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The Study of mpValCitGlyPro Linker for Alcohol Payload Release

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The Study of ValCitGlyPro Linker for Alcohol Payload Release

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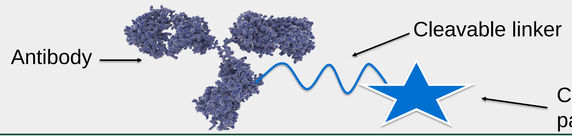
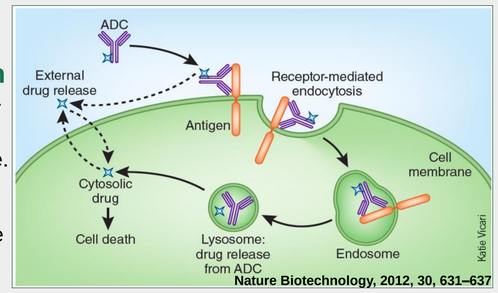
Abstract

Antibody-drug-conjugates(ADCs) are a class of drugs that deliver potent payloads to specific cells or tissues via antigen mediated uptake. ADCs are composed of an antibody that is specific to the intended target which is tethered to a payload by a cleavable linker. They are unique because they have the ability to deliver extremely cytotoxic payloads to only certain areas of the body without harming non-targeted tissues. There are currently ten ADCs that are FDA approved, most of which release amine-containing payloads. There is very limited technology that is effective for releasing alcohol-containing payloads. We have designed a tetrapeptide linker referred to as "ValCitGlyPro" that has shown success when compared to a simple ester linker ("mc"). We demonstrate that the mc linker is not readily cleaved by lysosomes, while the ValCitGlyPro linker is. Further, we demonstrate that this linker has modest stability in mouse and human serum. Finally, we have evaluated various breast-cancer targeting ADCs with both the ValCitGlyPro and the mc linker and have measured the intracellular payload concentration in a breast cancer cell line. Together, this data is being used to evaluate whether the ValCitGlyPro linker system is a suitable technology for the delivery of alcohol-containing payloads.



Introduction

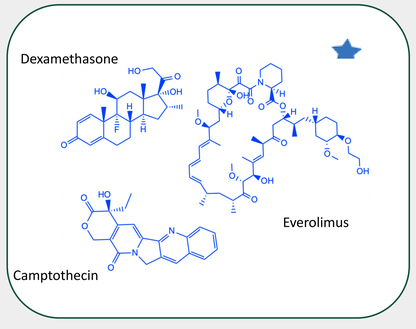
Cell internalization Process: The antibody binds to the antigen of choice on the cell surface. It is then absorbed into the cell where it is trafficked to the lysosome and the drug is released.



Nature Biotechnology, 2012, 30, 631–637

Why alcohol payloads?

Developing a linker for alcohol release is important because there are many OH containing drugs of interest, especially chemotherapeutic agents. ADCs allow for direct delivery of these agents, allowing for increased efficacy and decreased unwanted side effects.



Highly potent alcohol-containing payloads of interest:

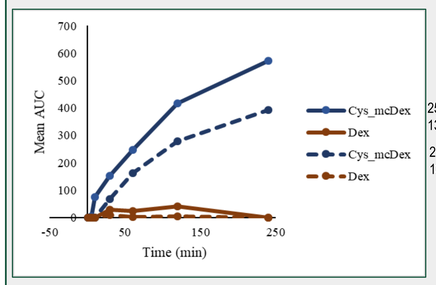
Dexamethasone (Dex):
Anti-inflammatory and immunosuppressant drug
Everolimus:
Immunosuppressant and anti-cancer drug
Camptothecin:
Anti-cancer drug and cytotoxic chemotherapeutic treatment

Overview of linker design:

Most ADCs utilize the ValCitPABC space seen above as a self immolative linker system to release amine containing payloads. This linker is not capable of releasing alcohol containing payloads. We initially explored a simple ester linker, hoping to take advantage of ubiquitous esterases that may cleave this functional group upon internalization. This simple ester linker is referred to as "mc" (maleimide caproyl). We selected dexamethasone as our model payload to test this linker in both serum stability assays and ADC catabolism studies.

