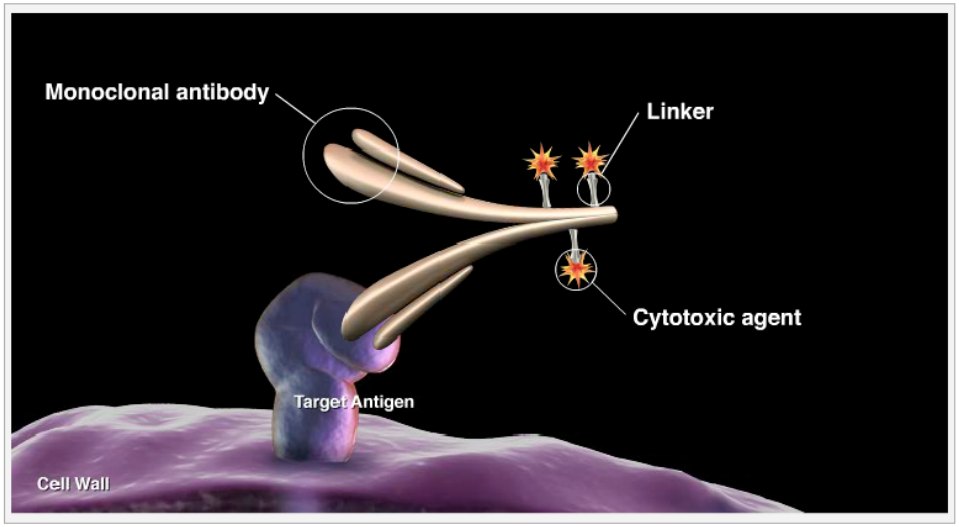
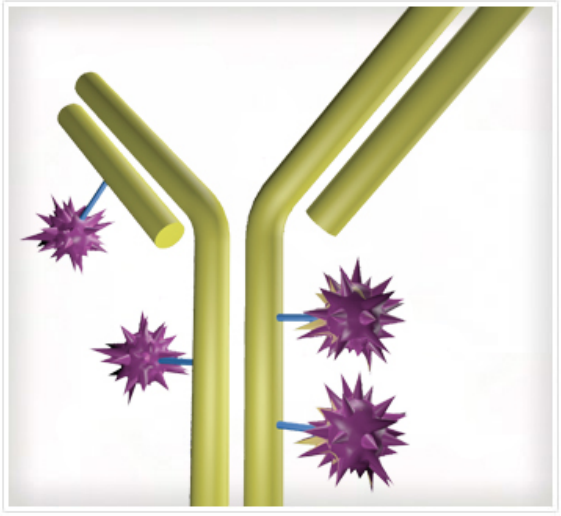
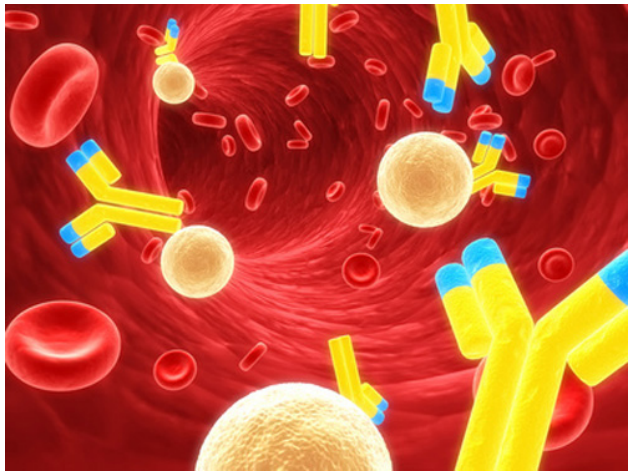


