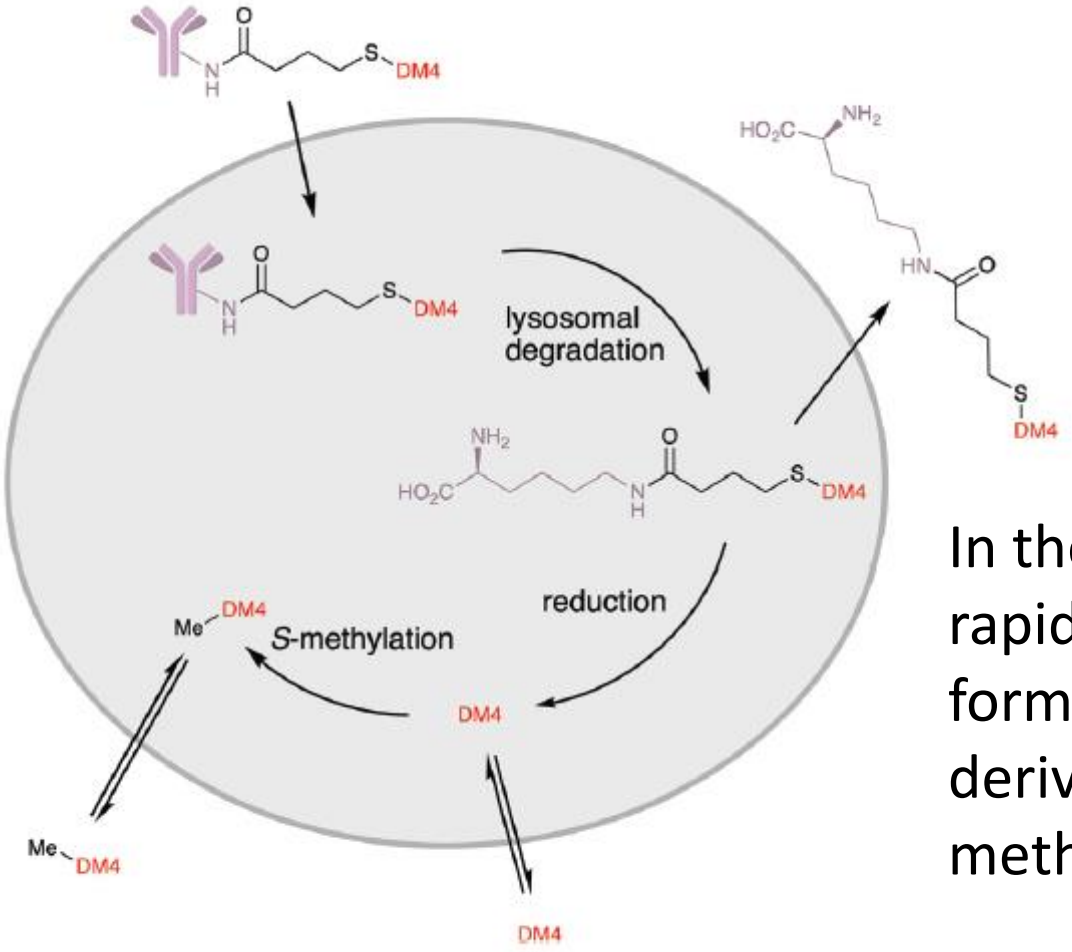


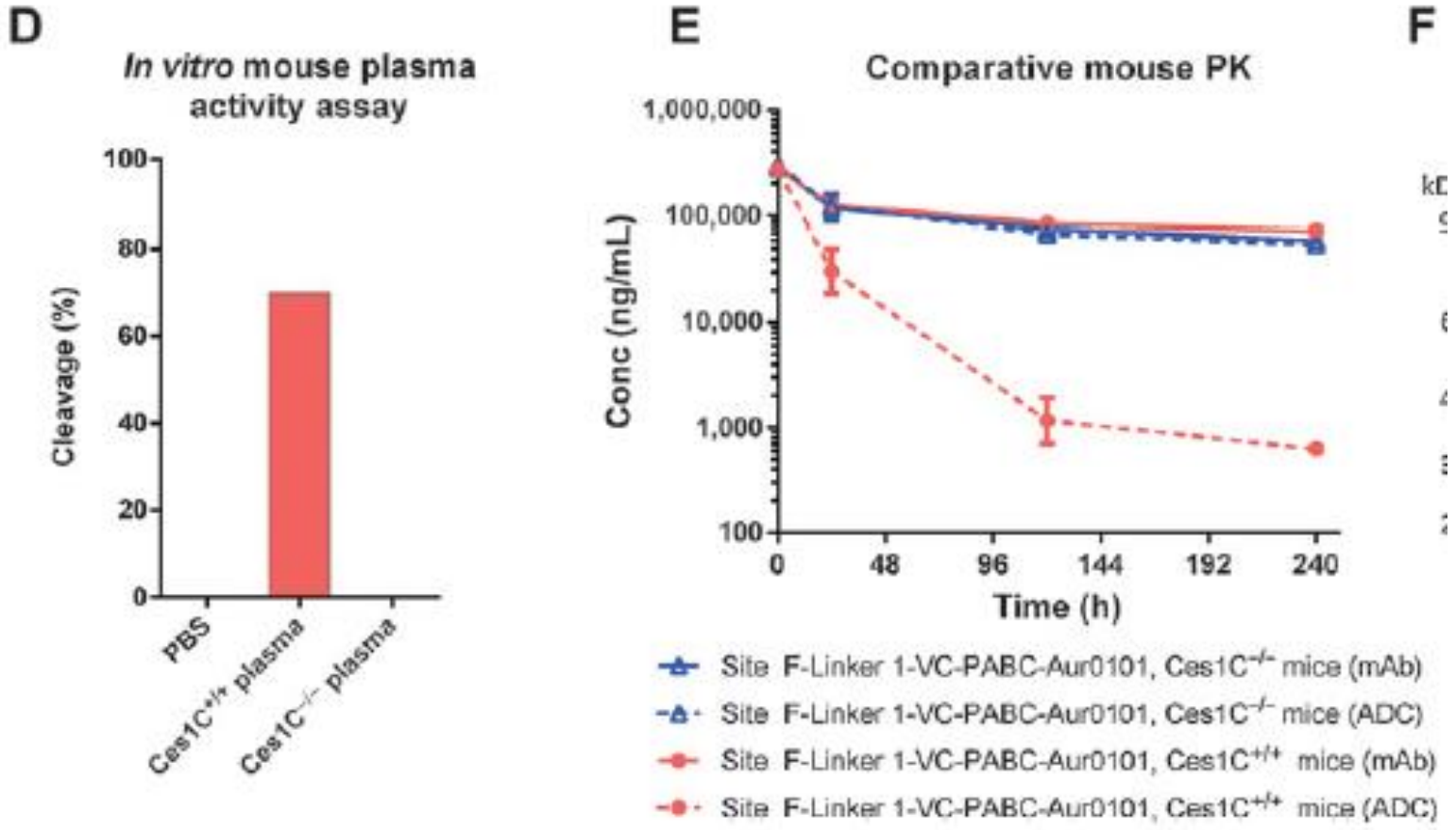
Fig. 8 Intracellular metabolism of a CanAg-DM4 ADC.

Bioconjugate Chem. **2010,** *21,* 84–92

In the case of DM4 payload, rapid *S*-methylation occurs to form the potent thioether derivative by an intracellular methyltransferase

DM1 payload is not significantly *S*-methylated after 8 hours

Ces1 is the enzyme responsible for the instability of Val-Cit linkers



Understanding the role of cathepsin B

Cathepsin S is the most active enzyme in the family to a Val-Cit containing ADC