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Mechanistic determination of mAb mediated internalisation of site specific, chemically labile linker technologies

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Glythera has developed a platform linker technology - PermaLink™ - for stable, site specific (cysteine) attachment. PermaLink™ is applicable to key product classes including Antibody Drug Conjugates (ADCs), conjugate vaccines and bi-specifics.

The global market for engineered protein products is estimated to be worth $168 billion by 2017, with monoclonal antibodies accounting for more than 50% market share. Ten of these blockbuster drugs generate more than $1 billion in annual revenue. Market share is difficult to predict for Antibody Drug Conjugates (ADCs), however the gain in effect means the market potential is considered to be large.

Given the specific requirements for the combination of product, cytotoxic payload and linker technologies we intend to fully elucidate the internalisation pathway for key antibody products and determine the specific benefits of our proprietary technology using in vitro studies.

PermaLink™

PermaLink™ is Glythera's proprietary platform linker technology. PermaLink™ uses novel chemistry to specifically functionalise surface cysteine residues through a stable thioether bond. A range of PermaLink™ linkers are available for use.

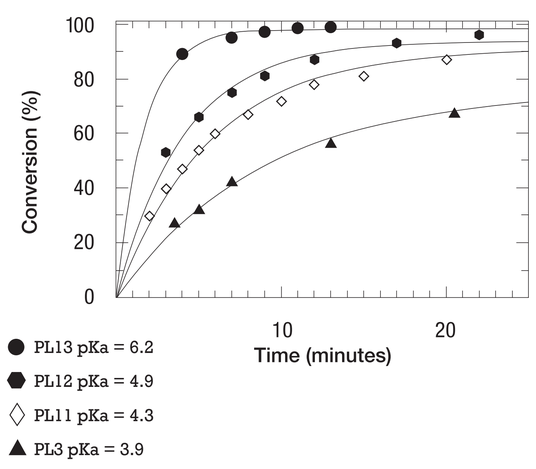

FIGURE 1: *pKa affects reaction rate. Reactions carried out at pH 7*

PermaLink™ reactivity is related to pKa (Figure 1) and the rate of conjugation for each linker is pH dependent (Figure 2).

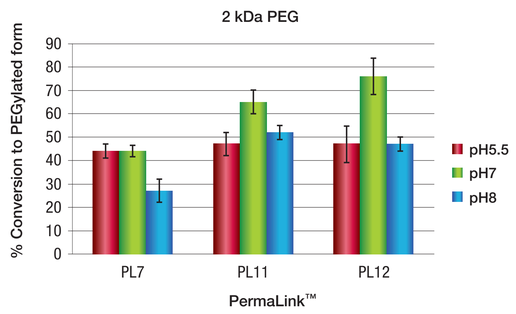

FIGURE 2: *Method development for linker conjugation. 2 kDa PEG was used to exemplify the conjugation method. The three PermaLink™ linkers used in this example show varying reactivity which is pH dependent.*

Specificity for cysteine has been shown via an amino acid challenge experiment. A preliminary incubation study of PermaLink™ with a cysteine thiol (N-acetyl cysteine, NAC) was undertaken at pH 7 in a methanol:PBS buffered solution at ambient temperature. RP-HPLC was used to show addition of free thiol to PermaLink™ over time. The 24 hour time point shows a new peak appearing at 5.04 minutes (Figure 3A) which LC-MS has confirmed as the PermaLink™-NAC adduct (data not shown).

To show that PermaLink™ is selective for thiol addition and forms a stable thioether, the linker was challenged by addition of a 2 molar equivalent mixture of tyrosine/histidine/lysine/NAC. A time course was performed at pH 7 and ambient temperature (Figure 3B). At 72 hours the elution profile shows the reaction has plateaued at < 25 % conversion indicating that PermaLink has unimolecular selectivity for free thiol and is not influenced by nucleophilic side chains from other amino acids.

