# Unlocking the unique potential of AvidiMAb® in fighting cancer

Bubacarr G Kaira, Foram Dave, Elena Dubinina, Omar Mohammed, Bryony Heath, Poonam Vaghela, Ruhul Choudhury, Tina Parsons, Samantha Paston and Mireille Vankemmelbeke and Lindy G. Durrant

[1]Scancell Ltd, University of Nottingham Biodiscovery Institute

## INTRODUCTION

- Avidity, the combined binding strength of individual interactions, is a key aspect of cancer-targeting by therapeutic antibodies.
- Fc-engineering AvidiMab® technology from Scancell improves and enhances antibody function via non-covalent Fc:Fc associations by neighboring target-bound mAbs.
- Over the past few years, CD40 agonist have been recognized show promising potential in mimicking CD40L by crosslinking CD40 to activate and promote APCs maturation and their antigen presentation capabilities.
- CD40 agonists induced clustering of CD40 expanse tumor antigen-specific cytotoxic T cells to effectively kill cancer cells.
- SEA-CD40 is a CD40 agonist and recently entered phase 2 clinical trial. The Fc-engineered AvidiMab® technology was introduced into SEA-CD40 in an IgG1 format to improve it functional and cytototic capabilities in cancer.

### Fc-engineering AvidiMab® technology in SEA-CD40 antibody

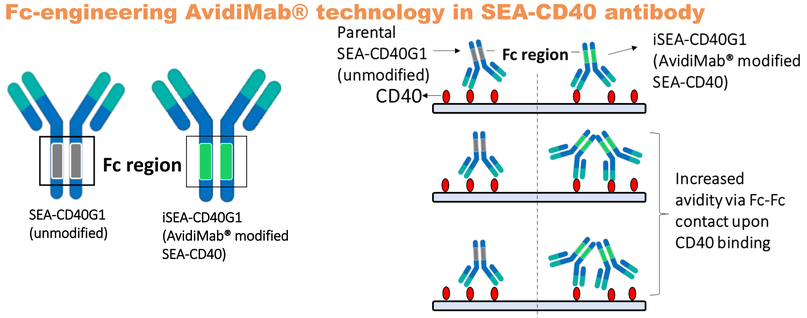
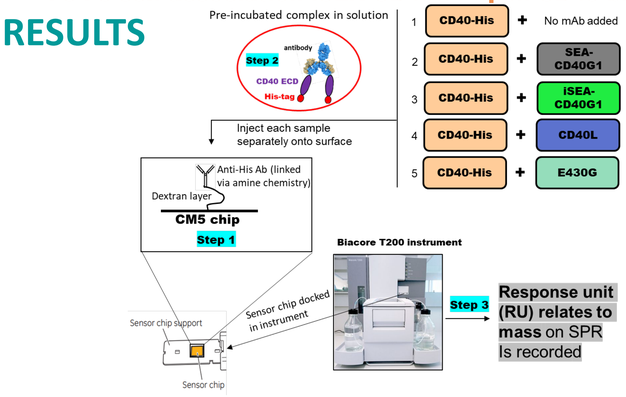


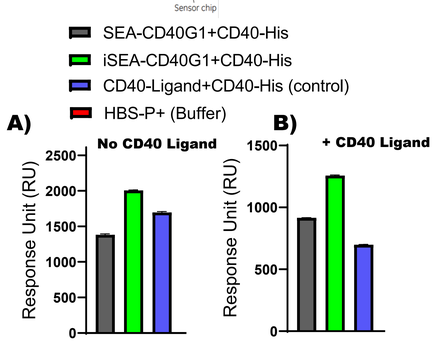

**Figure 1:** Key residues from murine IgG3 transferred into human SEA-CD40 IgG1 Fc region through Fc-engineering AvidiMab® technology to produce iSEA-CD40G1. Upon CD40 binding to iSEA-CD40G1 self-association increases avidity and functional enhancement.

