HOMOGENEOUS ADCs BEARING TWO DIFFERENT PAYLOADS SHOW STRONG SYNERGY IN TUMOR KILLING

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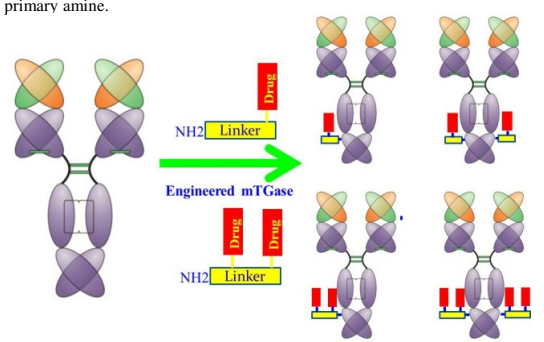
INTRODUCTION

Using engineered microbial transglutaminase (mTGase), we are able to conjugate any given toxin with an amine group to any mAB without the need of antibody re-engineering. Such a simple and quick site-specific conjugation method has enabled us to screen out endosome escaping & non-cleavable (EENC) linkers. These EENC linkers need an optimal length to achieve the highest ADC activity. ADCs made with EENC linkers are highly stable in human, rat and mouse plasma and potent. When two different payload toxins were conjugated site-specifically to one mAB molecule, the homogeneous hybrid ADC (DAR 2) prepared shows synergy towards tumor cell killing while mixtures of the respective two homogeneous ADCs (DAR 2) bearing the same toxins do not. The pM Ic50 in assay and high in-vivo potency challenges the widely accepted ADC concept of an activation pathway via lysosomal destruction to release free toxin. On the contrary, our data imply that ADC can be directly active after escaping from an endosome and as a result of multiple interactions of the different payloads, make the new ADCs more potent.

METHODS

Through rational design, we have engineered a mTgase with an enlarged active center that allows one specific endogenous Gln of each heavy chain in Fc region of **any** IgG1, 2, and 4 to be conjugated with a toxin containing a primary amine.

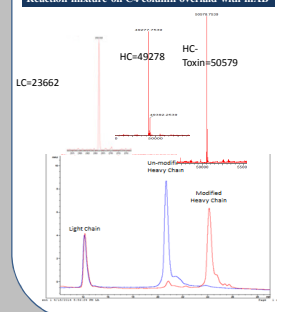


ADC is made in a one-pot reaction, where mAB and toxin are mixed with mTgase directly or loaded to a mTgase immobilized column. Yield of conjugation between 90 to 95% of DAR2 has been routinely obtained. Thus, purification of ADC can be simplified as a one-step removal of excess toxins.

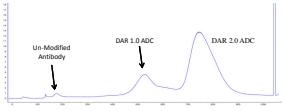
RESULTS

1. ADC HPLC & MS Analysis

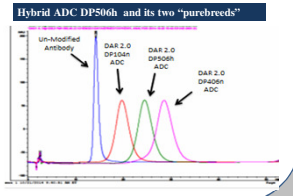
Reaction mixture on C4 column overlaid with mAB



Reaction mixture on Phenyl column

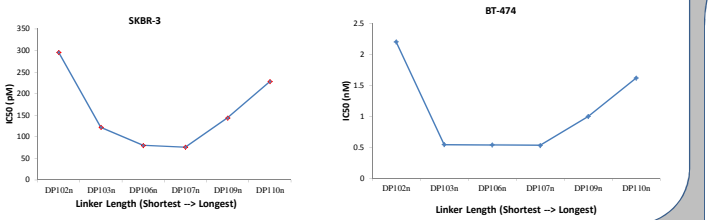


Hybrid ADC DP506h and its two "purebreeds"

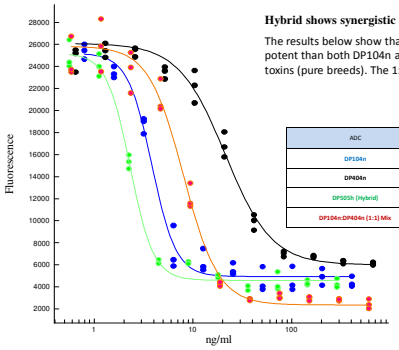


2. Optimal Linker Length

EENC linkers of different lengths were used to conjugate a same payload (tubulin inhibitor) to Trastuzumab site-specifically and the resulting ADCs were assayed in different cell lines for Ic50. Even the shortest linker tested here would be long enough to allow free interactions between toxin on mAB and microtubule. The optimal linker length may allow multiple toxins of one ADC to bind microtubule simultaneously.



3. *In Vitro* Potency

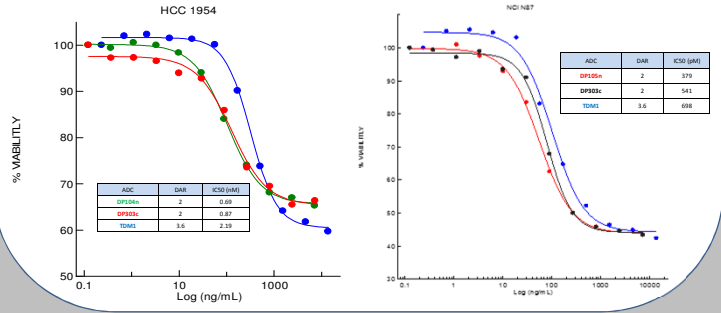


Hybrid shows synergistic effect in SKBR-3 Cell Based Assay

The results below show that the hybrid ADC(DP505h) of Trastuzumab is more potent than both DP104n and DP404n, the two homogeneous ADCs of the same toxins (pure breeds). The 1:1 mixture of the two ADCs is also lesser potent.