Antibody-Drug Conjugates



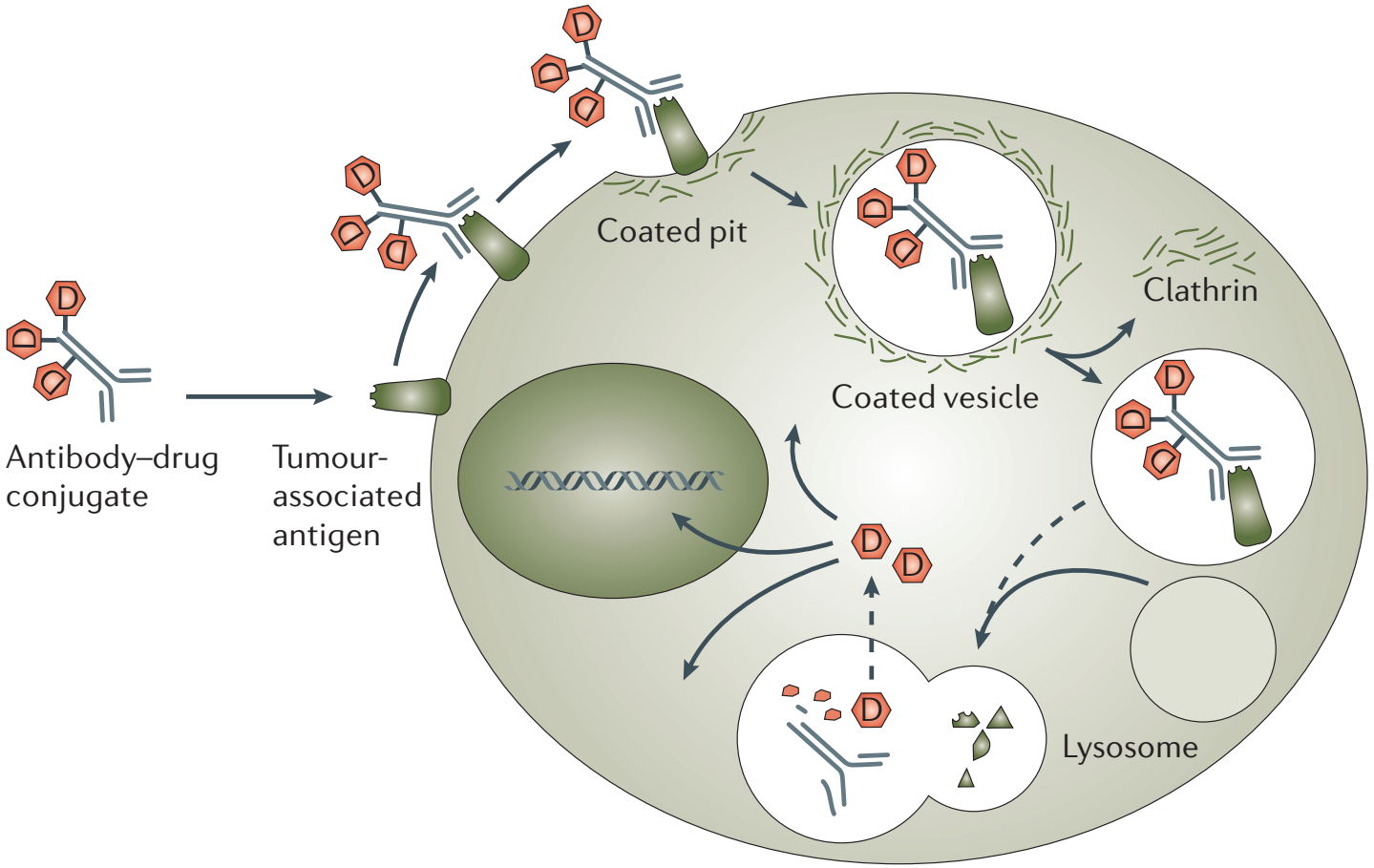


Immunoconjugates

- Monoclonal antibodies (mAbs) coupled to highly toxic agents (radioisotopes and toxic drugs) that bind to specific antigens on cells and deliver chemotherapy
- Radioimmunoscintigraphy uses gamma carriers to target delivery of beta emmiters. Radioisotopes include ^{90}Y and ^{131}I
- ADCs are combinations of a cytotoxic drug, a target-specific mAb, and a linker connecting the drug to the antibody
  - Proposed in the 1970s and pursued with moderate success
  - renewed interest and greater success in the last five years
  - About 25 ADCs currently in clinical trials[1]
- Designed to optimise the therapeutic window (gap between the effective dose and the toxic dose)
- Mostly directed against solid tumours (but some hematologial)

1. Teicher, B. A.; Doroshow, J. H. **The Promise of Antibody–Drug Conjugates** *N Engl J Med* **2012**, *367*, 1847–1848.