### Fc-Fc self-association iSEA-CD40G1 upon CD40 binding

## RESULTS




Pre-incubated AvidiMab iSEA-CD40G1 + CD40 shows higher RU suggesting CD40 induced Fc-Fc self-association over parental SEA-CD40G1 + CD40.




**Take home message:**
Pre-formed AvidiMab iSEA-CD40G1 + CD40 (or plus CD40 Ligand) showed higher RU suggesting CD40 induced AvidiMab iSEA-CD40G1 oligomer formation and that CD40L and AvidiMab iSEA-CD40G1 can co-bind to CD40.

**Figure 2:** Pre-incubated SEA-CD40G1 vs AvidiMab (iSEA-CD40G1) in the presence of CD40 (his tagged) captured on a CM5 chip surface (immobilised anti-His antibody).

### iSEA-CD40+CD40+CD40 Ligand forms larger sized oligomer than parental SEA-CD40 using DLS

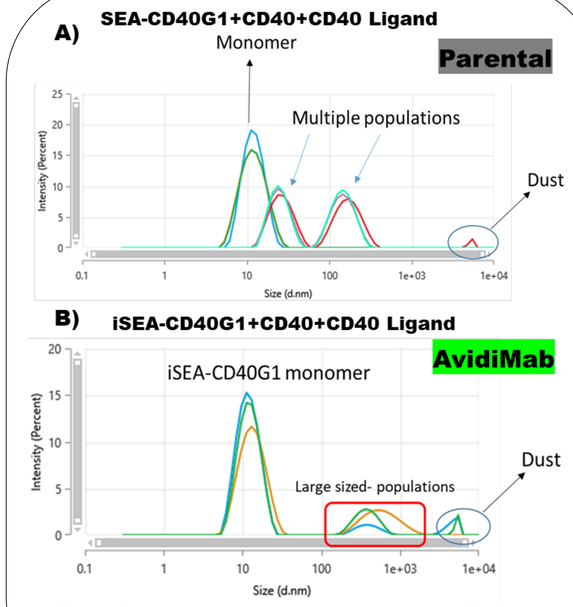
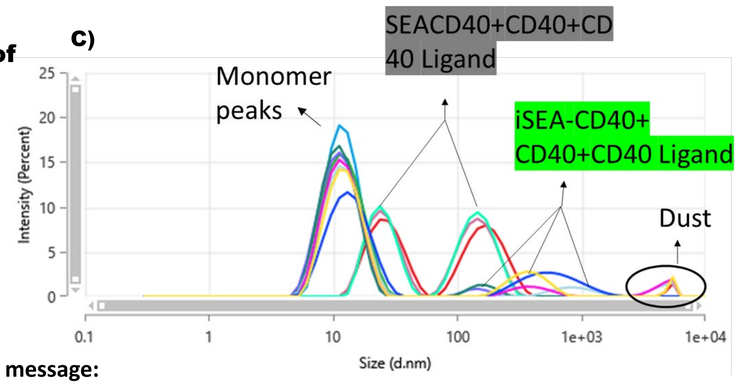


