Ynamines as Bioorthogonal Click Handles in ADC Synthesis

ADCs kill cancer cells...

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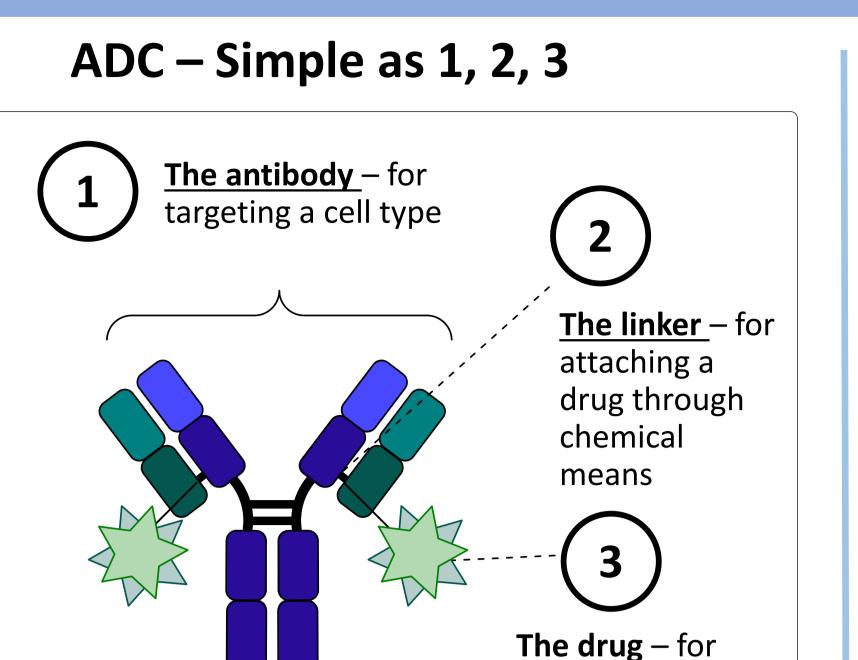
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therapeutic effect

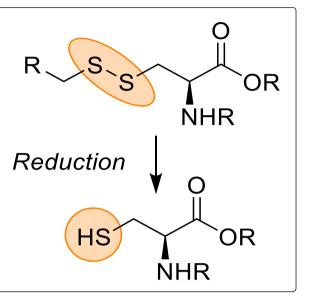




ADCs are a 'Magic Bullet' - The Future of Cancer Treatment



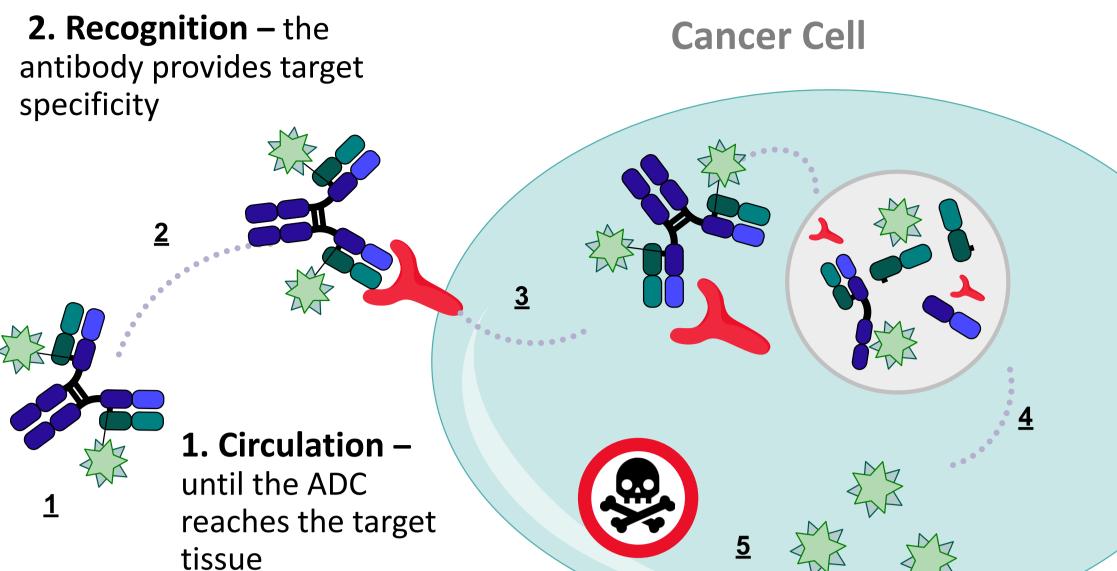
• Antibody Drug-Conjugates (ADCs) are a cutting-edge therapeutic – blending biological targeting with traditional medicinal chemistry



Amino Acid Manipulation

- Amino acids are subunits of all proteins, including antibodies
- Can be modified with biological chemistry

How does an ADC work?