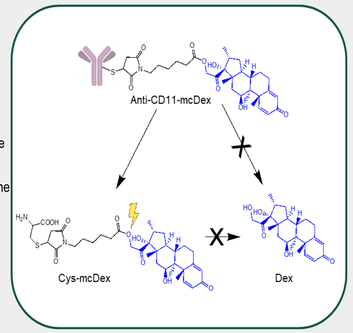
ADC Catabolism:

ADC Lysosomal Catabolism

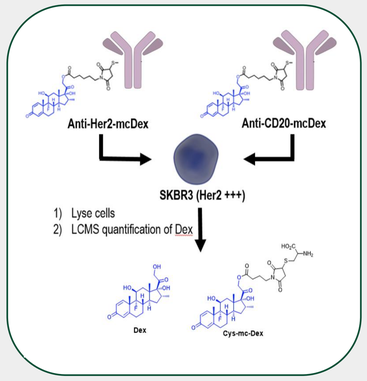


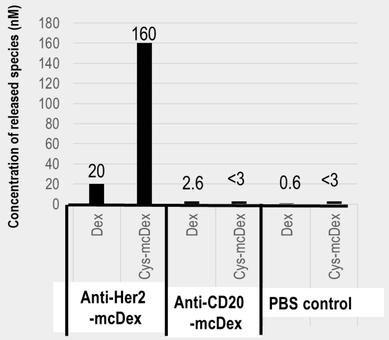
25 ug lysosome
13 ug ADC

2.5 ug lysosome
13 ug ADC



ADC Cellular Catabolism

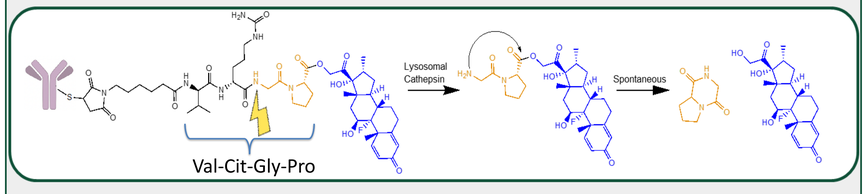




- **Delivery is mediated by antibody**
- **Dex is not efficiently released**
- **Need improved linkers to release alcohols**

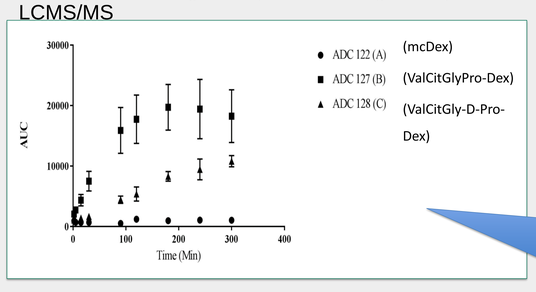
Conclusion: Esters are not efficiently cleaved in the lysosome and are not readily cleaved upon ADC uptake. This is likely due to the absence of esterases in the lysosome. For this reason, we re-designed the linker to incorporate a "self-immolative" spacer that results in spontaneous ester cleavage upon proteolysis.