Receptor mediated endocytosis



Schrama, D.; Reisfeld, R. A.; Becker, J. C. *Nature Reviews Drug Discovery* **2006**, *5*, 147–159.

Challenges

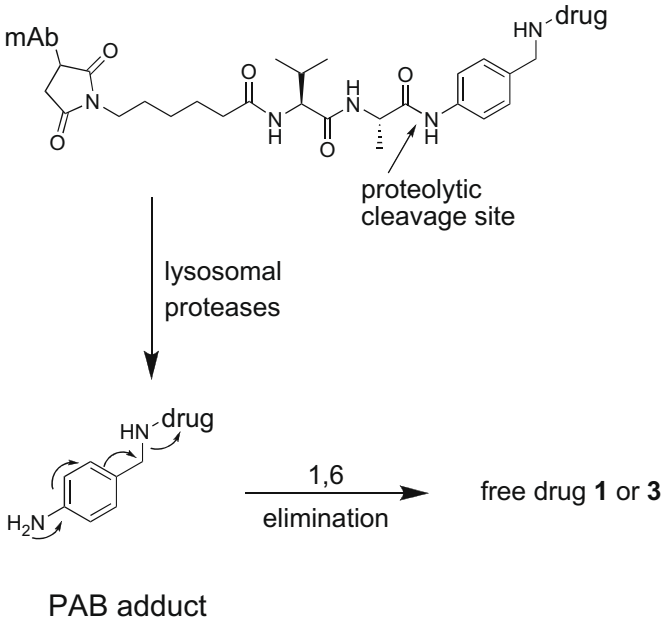
- Physiological barriers: tumour extravasion and penetration[2]
  - More time in the blood means increased likelihood of cleavage occurring early
- mAb immunogenicity: body's reaction to non-human antibodies
  - Use fully human mAbs that are protein engineered or created in transgenic mice
- Normal tissue expression of the targeted antigen: killing host cells in addition to cancer
  - Need to to identify antigens overexpressed specifically on cancer cells
- Low drug potency: inefficiency of targeting the tumour necessitates the use of very toxic drugs
  - requires specific dosing and linkage as early release of the drug in blood can cause toxicity
- Drug release: inefficient and drugs not in active states
  - mAbs can have short half-lives or may lack efficient interaction with the target
- Linker choices: need correct stoichiometry, location and chemistry of linker--cleavage by enzymes in the cell or in certain acidic or reductive conditions.
  - conjugation can change the activity of the antibody.

2. Wu, A. M.; Senter, P. D. **Arming Antibodies: prospects and challenges for immunoconjugates.** *Nature biotechnology* **2005**, *23*, 1137–1146.

ADC Linkers-types

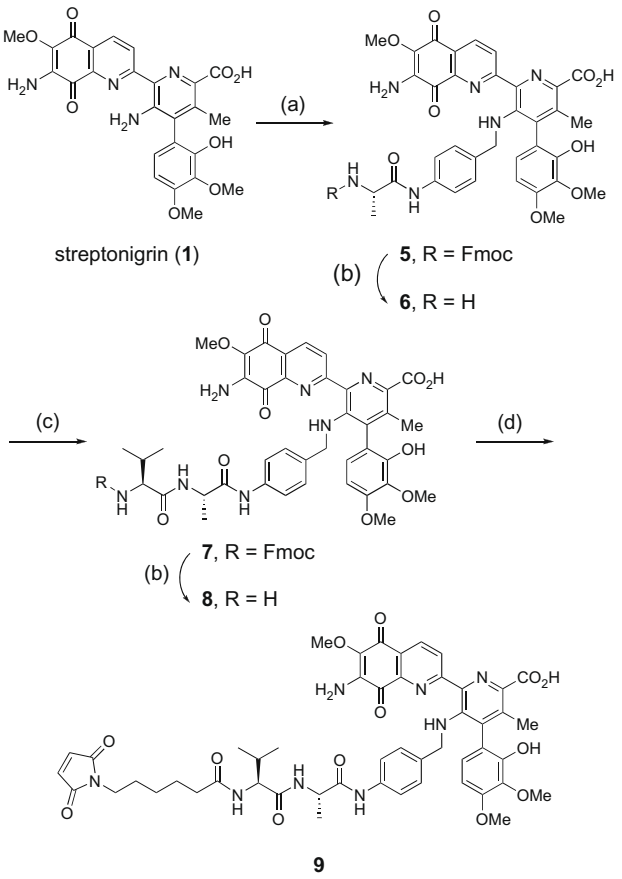
Disulfide: intracellular concentration of thiols, such as glutathione and cysteine, are much higher than those in plasma. Selectively cleaved due to a more reductive intracellular environment

