

The effect of linker on target cell catabolism and PK/PD of trastuzumab-maytansinoid conjugates

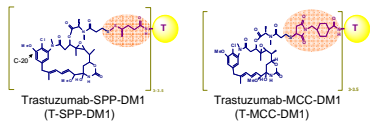
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Abstract A149

INTRODUCTION

Trastuzumab-DM1 (T-DM1) is an antibody-drug conjugate (ADC) in clinical development for the treatment of HER2+ metastatic breast cancer. It consists of a maytansinoid cytotoxic agent, DM1, conjugated via a non-reducible ("uncleavable") thioether linker, SMCC, to the humanized HER2-binding antibody, trastuzumab (T). Nonclinical studies of T-DM1 showed slightly greater efficacy in mouse models of HER2+ breast cancer and greater tolerability in rodents than an alternative design, T-SPP-DM1, that employs a reducible ("cleavable") disulfide linker. The efficacy findings were unexpected as, for several other targets, disulfide-linked conjugates showed greater activity in xenograft models than thioether-linked designs. To explore the impact of linker reducibility, T-SPP-DM1 and thioether-linked T-DM1 (T-MCC-DM1) were compared for *in vitro* and *in vivo* efficacy, pharmacokinetics, and target cell metabolism.

Figure 1. Structures of conjugates evaluated



METHODS

Clonogenic cytotoxicity assay: Cells were plated at low density (100 cells/well for SK-BR-3 and BT-474EEI; 200/well for BT-474) in Ham's F-12 DMEM (50:50) + 10% FBS + 2 mM L-glutamine and allowed to adhere overnight. The conjugates were added the next day and the cells were incubated until colony formation was determined to be maximal. The medium was removed and the colonies were counted after staining with crystal violet dye (0.5% in methanol).

In vivo efficacy studies: BT-474EEI cells (2×10^7) were injected into the mammary fat pad of beige nude XID mice and tumors were grown to ~250 mm³. Animals were randomized into nine groups (n=10/group) and treated with single bolus i.v. injections of the conjugates. Tumors were measured with calipers twice weekly for 24 d then weekly for 120 d following dosing or until tumors reached a volume of 3000 mm³. Tumor volumes were determined using the formula: Tumor volume (mm³) = (longer diameter X shorter diameter)² X 0.5.

In vivo metabolism studies: Mice bearing BT-474EEI tumors (as described above) were randomized into 2 groups (n = 15/group) and treated with a single 10 mg/kg bolus i.v. dose of T-MCC-[³H]DM1 or T-SPP-[³H]DM1. Two additional control groups (n = 3/group) were treated with matching doses of non-targeting antibody (NT)-MCC-[³H]DM1, or NT-SPP-[³H]DM1. Three mice were sacrificed after each time interval, plasma was collected, systemic circulation was flushed with PBS, and tumors were collected. The specific radioactivity of [³H]DM1 and the total radioactivity associated with plasma and tumor homogenates were used to determine the concentration of conjugate and the %ID/g, respectively.

Analysis of plasma and tumors: Tumors were homogenized in TBS containing 5 mM Tris-HCl maleimide and analyzed for radioactivity by solubilization and LSC, and tumor metabolites by organic extraction, LC/MS, HPLC and LSC. Plasma samples were analyzed for [³H]ADCs by LSC. Plasma samples from PK studies were collected and analyzed for total antibody (Tab) or conjugate (ADC) by ELISA.

In vitro cytotoxicity: T-MCC-DM1 vs. T-SPP-DM1

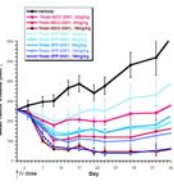
Figure 2. In vitro cytotoxicity on BT-474EEI and SK-BR-3 cells



T-SPP-DM1 and T-MCC-DM1 have similar cytotoxic potency

Anti-tumor efficacy and pharmacokinetics

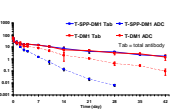
Figure 3. Efficacy in BT-474EEI tumor-bearing mice



Mice were administered a single i.v. bolus dose of T-MCC-DM1 or T-SPP-DM1

T-MCC-DM1 and T-SPP-DM1 have similar efficacy by dose

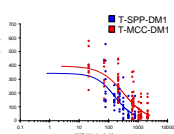
Figure 4. Pharmacokinetics of T-SPP-DM1 and T-MCC-DM1



Mice were administered a single i.v. bolus dose (3 mg/kg) of T-MCC-DM1 or T-SPP-DM1.

T-MCC-DM1 exposure is greater than T-SPP-DM1

Figure 5. Relationship between BT-474EEI tumor volume (at 21 d) and AUC



Molecule	ED50	AUC Estimate
T-MCC-DM1	324 (4.6 mg/kg)	
T-SPP-DM1	161 (6.2 mg/kg)	

* Dose based on CL₀ estimates of 14.2 and 38.3 mL/day/kg for T-MCC-DM1 and T-SPP-DM1, respectively.

BT-474EEI tumor volumes of mice at day 21 (Fig. 3) were plotted against the dose adjusted plasma AUC (Fig. 4).