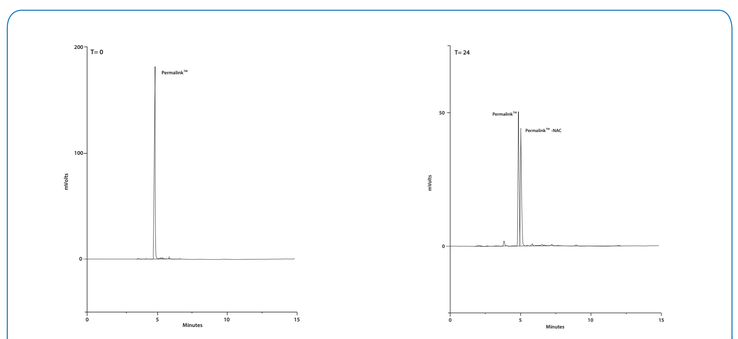

Figure 3A: *Amino acid challenge study. PermaLink™ in methanol treated with 2 molar equivalents of a 50mM N-acetyl cysteine (NAC) solution at pH 7 in PBS buffer, ambient temperature.*

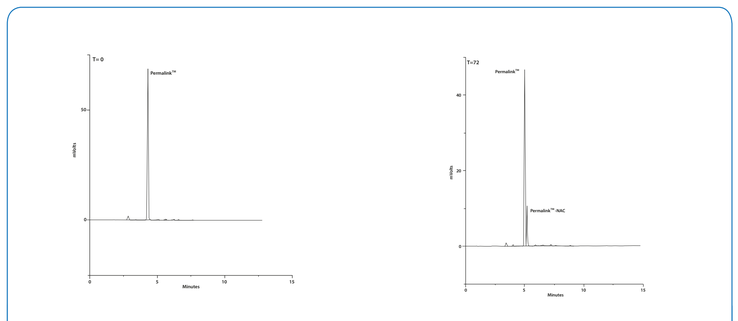

Figure 3B: *PermaLink™ in methanol challenged with a 2 molar equivalent mixture of tyrosine/histidine/lysine/NAC in PBS buffer.*

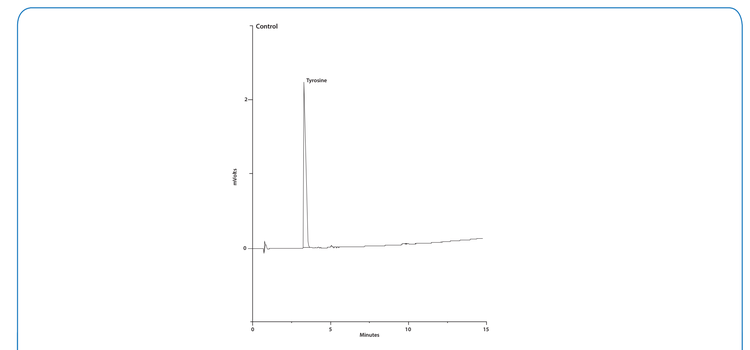

Figure 3C: *MeOH:PBS buffer containing the 4 amino acids was used as a control. This identifies a minor peak component resulting from absorbance of tyrosine.*

Antibody Drug Conjugates (ADCs)

Antibody Drug Conjugates (ADCs) enhance the anti-tumour effects of monoclonal antibodies (mAbs) by combining the mAb with a cytotoxic drug. ADCs have the added advantage of being able to reduce the adverse systemic effects of the cytotoxic by sequestering the drug until it reaches the target site permitting in effect a higher dosage of cytotoxic to be administered.

The EMA and FDA approval of Adcetris® and approval of Kadcyla® by the FDA has paved the way for increased research into this class of biotherapeutic.

ADCs will be generated for comparison with currently marketed products by employing Glythera's PermaLink™ technology to attach a cytotoxic payload.

mAbs typically contain 8 cysteine residues which form 4 disulphide bonds. A reduction reaction will be carried out followed by conjugation of the PermaLink™-payload. Method development will be required to optimise the reduction and conjugation steps. Literature suggests that generally 2-4 cytotoxic molecules per mAb give the best therapeutic window[1,2].

Discussion

Glythera's PermaLink™ technology has already shown efficacy in a vaccine model (data not shown) and we are now further developing the platform for rapid deployment into ADC manufacture. A range of commercially available and early stage mAbs are being investigated to show the utility of the platform in ADCs and demonstrating the site specific and stability benefits of the technology as well as effective internalisation and release of the active payload.

References

1. Ducry, L. and Stump, B., Bioconj. Chem., 2010, 21, 5-13
2. Junutula, J.R., et al, Nat. Biotechnol., 2008, 8, 925-932

Acknowledgements

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