Hydrazone: selectively cleaved within the intracellular compartment of lysosomes (lower pH compared to the systemic blood circulation). Hydrazones have typically been linked to antibody thiol groups generated through interchain disulfide bone reduction.



Peptide linkers: selectively cleaved by lysosomal proteases (ex: cathepsin-B). Increased serum stability and improved anti-tumor effects compared to hydrazone linkers.

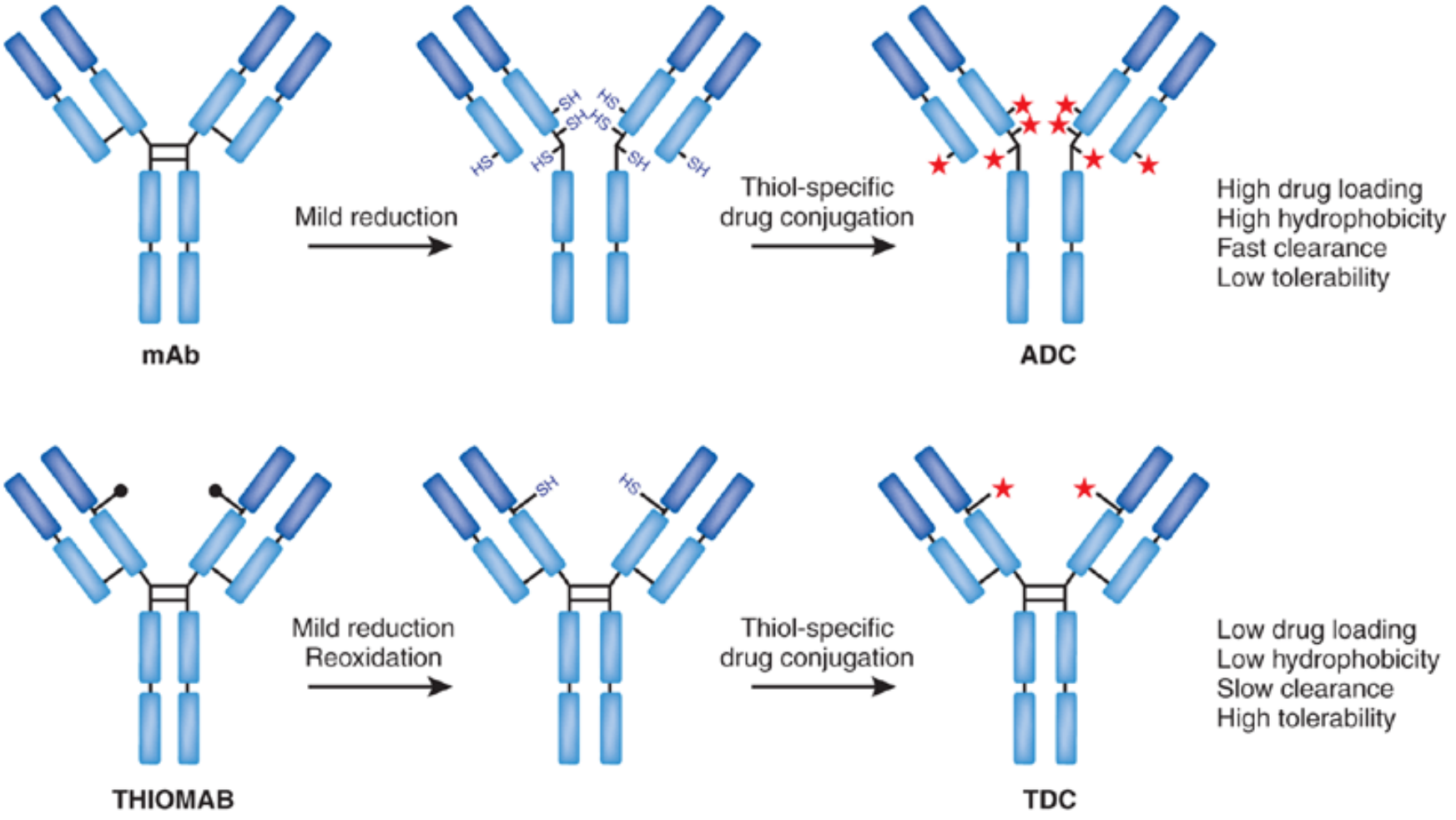
ADC linkers



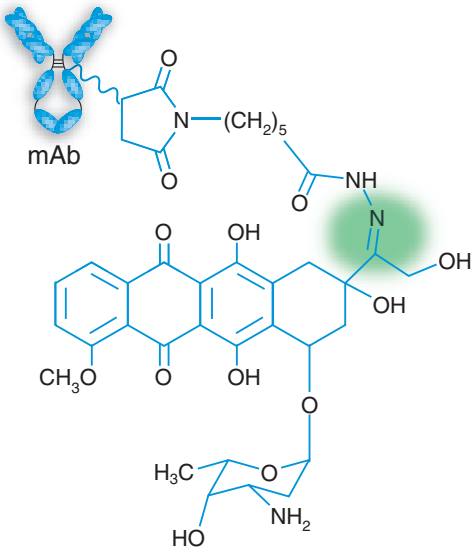
Scheme 2. Synthesis of a streptonigrin drug linker. Reagents and conditions: (a) Fmoc-Ala-PAB-Br, DIPEA, DMF, rt, 18 h, 44%; (b) DMF:piperidine (4:1), quant.; (c) Fmoc-Val-OSu, DIPEA, DMF, rt, 88%; (d) MC-OSu, DIPEA, DMF, rt, 86%.

Burke, P. J.; Toki, B. E.; Meyer, D. W.; Miyamoto, J. B.; Kissler, K. M.; Anderson, M.; Senter, P. D.; Jeffrey, S. C. *Bioorg Med Chem Lett* **2009**, *19*, 2650–2653.

