Correlation of Discrete Drug-linker Variation on Antibody Drug Conjugates (ADC) to Binding Activity Using Surface Plasmon Resonance

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Introduction

Antibody-drug Conjugates (ADCs)

Antibody-drug conjugates are one of the fastest growing classes of oncology therapeutics. ADCs are hybrid innovative biotherapeutics, that combine the specificity of monoclonal antibodies (mAb) with highly potent cytotoxic small molecules conjugated via a bifunctional linker, which is a critical factor in determining the effectiveness of the ADC therapy. Characterization of ADCs, which includes site-specificity, potency of the drug, conjugation sites, binding properties, and Drug to Antibody Ratio (DAR) can be challenging owing to the complexity of this class of biologic.

ADCs rely on the target-binding specificity of a mAb to selectively deliver potent drugs to target cells. Binding interactions between IgG antibodies and Neonatal Fc receptor (FcRn) regulates half-life in vivo and is associated with the improvement of therapeutic efficacy. The efficacy of tumour targeting ADCs may also depend on antibody effector functions, including antibody-dependent cell-mediated cytotoxicity (ADCC), that are mediated via interactions between the antibody Fc region and components of the immune system, such as immune cell surface Fcγ receptors, and complement.

Trastuzumab (Tmba), a recombinant humanized IgG1 monoclonal antibody against the Receptor tyrosine-protein kinase erbB-2 (HER-2), is known to mediate ADC activity by attracting the immune cells to tumour sites that overexpress HER-2 antigen. The most important contributors to ADC activity in vivo are thought to be Natural killer (NK) cells, which express only Fc gamma receptor IIIa (FcγRIIIa).

In our previous study, we have investigated the relationship between the nature of PEG linkers and Fc region mediated binding interactions using an ADC mimic. In this study we expanded the investigation into discreet linker and DM1 taxin variation, and the Fcn and FcγRIIIa binding activities using Surface Plasmon Resonance (SPR).

Library Conjugation

PEG-DM1 Procedure:

Tmba was reacted with the desired NHS-linker at 2 loadings (5 and 7 mol eq) for 90 min. DM1 was then added at the same equivalents of linker and reacted for 60 min. Reaction solutions were then transferred to an Amicon 30kD spin filter and exchanged 3 times with 1x PBS pH 7.4 to remove impurities. Retentates were removed and submitted for analyses.

Table 1. Analytical Characterization Results

<table>
<thead>
<tr>
<th>Sample</th>
<th>Loading</th>
<th>FcγRIIIa</th>
<th>FcRn</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A_P_PEG2_5X</td>
<td>7.25 100</td>
<td>91.74</td>
<td>91.74</td>
</tr>
<tr>
<td>1A_P_PEG4_5X</td>
<td>7.38 97.35</td>
<td>91.74</td>
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<tr>
<td>1B_P_PEG2_5X</td>
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<td>92.28</td>
<td>92.28</td>
</tr>
<tr>
<td>1B_P_PEG4_5X</td>
<td>7.2 92.28</td>
<td>92.28</td>
<td>92.28</td>
</tr>
<tr>
<td>3A_P_PEG2_5X</td>
<td>7.33 91.86</td>
<td>91.86</td>
<td>91.86</td>
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<tr>
<td>3A_P_PEG4_5X</td>
<td>7.25 91.86</td>
<td>91.86</td>
<td>91.86</td>
</tr>
</tbody>
</table>

Table 2. Result summary of SPR Binding Activity of Taxin Variants.

PEG-DM1 Procedure:

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Figure 1. PEG-DM1 Conjugation Process

Summary

SPR binding assays allowed real time kinetic analysis of interactions between Fc receptors and mAb-based therapeutics including ADCs. We have demonstrated rapid analysis of different constructs using SPR which provides preliminary indication of linker selection on Fc binding activity. Our results suggested some loss in affinity for FcRn but a general increase in FcγRIIIa binding activity for Tmba using PEG linkers. Further investigation is required to understand the impact on the linker conjugation to the sensitivity of target binding and effector function. The goal of ADC Expression™ is to deliver research-ready, ADC construct diversity sets to assist customers with the selection of appropriate candidates for clinical scale production. Working with our partners, we can provide any and all diversity concepts from varied mAb constructs to conjugation methodologies to linkers and payloads. These libraries can be used to promote confident, data-driven candidate selection at the pre-production development stage to optimize the desired outcomes in the clinic. Our aim is to offer a commercially viable diversity set(s) that can become the industry standard tool for pre-clinical candidate selection.

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