# Development of an Integrated Method to Quantify Receptor Occupancy for Agonist Immunotherapeutics that Stimulate Target Cells to Migrate from the Peripheral Blood

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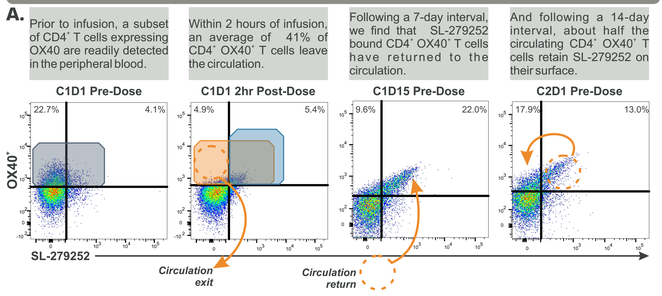
## Background

- Shattuck Labs' Agonist Redirected Checkpoint (ARC) platform adjoins the extracellular domain (ECD) of a Type 1 membrane protein to the ECD of a Type 2 membrane protein via a central Fc domain derived from an IgG4 antibody. These bi-functional proteins are engineered to block the transmission of an immune inhibitory signal from tumor cells while concomitantly delivering an immune-stimulatory signal to an immune cell within the tumor microenvironment.
- One of the pharmacodynamic measures of biologic compounds is an assessment of target receptor/ligand occupancy (RO). If the primary mechanism for drug clearance is through target binding, drug exposure may increase once RO is saturated.
- SL-172154 (SIRPa-Fc-CD40L; poster 429) and SL-279252 (PD1-Fc-OX40L; poster 494) are two bi-functional fusion proteins in phase I clinical trials (NCT04406623 and NCT03894618, respectively).
- The binding interaction between a biologic compound and its targets (CD47 or PD-L1, respectively) typically does not stimulate migration from the blood, allowing direct measurement of drug and target.
- Evaluation of RO for immune agonists (CD40L and OX40L, respectively) is challenging because agonists can stimulate leukocytes to rapidly extravasate from the peripheral blood following infusion, thus precluding direct RO measurement as target cells are no longer present in the blood (Figure 1A).

## Methods

Study design, sample collection, and assessment details have been described in posters 429 and 494. To assess full receptor engagement of CD40 or OX40, the formula shown in Figure 1B was derived. The formula captured cells that rapidly migrated from the blood, combined with those that remained in the blood within 2 hours post infusion using multiparameter FACS analysis. SAS JMP was used to calculate and visualize all parameters. FACS analysis was performed using FlowJo V10.8 (BD Biosciences) with UMAP (v3.1), XShift (v1.4.1), and ClusterExplorer (v1.6.3) implementations.

### Figure 1. CD4$^+$ OX40$^+$ T Cells and SL-279252




### Figure 1 *(continued)*. CD4+ OX40+ T Cells and SL-279252

B.

The proportion of target expressing cells that were present prior to drug administration but are absent post-infusion are termed the "marginators ".

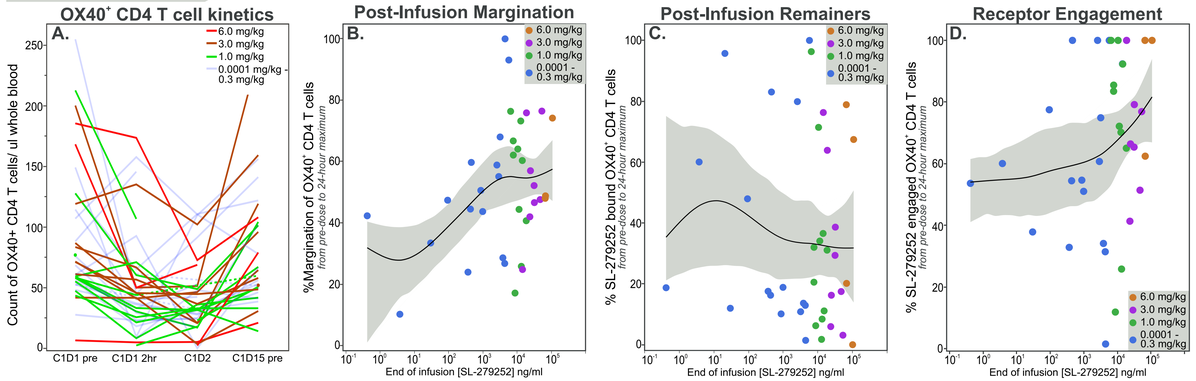
The proportion of drug-bound target expressing cells that have not exited the circulation at the selected post-infusion time point are termed the "remainers".