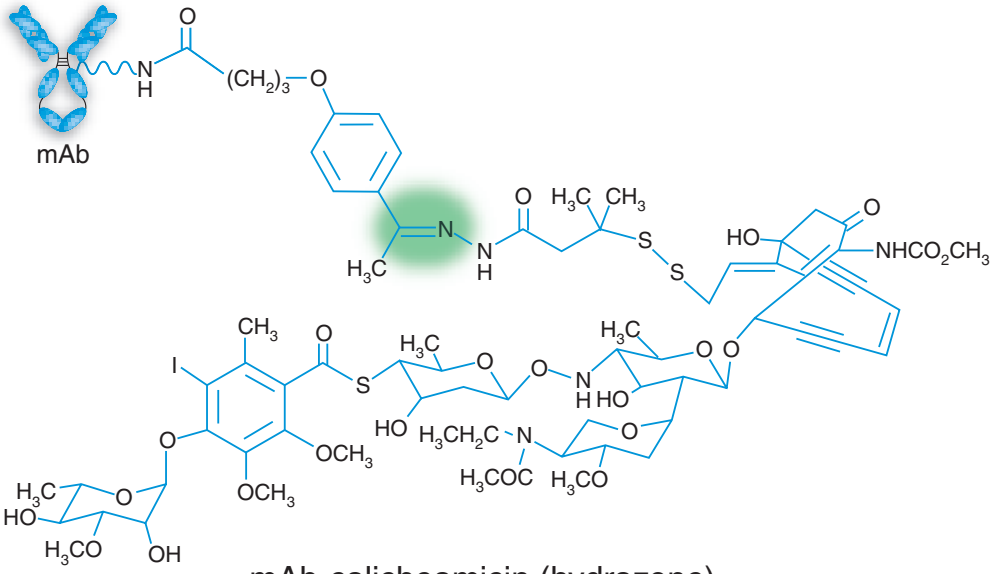
Conjugation



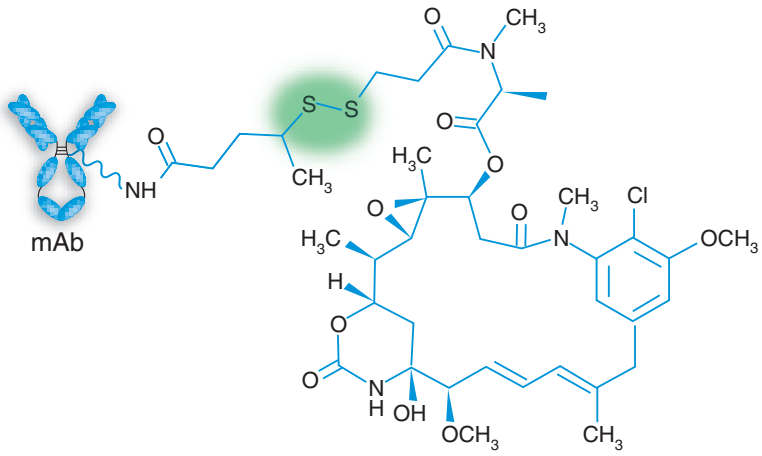
Damle, N. K. *Nat. Biotechnol.* **2008**, *26*, 884–885.



