

Introduction

Antibody-drug conjugates using different linker types (disulfide, thioether, peptide, and hydrazone) are in clinical testing for the treatment of various cancers. The antibody-maytansinoid conjugates (AMCs) now in clinical evaluation contain either a reducible ("cleavable") disulfide linker or a non-reducible ("non-cleavable") thioether linker. The linker selection is determined by *in vitro* and/or *in vivo* evaluation. AMC activation in target cancer cells is believed to occur via lysosomal processing, releasing lysine-linker-maytansinoid metabolite, that in the case of disulfide linker, is reduced to a maytansinoid thiol metabolite that can further undergo S-methylation (Erickson H. K. et al, *Cancer Research*, 2006, 66, 4426).

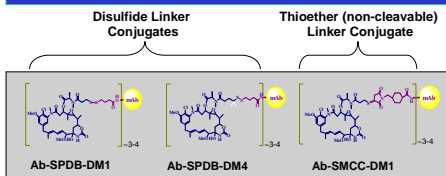
We investigated anti-EGFR AMCs in two high EGFR expressing cell lines (MDA-MB-468 and A431) as a model for mechanistic studies using two different anti-EGFR antibodies. We observed that conjugates with both disulfide (SPDB-DM4) and non-cleavable (SMCC-DM1) linker designs had similar *in vitro* cytotoxicity towards MDA-MB-468 cells, whereas the disulfide-linked conjugates were much more potent than the non-cleavable linker conjugates towards A431 cells. We also prepared a disulfide-linked conjugate with a more cleavable disulfide linker (SPDB-DM1) and found that this conjugate was more active against A431 cells.

To understand how linker cleavability affects *in vitro* cytotoxicity, we examined the intracellular trafficking of the conjugates and investigated the cellular accumulation of metabolites.

Objective

- To investigate the role of lysosomal processing in cytotoxic activities of AMCs with disulfide and non-cleavable linkers.
- Anti-EGFR AMCs were tested in high EGFR expressing cell lines and used as a model.

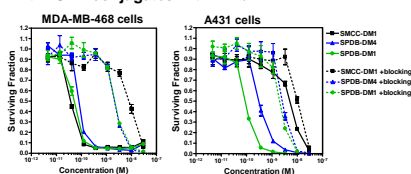
Structures



Increasing resistance to linker cleavage

In vitro Cytotoxicity

Anti-EGFR conjugates with mAb1



Relative potency: SPDB-DM1 > SPDB-DM4 = SMCC-DM1 > SPDB-DM1 > SPDB-DM4 > SMCC-DM1

Cell line	IC ₅₀ SPDB-DM1	IC ₅₀ SPDB-DM4	IC ₅₀ SMCC-DM1	Mean Fluorescence (Relative antigen density)
MDA-MB-468	0.04 nM	0.06 nM	0.03 nM	2240
A431	0.09 nM	0.40 nM	9.0 nM	2460

In vitro cytotoxicity: 2000 cells/well, 96-well plate. *For blocking, pre-incubation with 1 μM unconjugated mAb1, 4-day continuous exposure, WST-8 assay. Flow cytometry (antigen density): FITC-labeled Goat Anti-Mouse-IgG antibody treatment, formalin fixation.

- Both MDA-MB-468 and A431 cells express EGFR at strong and similar levels.
- In MDA-MB-468 cells, two disulfide-linked (SPDB-DM1, SPDB-DM4) and non-cleavable linker (SMCC-DM1) conjugate have similar cytotoxic activity.
- In A431 cells, disulfide-linked conjugates are much more potent than non-cleavable linker (SMCC-DM1) conjugate.
- In A431 cells, the less hindered disulfide SPDB-DM1 conjugate is slightly more active than SPDB-DM4 conjugate.

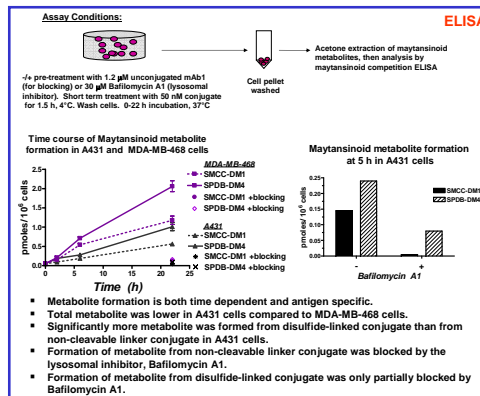
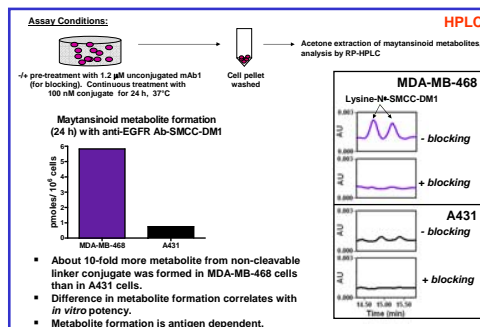
Anti-EGFR conjugates with mAb2

Cell line	IC ₅₀ SPDB-DM4	IC ₅₀ SMCC-DM1
MDA-MB-468	0.07 nM	0.06 nM
A431	0.20 nM	3.0 nM

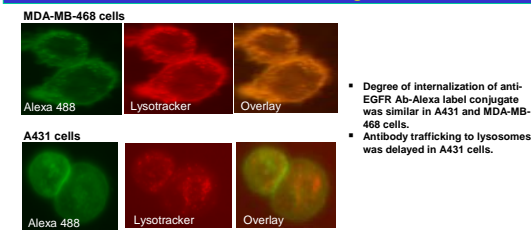
In vitro Cytotoxicity: 2000 cells/well, 96-well plate, 4-day continuous exposure, WST-8 assay

- Another anti-EGFR mAb2 with different binding epitope than mAb1 is used for conjugation.
- There is a similar trend of cytotoxic potency for mAb2-maytansinoid conjugates as mAb1-maytansinoid conjugates.
MDA-MB-468 cells: disulfide linker = non-cleavable linker
A431 cells: disulfide linker > non-cleavable linker

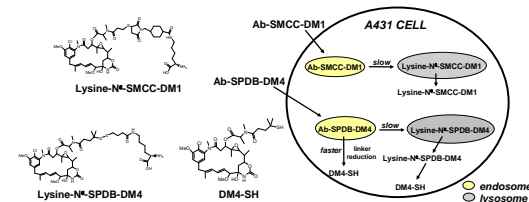
Cellular Metabolism



Intracellular Trafficking



Model for Conjugate Activation in A431 Cells (Inefficient Lysosomal Processing)



Conclusions

- In MDA-MB-468 cells (efficient Ab degradation), model anti-EGFR AMCs with both disulfide and non-cleavable linker designs have similar cytotoxic potency.
- In A431 cells (delayed Ab trafficking to lysosomes), AMCs with disulfide linkers are much more potent than those using a non-cleavable linker design.
- Maytansinoid metabolite formation from non-cleavable conjugate design appears to depend on lysosomal processing.
- In contrast, metabolite from disulfide-linked AMCs could form partly independent of lysosomal proteolysis in A431 cells.
- This non-lysosomal activation of disulfide-linked AMCs could be useful for targeting tumor cells that have inefficient lysosomal processing or tumor antigens that are poorly processed within lysosomes.