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A Cysteine Specific, Highly Stable Linker Technology Gives Improved Efficacy in an ADC Model

Jenny Thirlway, Justyna Mysliwy, David J. Mansell, Matthew Smith, Chris Birchall, and David J. Simpson

Glythera Ltd, Hershal Annex, King's Road, Newcastle Upon Tyne, NE1 7RU, UK jenny.thirlway@glythera.com

Glythera has developed a cysteine specific, highly stable conjugation platform - PermaLink™ - which outperforms an approved conjugation technology when compared directly in a Trastuzumab based ADC model.

PermaLink™ is applicable to key product classes including Antibody Drug Conjugates (ADCs), conjugate vaccines and bi-specifics.

Combining the potency of small molecule drugs with the target specificity of antibody therapeutics, ADCs have demonstrated remarkable clinical potential.

The significant clinical benefits ADCs demonstrate, aligned with an exciting development pipeline, has led to market predictions of $9 billion by 2023 for the ADC class.

Currently, there are only two approved ADC products – Adcetris® and Kadcyla®.

ADC innovation is moving forward rapidly and the need for novel, tailored, site-specific conjugation technologies to link potent drugs to specific antigen-targeting antibodies is well recognised.

PermaLink™

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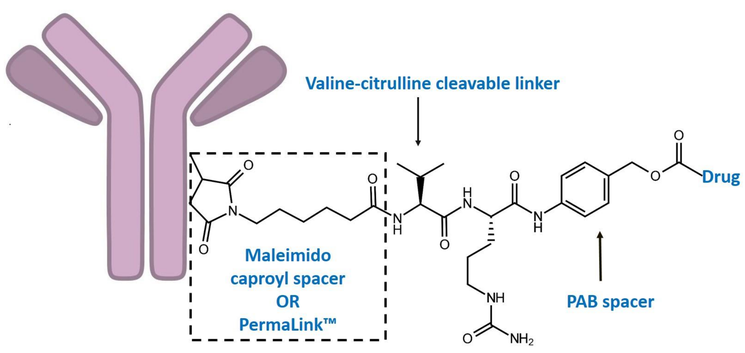
Figure 1: Substitution of a maleimide based linker technology with PermaLink™ technology

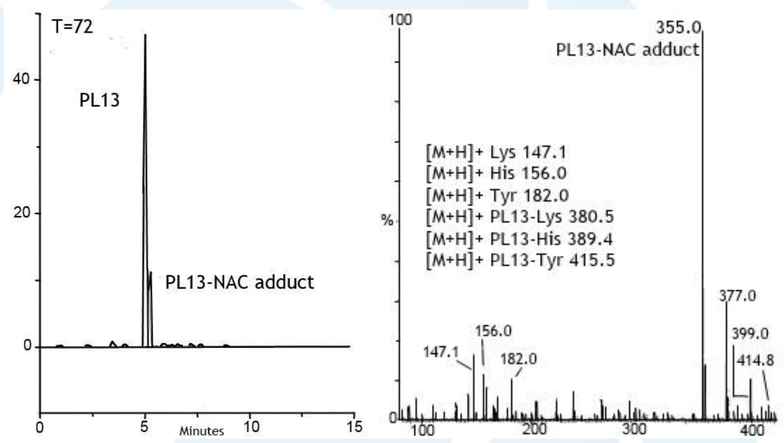


The model employs Trastuzumab plus a val-cit cleavable component and MMAE. PermaLink™ replaces the maleimido caproyl spacer. A maleimide version of the ADC was also created for comparison purposes.

Specificity for cysteine

Specificity for cysteine has been shown via an amino acid challenge experiment (Figure 2).

Figure 2: Cysteine specificity gives improved product homogeneity

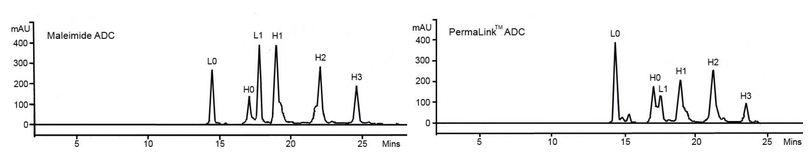


Amino acid competitive challenge – tyr/his/lys/*N*-Acetyl cysteine (NAC). Conversion to PermaLink™ (PL-13)-NAC adduct only, confirmed by LC-MS.

PermaLink™ generates appropriate drug loadings

A Drug to Antibody Ratio (DAR) of 4 was targeted (Figure 3).