3. Internalisation – the ADC is 'swallowed' into the cell

> **4. Degradation** – the ADC is broken apart to release the active drug

5. Therapeutic Action – the free drugs induce cell death

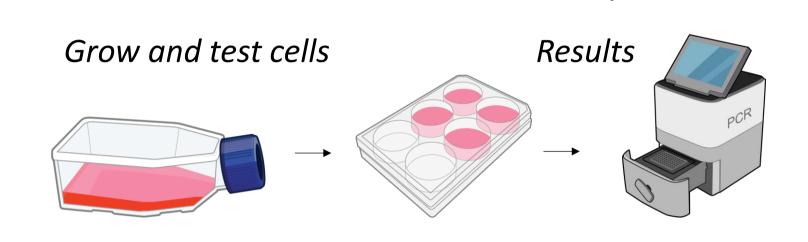
Chemotherapy



- Chemotherapy cannot distinguish between healthy and cancer cells.
- The death of healthy cells causes negative side effects for the patient

Project Aims

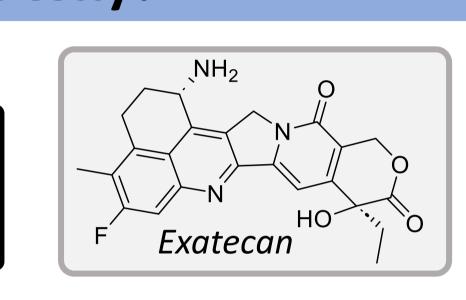
- Develop new methodology to produce ADCs
- Prove that these ADCs do not kill healthy cells



Constructing the ADC - How and Why?

Why are attachment methods important?

- The 2022 Nobel Prize winner was awarded to Meldal, Sharpless and Bertozzi for click chemistry. Morten Meldal compared click chemistry to lego building blocks - 'click them together, like snap, snap,'
- Click chemistry prevents messy reactions with undesired products and can be used in biological settings



....Healthy cells

survive

Choice of Drug

Highly potent cytotoxins are chosen for maximal cell-killing effects

Linker

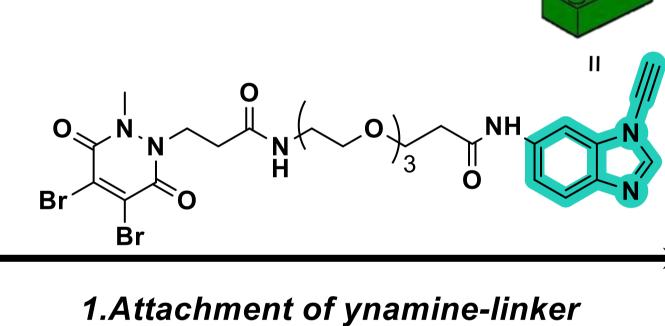
Exatecan is a chemotherapeutic which prevents DNA replication



Cysteine rebridging chemistry (reforming intermolecular bonds) to attach click handle

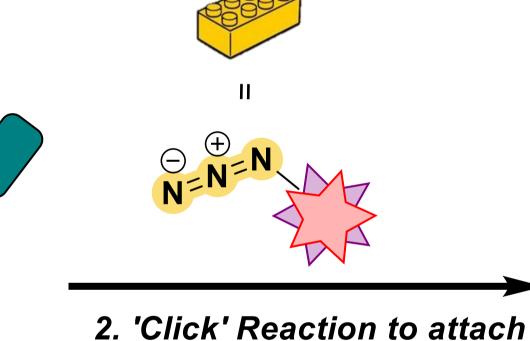
Click reaction of ynamine to attach drug