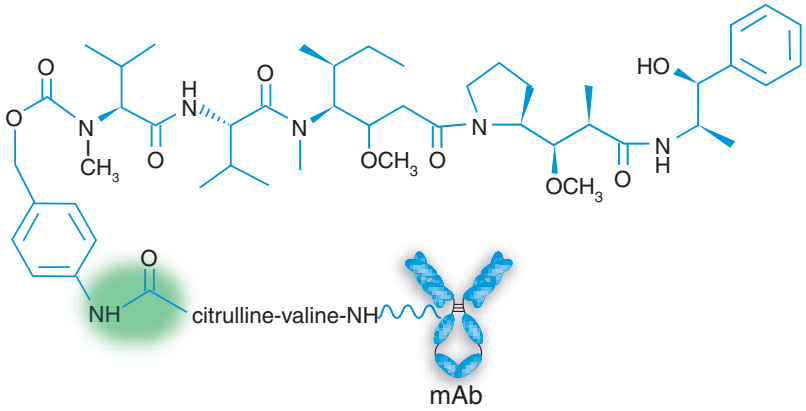
mAb-doxorubicin (hydrazone)



mAb-calicheamicin (hydrazone)



mAb-DM1 (disulfide)

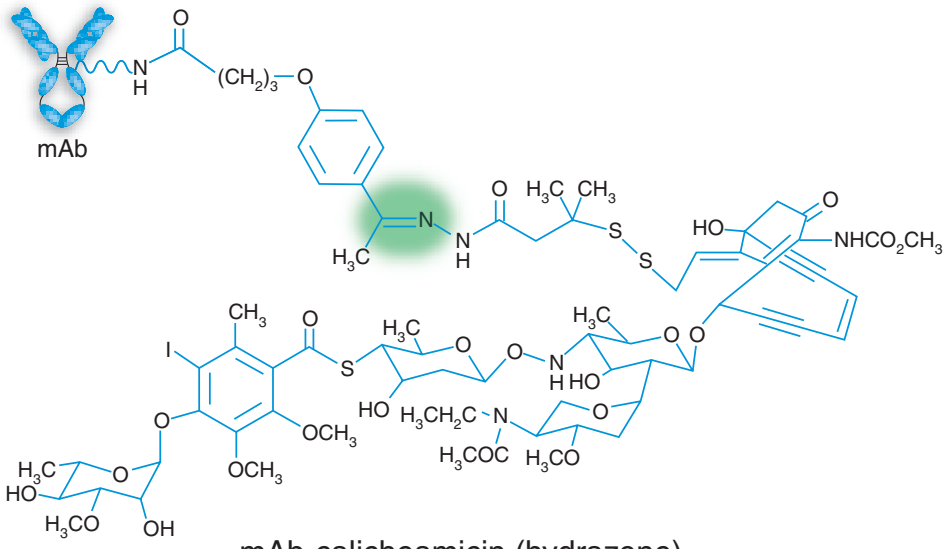


mAb-auristatin (peptide)

