

## Antibody maytansinoid conjugates with hydrophilic linkers: Cytotoxic therapeutics with enhanced potency *in vitro* and *in vivo*

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### 1. Introduction

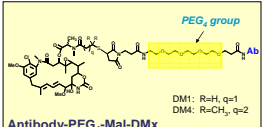
Several antibody-maytansinoid conjugates are in clinical testing for the treatment of different cancers. Each of these conjugates consists of a monoclonal antibody (Ab) with a proprietary maytansinoid cytotoxic agent (DMx) attached via either a reducible or a non-reducible linker. The conjugates in the clinic ("standard" conjugates) contain about four DMx molecules per antibody.

Each conjugate is designed to bind to the target antigen on a cancer cell, be internalized into the cell, and then release the DMx in a form that can inhibit microtubule function and kill the cell.

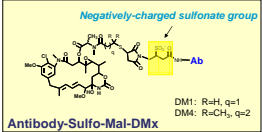
Herein we describe a new class of linkers - hydrophilic linkers - that allows the synthesis of conjugates with increased DMx molecules per antibody (D/A) without compromising conjugate solubility or pharmacokinetic behavior *in vivo*. Maytansinoid conjugates made with these new hydrophilic linkers are highly potent against both normal and multi-drug resistant (MDR) human tumor cell lines *in vitro* and *in vivo*.

### 2. Ab-DMx conjugates: Hydrophilic vs. standard linkers

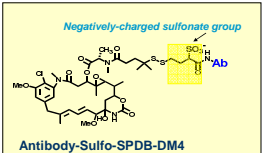
#### Hydrophilic Non-Reducible Linkers



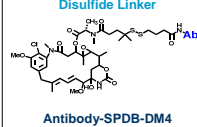
#### Standard Non-Reducible Linker



#### Hydrophilic Reducible Disulfide Linker



#### Standard Reducible Disulfide Linker



### 3. Conjugates with non-reducible hydrophilic linkers and standard DMx loads are highly active against MDR cells

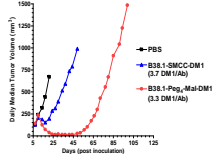
#### *in vitro* cytotoxicity data; EC50

conjugate	Colo 205 (nM)	Colo 205 MDR (nM)
B38.1-SMCC-DM1 (3.7 D/A)	0.11	0.48
B38.1-PEG <sub>n</sub> -Mal-DM1 (3.3 D/A)	0.06	0.11

- B38.1 Ab is a murine monoclonal Ab specific for EpcAM
- Colo 205 is a human colon carcinoma line; Colo 205 MDR was generated by transfection of Colo 205 cells with the human *MDR1* gene
- All *in vitro* cytotoxicity data carried out using 3-5 day continuous exposure; WST-8 assay; concentrations shown are based on antibody

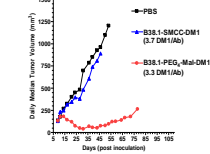
#### *in vivo* antitumor activity

##### B38.1 Ab conjugates/Colo 205 xenografts in SCID mice



Single i.v. dose of 10 mg Ab/kg

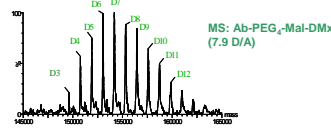
##### B38.1 Ab conjugates/Colo 205 MDR xenografts in SCID mice



Single i.v. dose of 20 mg Ab/kg

- B38.1 conjugates with hydrophilic PEG<sub>n</sub>-Mal linkers and standard DMx/Ab loads are highly active towards human Colo 205 MDR cells both *in vitro* and *in vivo*

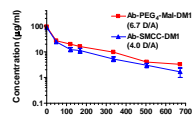
### 4. Hydrophilic linkers allow synthesis of conjugates with increased maytansinoid loads (> 4 DMx/Ab)



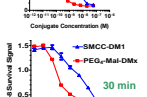
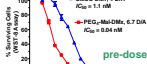
- Good mass spec. profile with normal distribution
- High % monomer (>99%); low free DMx (<1%)
- Good solubility; conjugate concentrations > 5 mg/ml