T-SPP-DM1 is slightly more efficacious than T-MCC-DM1 in mice bearing BT-474EEI tumors

In vitro metabolism studies

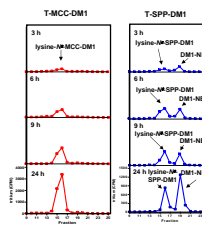
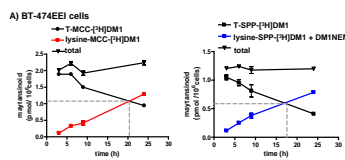


Figure 6. Target-cell metabolites (BT474 EEI cells)
 BT-474EEI cells (~5 x 10⁶) exposed to 20 nM T-DM1 or T-SPP-DM1 for 2-3 h on ice were washed and incubated in fresh medium at 37°C for 3-24 h. Metabolites were extracted from cells and medium and identified by HPLC and LSC. No metabolites were detected in the spent medium (data not shown).

Lysine-MCC-DM1 is the only metabolite of T-MCC-DM1 and Lysine-SPP-DM1 and DM1 observed for T-SPP-DM1
 Metabolites identical to those reported for other antibody-maytansinoid conjugates (AMCs) with disulfide linkers (1)

Figure 7. Rate of target-cell processing



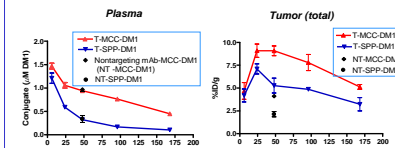
A) BT-474EEI cells
 B) SK-BR-3 cells

A) The radioactivity associated with the HPLC metabolites (Fig. 6) were converted to pmol/cell
 B) SK-BR-3 cells were exposed to T-MCC-[³H]DM1 and analyzed for metabolites as described for the BT-474EEI cells

Similar HER2-dependent processing for both conjugates
 Similar processing rates for BT-474EEI and SK-BR-3 cells
 No efflux of metabolites was observed from BT-474EEI cells
 Efflux was observed from SK-BR-3—possibly due to a higher intracellular concentration of metabolites

In vivo metabolism studies

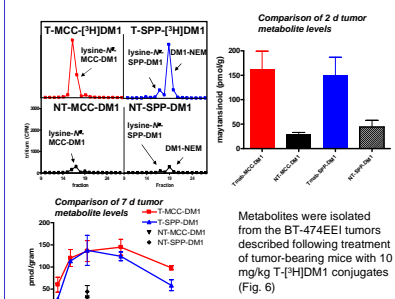
Figure 8. Plasma levels and tumor localization of trastuzumab and non-targeting (NT) antibody conjugates in BT-474EEI tumor-bearing mice



Mice were treated with a single bolus i.v. dose of [³H]ADC. Total radioactivity associated with the plasma and tumors was used to calculate the concentration of conjugate and %ID/g

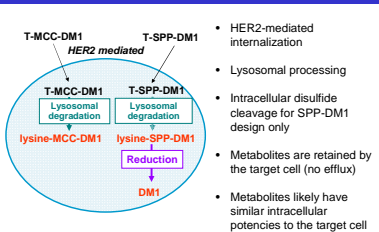
Consistent with the PK analysis (Fig. 4), the 7 d plasma AUC for T-SPP-DM1 is lower (2.7-fold) than T-MCC-DM1
 7 d AUC for total tumor maytansinoids (conjugate + catabolites) for T-MCC-DM1 is 1.5-fold higher than T-SPP-DM1

Figure 9. HER2-dependent tumor metabolites



Metabolites were isolated from the BT-474EEI tumors described following treatment of tumor-bearing mice with 10 mg/kg T-[³H]DM1 conjugates (Fig. 6)
 7 d AUC for T-SPP-DM1 and T-MCC-DM1 are similar
 More efficient processing of T-SPP-DM1
 Tumor delivery is HER2-dependent

Model for the activation of T-MCC-DM1 and T-SPP-DM1 by BT-474EEI cells



CONCLUSIONS

- T-MCC-DM1 and T-SPP-DM1 differed in intracellular processing, with lysine-linker-DM1 formed with both, but the DM1 metabolite formed only with T-SPP-DM1. This is consistent with findings with other AMCs.
- The two conjugates displayed similar processing kinetics *in vitro*, consistent with their similar *in vitro* activity.
- The tumor metabolite levels of T-SPP-DM1 and T-MCC-DM1 were found to be similar despite the faster plasma clearance and lower tumor exposure of T-SPP-DM1.
- The similar activity of T-MCC-DM1 and T-SPP-DM1 *in vivo* is consistent with their similar tumor metabolite levels.
- No efflux of metabolites from the HER2+ BT-474EEI cells was observed *in vitro*. With other AMCs that use a disulfide linker, efflux of non-lysine metabolites (e.g., DM1) from the antigen-positive cells can contribute significantly to efficacy, as these metabolites can kill other cancer cells in close proximity.
- Limited efflux from tumor cells *in vivo* may explain why T-SPP-DM1 is not significantly more active than T-MCC-DM1 in xenograft models

ACKNOWLEDGEMENTS / REFERENCES

(1) Erickson, H. K., Park, P. U., Widdison, W. C., Kovtun, et al. (2006) Cancer Res 66, 4426-33.