Figure 3: PLRP analysis to calculate DAR



	Maleimide ADC				PermaLink™ ADC			
Peak ID	Area sum	Drug count	Drug per chain	DAR	Area sum	Drug count	Drug per chain	DAR
L0	31.60	0.00	0.62	4.32	34.40	0.00	0.38	3.80
L1		0.62				0.38		
H0	68.40	0.00	1.54		65.61	0.00	1.52	
H1		0.40				0.35		
H2		0.64				0.78		
H3		0.50				0.40		
H4		0.00				0.00		
Sum	100	N/A	2.16		100	N/A	1.90	

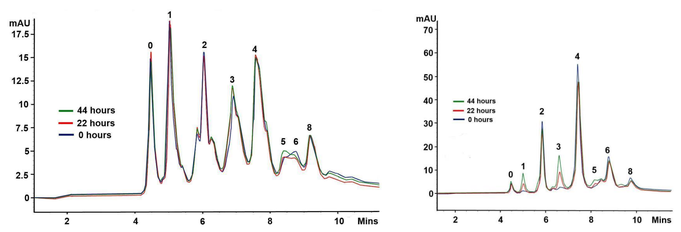
SEC analysis (not shown) gave % monomer values of 96.5 and 95.3 for the maleimide ADC and PermaLink™ ADC, respectively.

Enhanced stability of the PermaLink™ platform

To demonstrate the enhanced stability of the PermaLink™ system *in vitro*, a de-drugging experiment was performed and analysed by Hydrophobic Interaction Chromatography (HIC).

N-Acetyl cysteine was used as a competing thiol in 77 times excess. Figure 4 shows the results of this experiment.

Figure 4: De-drugging via a NAC challenge experiment (at 37 °C in PBS)

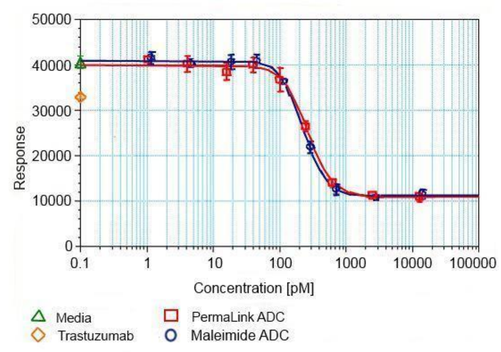


The PermaLink™ ADC shows no change in peak shape over time indicating that the construct is stable when challenged with thiol. This is in contrast to the maleimide ADC which shows an increase in the abundance of the odd numbered DAR species over 44 hours indicating drug loss.

Comparable *in vitro* activity

The relative potency of the maleimide and PermaLink™ ADCs are shown in Figure 5.

Figure 5: *in vitro* activity. SKBR3 cell line, time = 72 hours

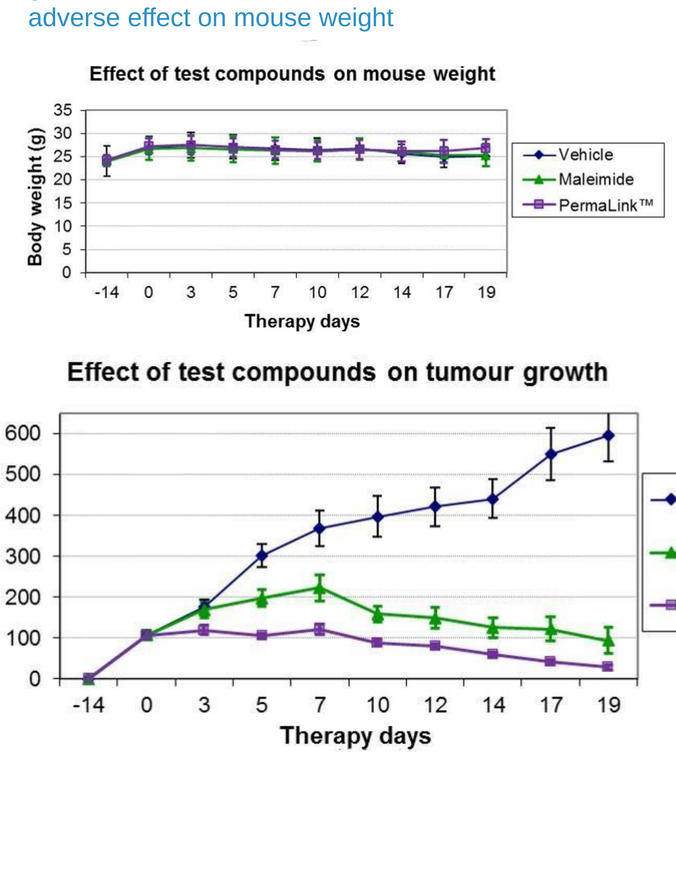


The ADC constructs show equivalent cell kill activity in the SKBR3 cell line

PermaLink™ outperforms a maleimide based technology in a xenograft model

The PermaLink™ ADC demonstrated enhanced *in vivo* activity in a mouse xenograft model. The human breast cancer cell line, BT474 was transplanted into athymic nude mice. A ~5 mg/Kg dose of ADC was given on day zero (dosing was weighted for DAR), tumours were measured and mouse weights recorded (Figure 6).

Figure 6: The PermaLink™ ADC reduces tumour volume to a greater extent than the maleimide ADC and does not have an adverse effect on mouse weight



Conclusion

Glythera's PermaLink™ technology gives greater specificity and enhanced stability when compared to maleimide based linkers. PermaLink™ has shown *in vitro* and *in vivo* efficacy in an ADC system, outperforming a maleimide based linker in a mouse xenograft model.

Acknowledgements

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