### 5. Plasma PK and bioactivity of 6.7 D/A Ab-PEG<sub>n</sub>-Mal-DMx conjugate

Single bolus i.v. dose at 5 mg/kg anti-CD56 Ab conjugate in CD-1 mice (3 mice per group)

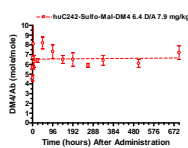
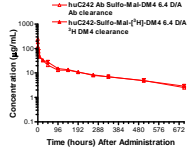


#### Plasma Bioactivity



- ELISA for total Ab measurement: capture with goat-anti-Hu IgG(Hu) → detection with HRP-Goat-anti-Hu IgG-Fc
- Bioactivity assay: cytotoxicity assay on RH30 cells with diluted plasma samples
- Plasma PK of 6.7 D/A PEG<sub>n</sub>-Mal-DM1 conjugate is favorable (similar to SMCC-DM1 conjugate with 4 D/A)
- The initial advantage in bioactivity of the 6.7 D/A PEG<sub>n</sub>-Mal-DM1 conjugate over the standard D/A load conjugate is maintained during circulation in mice

### 6. Clearance of Ab-Sulfo-Mal-[<sup>3</sup>H]-DM4 conjugates: High DMx load species remain in circulation up to 28 d

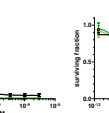
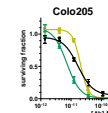


- Single bolus injection in CD1 mice of 7.9 mg Ab/kg; plasma from 3 mice sampled for each time point
- Total Ab concentration determined by ELISA (goat α-Hu IgG capture; HRP-donkey α-Hu IgG detection)
- Total DMx concentration determined by <sup>3</sup>H scintillation counting

- Conclusions:
- Rate of Ab clearance for high DMx load Sulfo-Mal-DM4 conjugate (t<sub>1/2β</sub> = 10 d) similar to that of naked Ab (t<sub>1/2β</sub> = 12 d), indicating high DMx load does not cause accelerated clearance of conjugate
- Rate of conjugate clearance matches rate of Ab clearance, indicating non-reducible linkage of DMx to Ab is stable in plasma

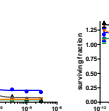
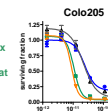
### 7. Conjugates with non-reducible hydrophilic linkers and high DMx loads are highly active against MDR cells

anti-CanAg Ab conjugates: standard cytotoxicity assay format



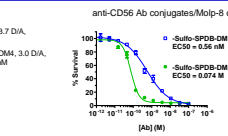
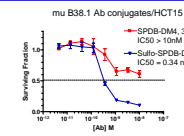
- Cytotoxicity assay on cell line with high Ag density: PEG<sub>n</sub>-Mal-DM1 conjugates at regular and high DMx loads are very active on a multi-drug resistant cell line

anti-CanAg Ab conjugates: 50x excess naked Ab assay format



- Cytotoxicity assay format using 50x excess of naked Ab simulates low Ag density conditions and differentiates conjugate potency: PEG<sub>n</sub>-Mal-DM1 and Sulfo-Mal-DM1 conjugates at regular and high DMx loads retain their high potency on an MDR cell line even under conditions of low antigen occupancy

### 8. Conjugates with reducible hydrophilic linkers are active against MDR cells and can support high DMx loads



- Conjugates with Sulfo-SPDB linkers are highly active on a natural MDR cell line (HCT-15)
- High DMx load Sulfo-SPDB-DM4 conjugate shows super-stoichiometric increase in potency compared to regular load Sulfo-SPDB-DM4 conjugate

### 9. Conclusions

- Ab-maytansinoid conjugates with hydrophilic linkers (non-reducible or disulfide linked) with standard DMx/Ab loads are highly potent against MDR cancer cells *in vitro* and *in vivo*.
- Ab-maytansinoid conjugates with increased DMx/Ab loads can be prepared using these new hydrophilic linkers. These conjugates maintain their desired binding activity and biochemical characteristics.
- Conjugates with high DMx/Ab loads made with hydrophilic linkers show favorable PK behavior *in vivo* and maintain their bioactivity advantage over conjugates with standard DMx/Ab loads during circulation in plasma.