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Esters in ADC linkers: Optimization of stability and lysosomal cleavage

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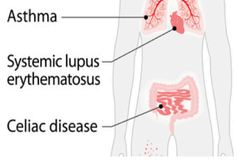
Abstract

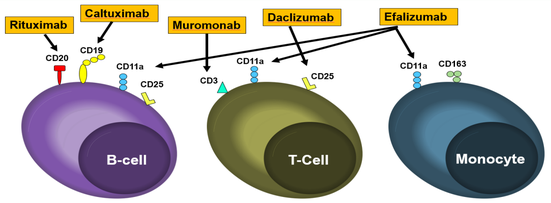
Antibody drug conjugates (ADCs) present a unique opportunity to increase the safety of highly toxic drugs by utilizing the specificity of antibodies to obtain targeted delivery of potent drugs to specific tissues. Classically, this is achieved by linking an amine-containing payload to an antibody using the well-studied mcValCitPABC linker. Yet, there are surprisingly few technologies for attaching alcohol containing payloads to an antibody. Theoretically, the simplest way to do this would be through an ester linkage. However, very little is known about the lysosomal and plasma stability of ester linkages on ADCs. Herein, we describe a number of experiments to evaluate this stability in both human/mouse plasma and in lysosomes. Furthermore, we describe the design of a peptide-linked ester that is rapidly cleaved in the lysosome.

The stability of ester linked payloads was evaluated by examining an anti-Her2 antibody bound to an ester linked dexamethasone payload. In the presence of lysosomes, the ester remained intact while the antibody backbone was proteolytically degraded. Upon incubation with SKBR3 cells, the major product released from the ADC was an ester-containing metabolite, cys-mc-Dex, thus confirming the lysosomal stability of this linkage in a cell-based system. Having demonstrated that simple esters are stable upon lysosomal uptake,we next undertook the design of a novel linker that undergoes immolation resulting in spontaneous ester-cleavage. In a model system designed to release dexamethasone, we have shown that this linker is rapidly cleaved by cathepsin B, thus resulting in the release of free dexamethasone. Furthermore, we show that the ester is stable in mouse plasma and only cleaved upon lysosomal uptake. With this research we hope to further our knowledge of ester linkages in ADCs as well as to develop a feasible way to release an unmodified alcohol-containing drug into a chosen cell type via a lysosomally-induced ester linkage.



Overall Goal

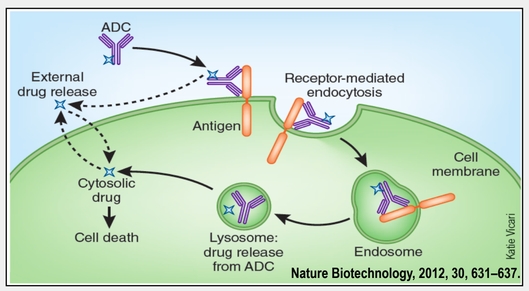
Deliver a glucocorticoid to lymphocytes using targeted drug delivery via antibody in order to increase efficacy and decrease side effects of drugs that treat autoimmune diseases



Introduction

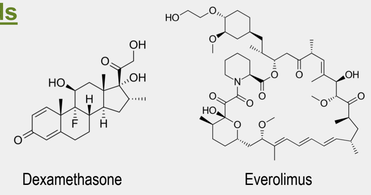
Cell Internalization

The antibody binds to the antigen of choice on the cell surface. It is then absorbed into the cell where it is trafficked to the lysosome and the drug is released.

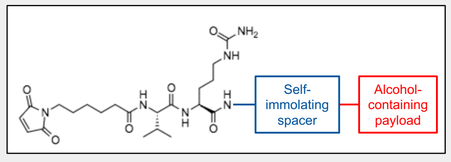


Release of alcohol containing payloads

Experimental payloads, dexamethasone and everolimus, are used to simulate glucocorticoids which typically have an alcohol handle rather than an amine handle. There is a demand for novel ways to release these alcohol-containing payloads upon lysosomal uptake.



Dexamethasone Everolimus



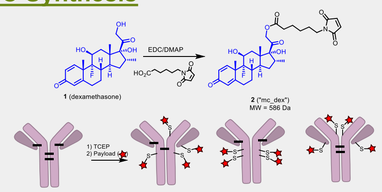
The proposed method for releasing alcohol-containing payloads is to introduce a self-immolating spacer that will spontaneously cyclize following lysosomal cleavage of the amide bond of the citrulline.

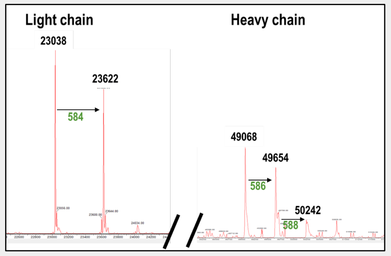
Questions

1. Can we make a linker that couples via an ester in order to deliver alcohol containing payloads?
2. Is the drug delivered to the cell and does it work?

