Supplementary Figure Legends

HER2-mediated processing of T-DM1 and T-SPP-DM1 is similar in other breast carcinoma lines

We investigated the processing of the conjugates by SK-BR-3 and MCF7-neo/HER2 cells to investigate whether the activation of the conjugates varies in different breast carcinoma cells. Exposure of the MCF7-neo/HER2 cell line to T- [^{3}H]DM1 and T-SPP-[^{3}H]DM1 yielded the same catabolites as observed with the BT-474EEI cells and the rates for the processing of both conjugates were also found to be similar with around 50% of the initially bound conjugates processed to their respective maytansinoid catabolites after 20 h (Supplementary Figure S1). Unlike what was observed following exposure of the BT-474EEI cells to the conjugates, high levels of the catabolites were observed in the spent medium of the SK-BR-3 and MCF7-neo/HER2 cells treated with the conjugates. Exclusion of trypan blue stain from the cells indicated that their cell membranes were intact and that the observed efflux was not from dying cells. The reason for the observed efflux from the SK-BR-3 and MCF7-neo/HER2 cells is likely due to a higher intracellular concentration of the maytansinoid catabolites in these cells as compared to the BT-474 EEI cells allowing for the diffusion of the catabolites out of the cell. High catabolite levels within the SK-BR-3 and MCF7-neo/HER2 most likely resulted from their higher HER2 levels (about $1.5x10^6$ receptors / cell, about 3-fold higher than for BT-474EEI cells) as compared to the BT-474EEI cells (Supplementary Figure S1). Efflux was also observed following exposure of COLO 205 cells, which express high amounts of CanAg antigen, to anti-CanAg ADCs (9).

Supplementary Figure S1

A) HPLC Radiograms of the target-cell catabolites of T-[^{3}H]DM1 and T-SPP-[^{3}H]DM1 following treatment of SK-BR-3 (A) . Cultures of SK-BR-3 or MCF7-neo/HER2 (B) cells were exposed to the ^{3}H-conjugates as described in Fig. 4. Spent medium was removed and cells were harvested at the indicated time points and precipitated with acetone following treatment with NEM to block all free thiols, preventing thiol-disulfide exchange, and trapping DM1 as the DM1-NEM adduct. Maytansinoid catabolites were isolated from the medium by solid phase extraction as described in Methods. The acetone precipitate was solubilized and measured for residual radioactivity by LSC. Maytansinoid catabolites in the acetone extract (cells) and eluant from solid phase cartridges (medium) were separated using reversed-phase HPLC and fractions from the HPLC (1 min) were collected and measured for radioactivity. The chromatograms show the fraction number on the abscissa and the counts per minute on the ordinate. B) Rates for the catabolism of conjugates show a correlation between the formation of maytansinoid catabolites of T-DM1 and T-SPP-DM1 formed within cells (■,■) and medium (□,□) and a decrease in the concentration of intact conjugate still associated with the cells (●). Concentrations of the catabolites were calculated from the radioactivities associated with the catabolites isolated by HPLC and the concentrations for the intact conjugates were determined from the radioactivity associated with the acetone precipitates. The total maytansinoid concentration associated with the cells (▲) was found to remain constant until the 24 h time-point where the lower levels may reflect a loss in yield for the catabolites during the extraction from medium. The dotted line in the figure shows that the rates for the formation of the total catabolites(●,●) for the

conjugates are similar for the two cell lines.