Using Surface Plasmon Resonance to investigate the multi-specific modalities of immunotherapeutic molecules

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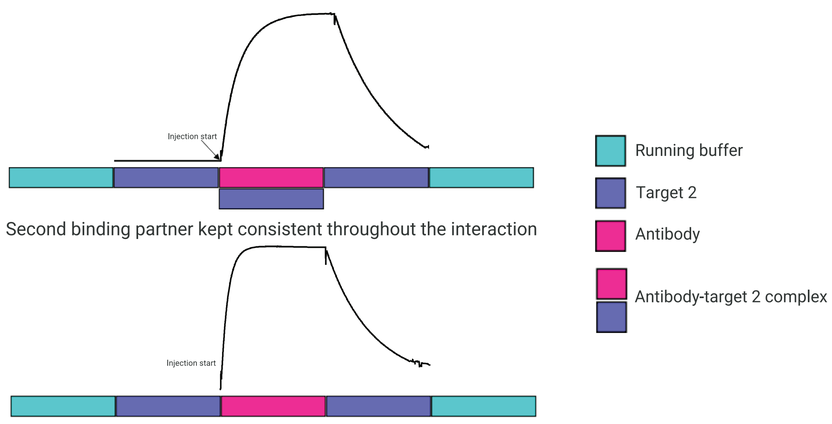
INTRODUCTION

Multi-specific modalities have expanded the functional capabilities of antibody-based therapeutics. Compared to monospecific antibodies, several individual interactions and their inter-dependency need to be considered for multi-specific antibodies. We have developed an SPR methodology that interrogates binding interdependency of a multi-specific antibody based therapeutic. We further expanded this approach to demonstrate the impact of Fab arm binding on FcR engagement. This methodology can be applied to a range of molecules from conventional mAbs, bispecific and multi-specific antibody-based therapeutics and fusion proteins as well to assess the effects of antigen binding on FcR function.

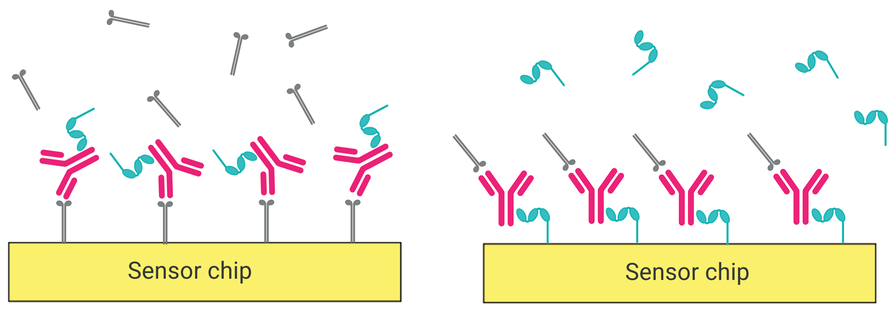
THE BINDING-DEPENDENCY ASSAY

Intitially, one target molecule is reversibly coupled to the chip surface. Following this, stable complexes of the antibody and a second target molecule are injected over the surface – these injections are flanked by injections of the second target molecule alone. The concentration of the second target molecule is maintained throughout the binding assay while the concentration of antibody is varied in a dose dependent manner and the interaction analysed using the multi-cycle kinetic mode. Keeping the second target molecule at a consistent level ensures the measurements are solely due to the interaction of the antibody and the first target.



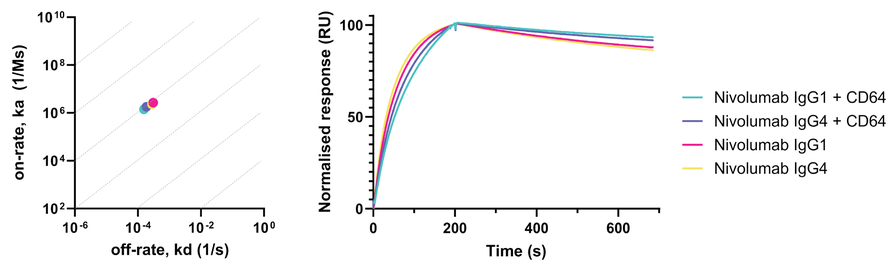
FAB-ANTIGEN-FcR DEPENDENCY

Nivolumab, an IgG4 immune check-point inhibitor targeting the PD1:PD-L1 axis, displays minimal FcγR binding thereby reducing antibody-mediated interactions such as ADCC and ADCP when compared to the IgG1 subclass of mAbs. It has been established that IgG4 antibodies are not completely shielded from FcγR interaction and bind under high avidity or aggregation conditions with FcγRI (CD64) and to a lesser extent FcγRIIA (CD32a), FcγRIIB (CD32b) and FcγRIIIA (CD16a). Moreover, IgG4 antibodies that are complexed can bind CD64 with a binding affinity on the same order of magnitude as IgG1 and IgG3 antibodies. We set out to use the dependency assay to investigate this further.