Katie Ris

Examples of ADCs

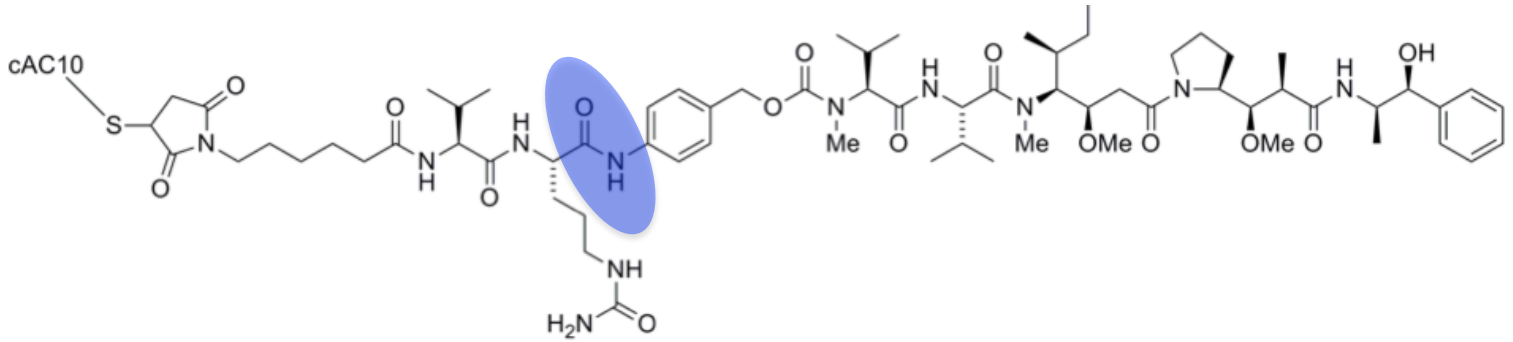
- gemtuzumab ozogamicin (Mylotarg by Wyeth)
  - Approved for acute myelogenous leukemia in 2001
  - Conjugate of gemtuzumab (CD33-specific antibody) linked to cytotoxic agent from class of calicheamicins
    - One of most potent antitumor agents known
    - Induce double-strand breaks
    - Linker is 2 labile bonds-hydrazone and sterically hindered disulfide
  - Mylotarg was withdrawn from the market in the US in 2010, but it has remained available in other markets



mAb-calicheamicin (hydrazone)

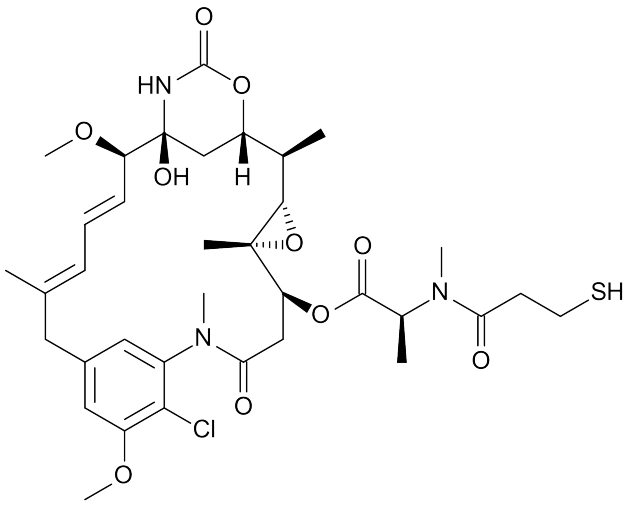
Examples of ADCs

- brentuximab vedotin (Adcetris)
  - Approved August 2011 for relapsed and refractory hodgkin lymphoma and malignant large cell lymphoma.
  - Contains brentuximab (antibody targets CD30) linked to 3-5 units of monomethyl auristatin E (antimitotic agent)
    - totally synthetic, stable, very potent, and is ideally suited for chemical modification
  - 2010 clinical trial, 34% of patients with refractory Hodgkin Lymphoma achieved complete remission and another 40% had partial remission. Tumor reductions were achieved in 94% of patients. In ALCL, 87% of patients had tumors shrink at least 50% and 97% of patients had some tumors shrinkage