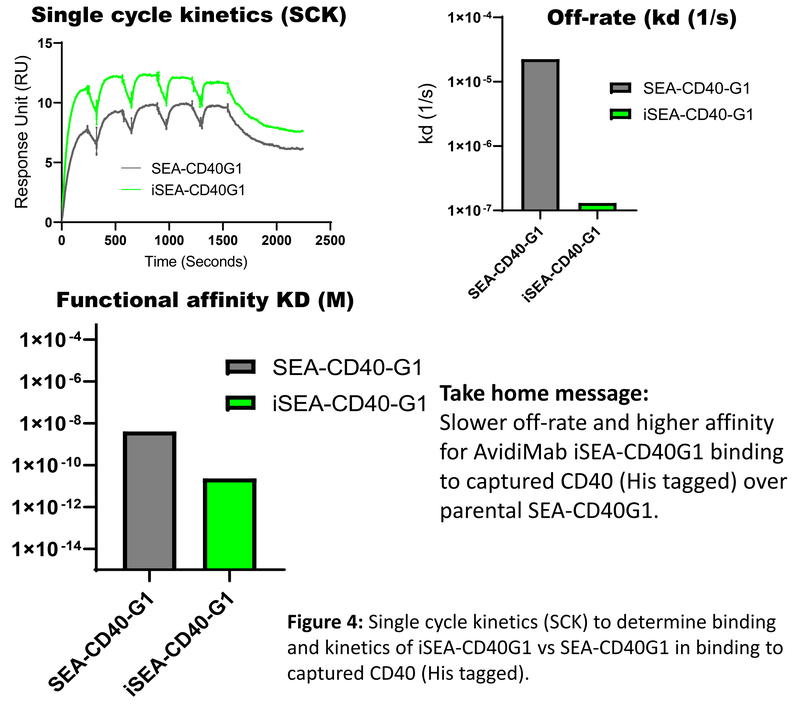

**Figure 3:** Size measurement for pre-incubated CD40 + CD40 Ligand in the presence of parental SEA-CD40G1 vs AvidiMab (iSEA-CD40G1).

**Overlay of A and B**




**Take home message:**
iSEA-CD40G1 vs SEA-CD40G1 forms large-sized populations following pre-incubation with CD40+CD40 Ligand suggesting co-binding of iSEA-CD40G1 and CD40 Ligand to CD40.

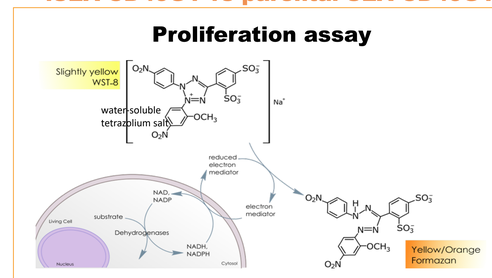
### Improved binding of iSEA-CD40G1 to CD40 over parental SEA-CD40G1




**Take home message:**
Slower off-rate and higher affinity for AvidiMab iSEA-CD40G1 binding to captured CD40 (His tagged) over parental SEA-CD40G1.

**Figure 4:** Single cycle kinetics (SCK) to determine binding and kinetics of iSEA-CD40G1 vs SEA-CD40G1 in binding to captured CD40 (His tagged).

### iSEA-CD40G1 vs parental SEA-CD40G1 in B cell proliferation



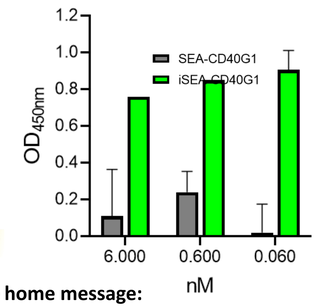




**Figure 5: Proliferation assay.** B cells treated AvidiMab iSEA-CD40 vs parental SEA-CD40 showed led to B cell expansion.

**Take home message:**
B cells treated with AvidiMab iSEA-CD40 vs parental SEA-CD40 showed B cell proliferation at increasing concentrations.

## CONCLUSIONS

- iSEA-CD40G1 (Fc-engineered AvidiMab® technology) formed Fc-Fc self association upon CD40 binding and form oligomers
- iSEA-CD40G1 displayed improved functional affinity over parental SEA-CD40G1.
- iSEA-CD40G1 exhibited superior B cell proliferative capability, compared to SEA-CD40.
- Overall, Fc-engineered AvidiMab® iSEA-CD40 clusters independent of Fc gamma receptors ((FcγRs) and therefore increases avidity for functional outcome.

## Future work

- validation in other immune co-agonists that rely on clustering for activity and/or checkpoint inhibitors (CPIs) that rely on increased residence time.

## References

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3. Yu X, Felce JH, Kellermayer B, Johnston DA, Chan HTC, Penfold CA, Kim J, Inzhelevskaya T, Mockridge CI, Watanabe Y,Crispin M, French RR, Duriez PJ, Douglas LR, Glennie MJ & Cragg MS. TNF receptor agonists induce distinct receptor clusters to mediate differential agonistic activity. Communications Biology 2021;4(1):772.