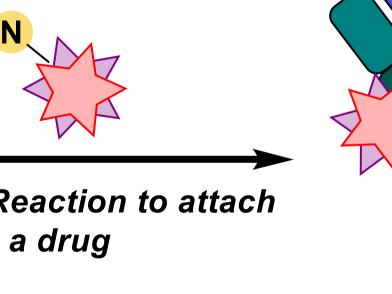
Antibody

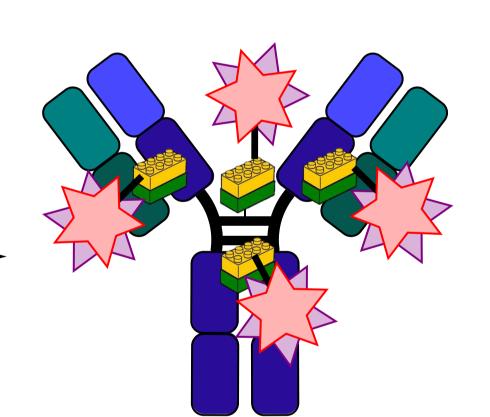


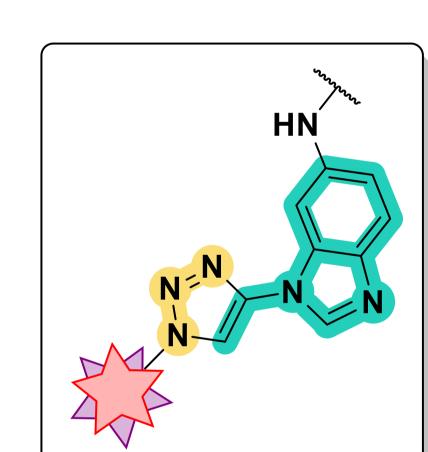
Through dibromopyridazinedione rebridging chemistry¹

Bioconjugation Strategy









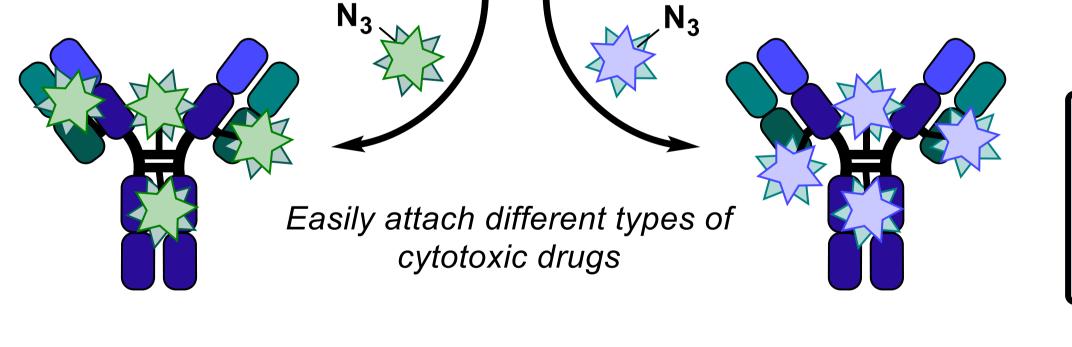
The click reaction creates a very

stable drug-antibody linker

Stable triazole linker

Bioconjugation

'a chemical strategy to form a stable link between a biomolecule and something else'