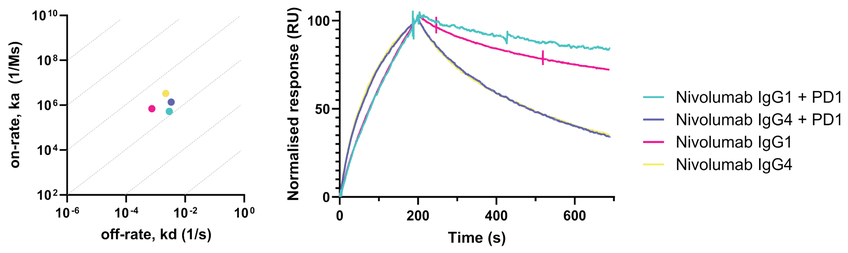
Left: precomplexed mAb-FcR complexes passed over PD1 surface; Right: precomplexed mAb-PD1 complexes passed over FcR surface

Nivolumab-CD64 complexes bind PD-1 with similar KD



On-off rate plot (left) and representative sensorgrams (right) for the interaction of Nivolumab with PD1 in the absence and presence of CD64

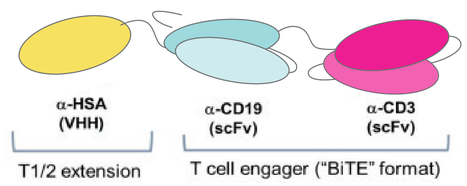
Nivolumab-PD1 complexes bind CD64 with differential affinity



On-off rate plot (left) and representative sensorgrams (right) for the interaction of Nivolumab with CD64 in the absence and presence of PD1

T-CELL ENGAGER DEPENDENCY

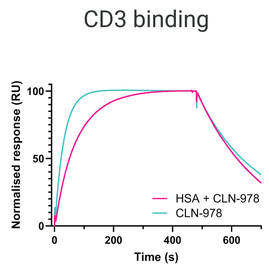
CLN-978 is a tri-specific T-cell engager (TCE) designed to bind T-cells and target them to malignant B cells. This molecule has a single-chain variable fragment (scFv) targeting CD3, a unique scFv with picomolar affinity for CD19 and a human serum albumin (HSA)- binding domain for half-life extension, eliminating the need for continuous infusion.



In addition to characterising the binding kinetics of CLN-978 to each target, the effects of the HSA binding domain on CLN-978 target affinity was investigated.

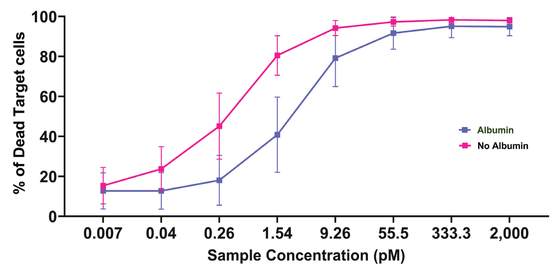
A decrease in affinity in the presence of albumin is driven by slower on-rates

Species	Protein	Serum Albumin	ka (1/Ms)	kd (1/s)	KD (nM)
Human	CD19	-	1.9×10^{5}	1.39×10^{-5}	0.0738
		+	ND	ND	ND
	CD3	-	6.98×10^{5}	4.99×10^{-3}	7.18
		+	2.23×10^{5}	3.73×10^{-3}	16.79
Cynomolgus	CD19	-	9.26×10^{4}	1.78×10^{-3}	19.22
		+	3.08×10^{4}	1.38×10^{-3}	44.98
	CD3	-	7.30×10^{5}	4.41×10^{-3}	6.04
		+	2.05×10^{5}	3.37×10^{-3}	16.45



Kinetic characterisation of the CLN-978/CD19/CD3 interaction in the absence and presence of serum albumin. Sensorgram shows CLN-978 binding to CD3 in the absence and presence of human serum albumin.

Minor shift in half-maximal CLN-978 concentration in the presence of HSA



Impact of HSA binding on T-cell mediated target cell cytolysis induced by CLN-978. Binding of albumin reduces the potency of CLN-978 in a TDCC assay employing the CD19 expressing B lymphocyte cell line Ramos.

WHAT ARE THE BENEFITS OF OUR METHOD?

Functional relevance

The assay aims to mimic the formation of the immune-complex between antibody and target followed by the binding of a second target allowing for the delineation of how differently the therapeutic may behave when one binding arm is engaged.

Gold-standard equipment

The unique A-B-A functionality of the Biacore 8K allows for the injection of a flanking molecule during sample injection which negates the need for the molecule in the running buffer.

Precise and accurate data

The assay set-up maintains a constant saturating concentration of one of the binding partners during the multi-cycle kinetic analysis ensuring the data truly represents association and dissociation of only one binding pair.

SUMMARY

We have developed a binding dependency assay which allows for the investigation of the multi-specific modalities of immunotherapeutic molecules. The examples presented demonstrate how single occupation of an antibody binding domain may affect a *'cis'* interaction. Understanding binding dependencies is critical for the prediction of functional activity and extends beyond immune cell engagers to all classes of bispecific and multispecific antibodies and even conventional monoclonal antibodies.

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