Examples of ADCs

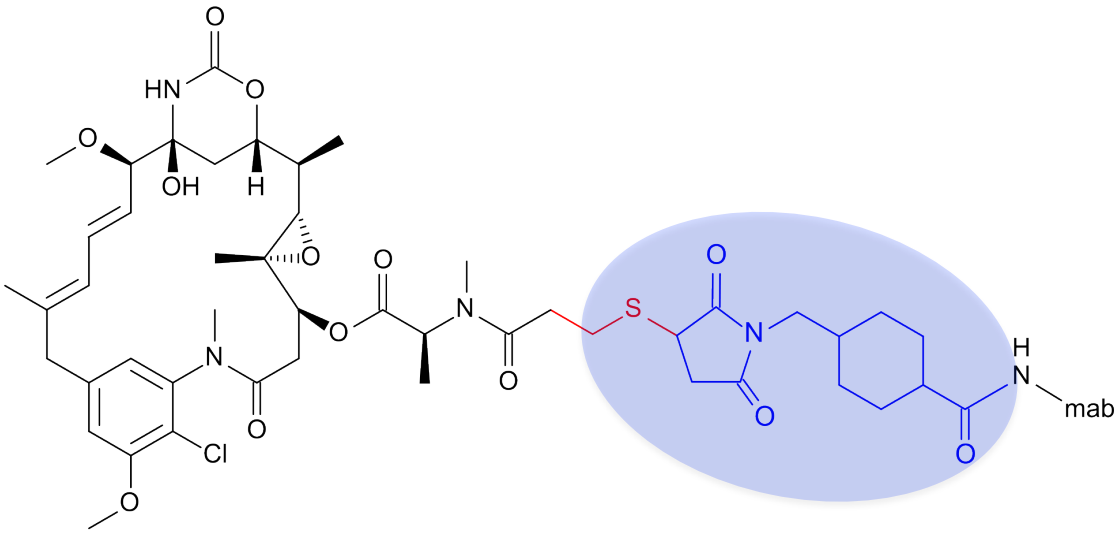
- Mertansines: derivatives of maytansine, an ansamycin antibiotic originally isolated from the Ethiopian shrub *Maytenus serrata*.
- 1000 times more potent than most clinical chemotherapies
- Analogues with labile disulfides allow reversible drug attachment and selectivity by attachment to modified lysines residues



6. Chari, R. V. J. *Acc. Chem. Res.* **2007**, *41*, 98–107.

Examples of ADCs

Trastuzumab emtansine (T-DM1)



- Roche completed Phase III Trials comparing use of T-DM1 to lapatinib plus capecitabine (reported last week) with very positive results
- Conjugate of trastuzumab (antibody) and mertansine (cytotoxic agent)
- Trastuzumab is specific for HER2 which binds to epidermal growth factor (EGF) and induces cell growth.
- HER2 is overexpressed in 20-30% of breast cancer cases and this ADC has been approved for advanced cases (where trastuzumab has already been administered).

Verma, S. S.; Miles, D. D.; Gianni, L. L.; Krop, I. E. I.; Welslau, M. M.; Baselga, J. J.; Pegram, M. M.; Oh, D.-Y. D.; Diéras, V. V.; Guardino, E. E.; Fang, L. L.; Lu, M. W. M.; Olsen, S. S.; Blackwell, K. K. *N Engl J Med* **2012**, *367*, 1783–1791.

What can we do?

- Novel linkers, new cleaving mechanisms?
  - Thiol, amide, hydrazone methodology or alternative linkage
- New, interesting drug targets?

- Collaboration and communication to enable production of new and more potent ADCs