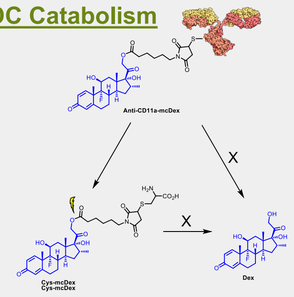
Simple Esters are Not Cleaved Upon Lysosomal Uptake

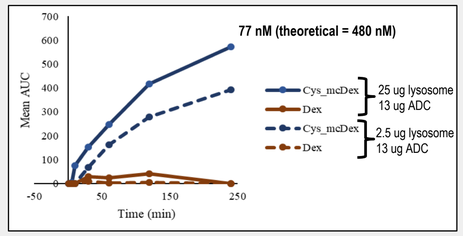
ADC Synthesis





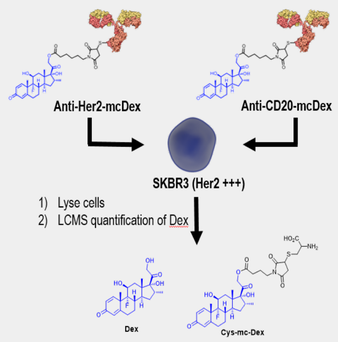
ADC Catabolism

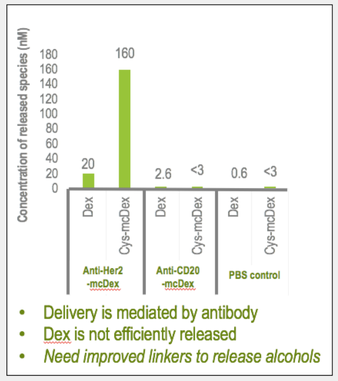




Lysosomes have little or no esterase activity.

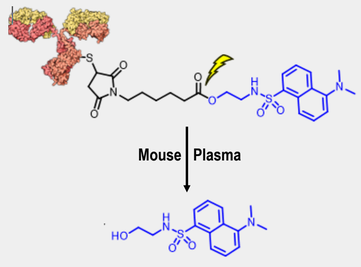
In Vitro Drug Release

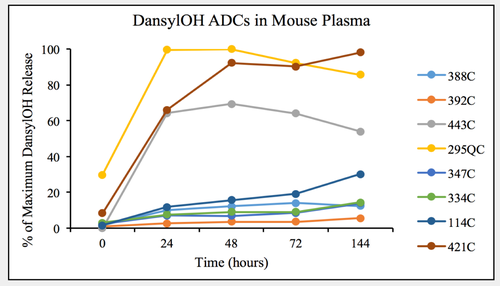




- Delivery is mediated by antibody
- Dex is not efficiently released
- *Need improved linkers to release alcohols*

Site-Specific Stability of Ester Linkages in ADCs



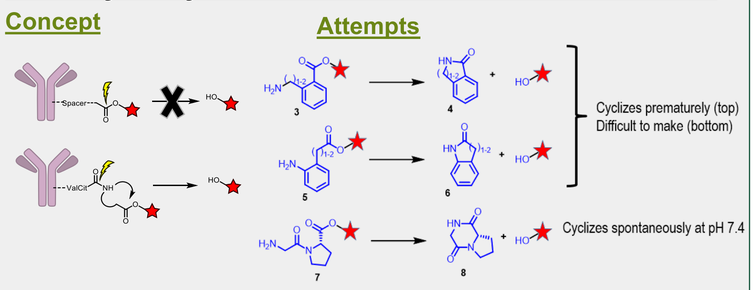


The stability of the ester containing ADCs is dependent on the site at which it is conjugated.

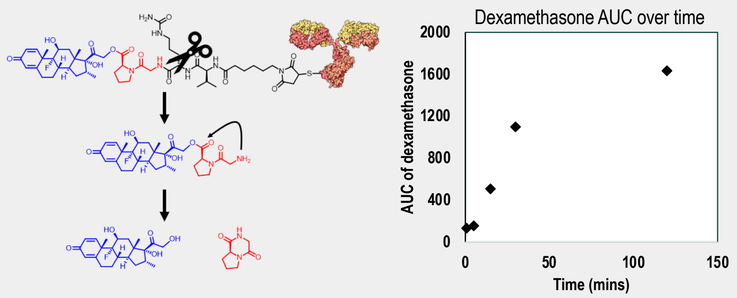
Proteolytically Cleavable Esters to Release Alcohols

Concept

Attempts



Lysosomal cleavage of the Gly-Pro Linker



This sterically constrained linker rapidly releases dexamethasone in the presence of the lysosome. This could be widely useful for the release of unmodified alcohol containing payloads.

Future Work

- Are the cleavable linkers stable in plasma?
- Is dexamethasone the best GC? (betamethasone?)
- *Can we demonstrate selective functional activity?*

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