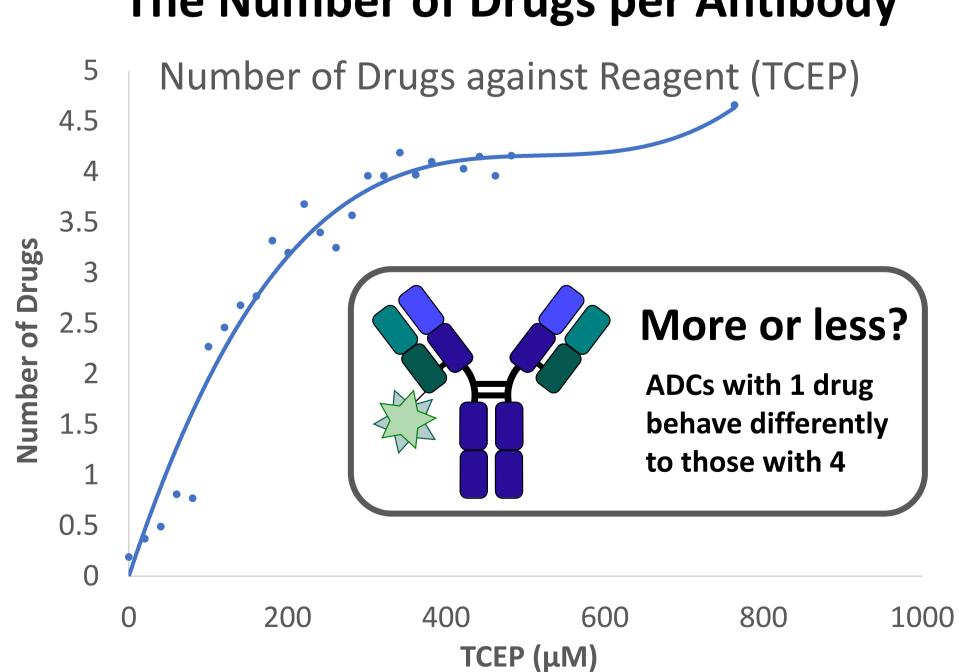
The Final ADC

• Full characterisation needed before testing in cells

Click Reactions

Require complementary reactive groups – such as the ynamine and an azide

The Number of Drugs per Antibody

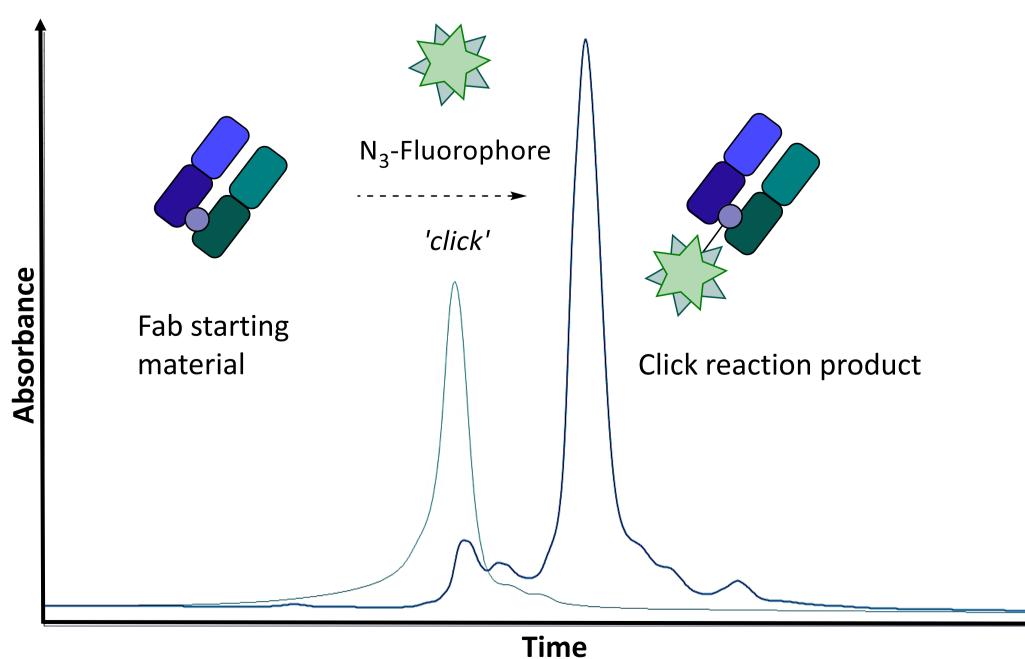


- The number of drugs per antibody has important effects on dosage, stability and therapeutic effects of the ADC
- This can be controlled by concentration of reactants during synthesis

Bioconjugation kinetics of full length mAbs Best ynamines from Fab studies used on full length antibodies (mAbs) **Fabs** Fabs - antibody fragments - provide a 'model' system for optimising reactions

Visualising Click Reactions

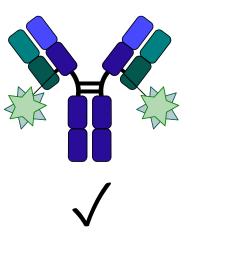
- Reaction progress can be visualised by chromatography
- ADCs have different retention times to unmodified antibodies and Fab fragments

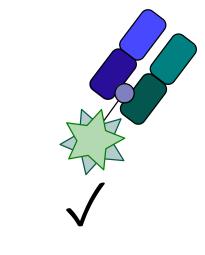


Conclusions and Future Work

Conclusions

- Development of new method to construct ADCs achieved
- Multiple ADCs and Fab conjugates synthesised, ready for testing





Testing on Live Cells

- Testing of the ADCs on both healthy cells and cancer cells.
- Cancer cells should die, healthy cells should keep growing