Proposed GlyPro self-immolative spcaer



ADC Lysosomal Cleavage

ADC +lysosomes (ph=4.7)→add PBS(ph=7.4) → measure Dex concentration by LCMS/MS

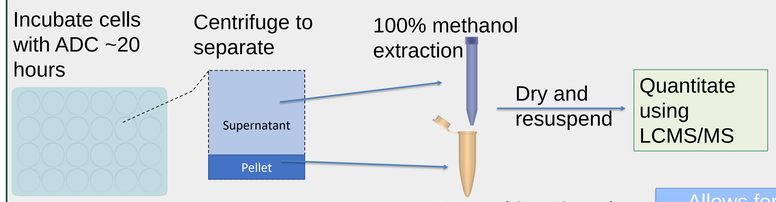


30 ug of ADC incubated with 5ug of lysosomes

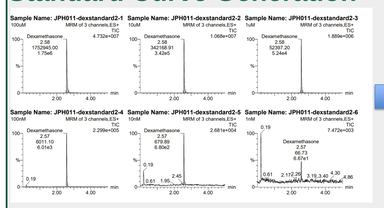
The ADC containing the ValCitGlyPro linker(B) showed the greatest amount of cleavage compared to a simple ester linker (A).

Assessing the dex release in a high-Her2 expressing cell line:

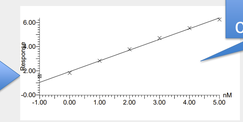
Methodology



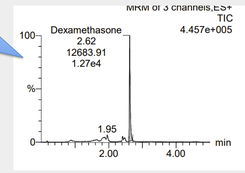
Standard Curve Generation



Allows for quantification of dex concentrations



Scan of a media sample showing dex



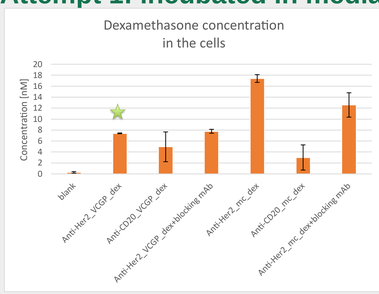
Experimental design

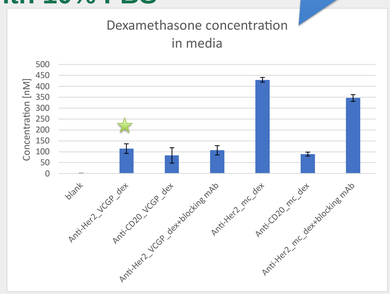
SKBR3 cells (over express HER2 receptor)
ADCs:
Anit-Her2_ValCitGlyPro_Dex
Anti-CD20_ValCitGlyPro_dex
Anti-Her2_ValCitGlyPro_Dex + blocking mAbs
Anti-Her2_mc_dex
Anti-CD20_mc_dex
Anti-Her2_mc_dex+blocking mAbs

Anti-CD20(non targeted control) and blocking antibody controls serve to demonstrate antigen mediated uptake.

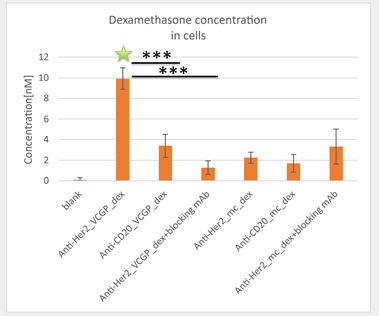
High levels of Dex in media were suggestive of esterase mediated cleavage by FBS-esterases

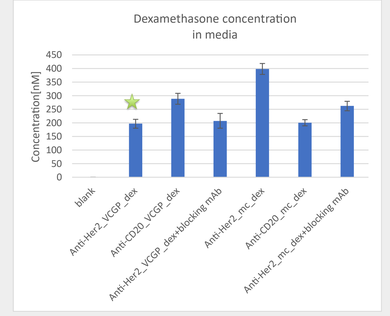
Attempt 1: incubated in media with 10% FBS





Attempt 2: incubated in media with 1% FBS





Conclusion: VCGP-Dex more efficiently delivers Dex to cells than mc-Dex. Delivery is specific to Her2, as evidenced by the null control (anti-CD20) and by the blocking antibody.

Final remarks and acknowledgements

Even with reduced FBS extracellular cleavage still remains a problem. Moving forward we will need to work on stabilizing the linker to prevent esterase cleavage.

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