ADC	DAR	IC50 (pM)
DP104n	2.0	26
DP404n	2.0	145
DP505h (Hybrid)	2.0	16
DP104n:DP404n (1:1) Mix	2.0	54

Homogenous ADCs are also potent in other Her2 + tumor cell lines

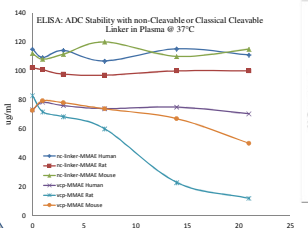


ADC	DAR	IC50 (nM)
DP104n	2	0.69
DP303c	2	0.87
TDM1	3.6	2.19

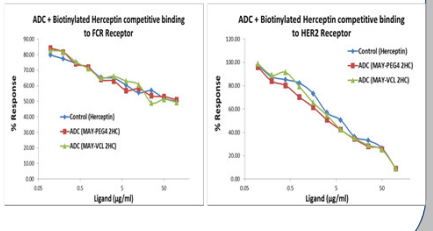
ADC	DAR	IC50 (pM)
DP105n	2	379
DP303c	2	541
TDM1	3.6	698

4. Stable in Plasma and No effect on Receptor Binding

EENC-linker is stable in Human, rat and mouse plasma @37°C
However, the classical cleavable linker is unstable in Rat plasma and moderate stable in mouse plasma.

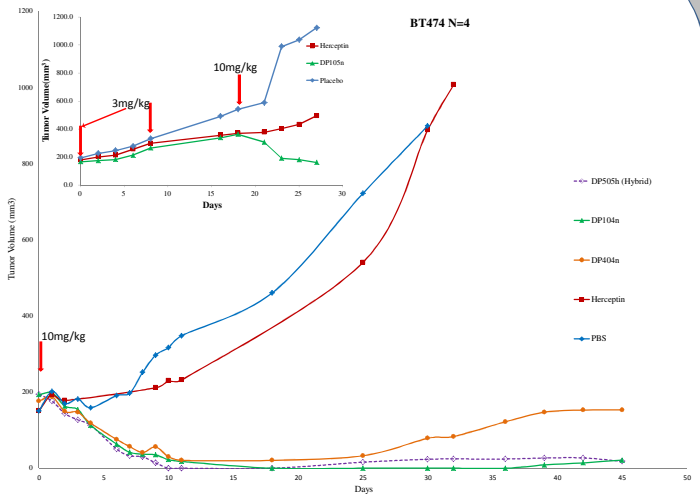


Site-Specific Conjugation does not affect FcR and Her2R binding affinity
Trastuzumab conjugates of Maytansine with a classical cleavable linker and /or non-cleavable PEG linker were tested in competitive binding assay.

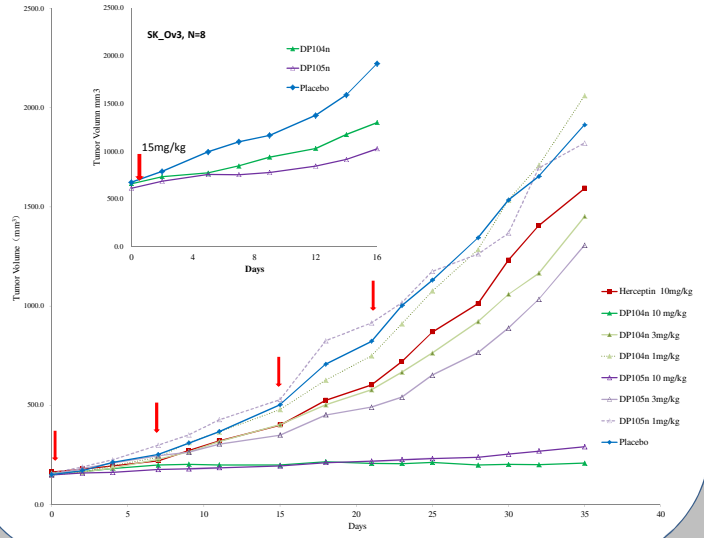


5. IN VIVO DATA

ADCs with EENC linker are efficacious at 10 mg/kg in Xenografts of BT474 cell, very little effect at 3mg/kg. The Hybrid (DP505h) is as potent as the highly active ADC of the same toxin (DP104n). Dose and injections are marked by red arrows.



At 10mg/kg, ADCs (DP104n and DP105n) can stop tumor growth in SK_OV3 Xenografts at volume of 200 mm3. At larger tumor volume (as large as 900mm3), DP105n achieves total regression with single 15mg/kg injection in 2 out of eight mice.



CONCLUSION

We presented a one-pot reaction to prepare site-specific ADCs. We have identified a novel non-cleavable linker, which increases not only ADC stability, but also potency. We also demonstrate synergy in cancer cell killing of hybrid ADC loaded with two different toxins..

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