$$\left[1 - \frac{\%\text{Total Target Cells @ Time } X_{Post}}{\%\text{Total Target Cells @ Pre}}\right] + \frac{\%\text{Drug Bound Target Cells @ Time } X_{Post}}{\%\text{Total Target Cells @ Pre}}$$

**Marginators** **Remainers**

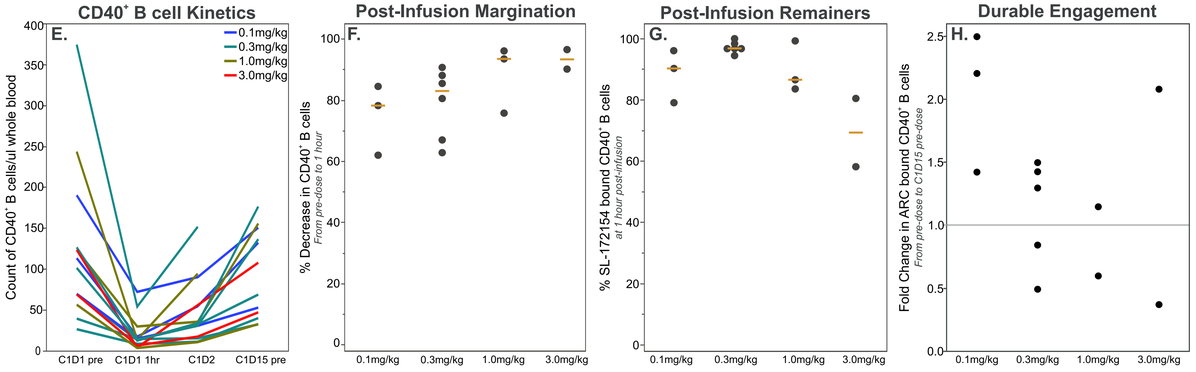
### Figure 2. ARC Infusion Leads To Rapid, Transient Target Cell Margination

SL-279252




Within 24 hours of SL-279252 infusion at 1 mg/kg (n=11), CD4$^+$OX40$^+$ T cell counts decreased from pre-dose by a median 57.8% (range 7.7 – 73.7%) (A & B). Of the CD4$^+$OX40$^+$ T cells remaining in the blood during that 24-hour post-infusion period at 1mg/kg and above, a median 40.5% were bound with SL-279252 (C). CD4$^+$OX40$^+$ T cell receptor engagement was then calculated as described and can be seen to trend upward along with increasing drug concentration (D). Lastly, CD4$^+$OX40$^+$ cells returned to pre-treatment numbers over a 7-day interval (A).

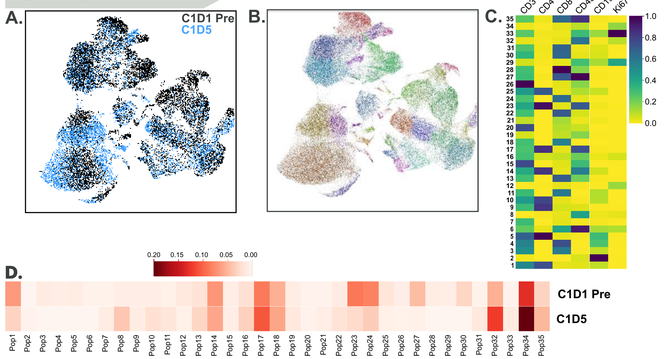
SL-172154




Similarly, within an hour of SL-172154 infusion, CD40$^+$ B cell counts decreased relative to pre-infusion amounts by a median 80% (E & F). Of the CD40$^+$ B cells remaining in the blood, a majority were bound with SL-172154 (G). CD40$^+$ B cells bound with SL-172154 detected in the blood prior to the next dose (H).

### Figure 3: ARC Administration Induces Target-specific Durable Phenotypic Changes

SL-279252




A randomly selected set of subjects receiving SL-279252 from the 0.3 mg/kg through 6 mg/kg dose groups were chosen for this analysis. C1D1 Pre and C1D5 FACS data, pre-gated on CD3$^+$ cells, from the 10-member T cell activation and proliferation panel, were analyzed with the hi-dimensionality reduction algorithm UMAP. The UMAP continent was shaded to indicate nominal sampling time, with black corresponding to the C1D1 pre time point and blue corresponding to C1D5 (A). Phenotypic segregation between C1D1 Pre and C1D5 post infusion samples was readily observed, suggesting that receptor engagement leads to sustained differentiation. Next, a K nearest neighbors method (XShift) was used to identify phenotypically distinct populations on the continent in an unbiased manner (B). 35 populations were identified, a heat map corresponding to select marker expression levels was generated (C). Lastly, a second heat map comparing the population frequencies identified in B, was generated to illustrate the phenotypic changes induced because of SL-279252 administration (D). Taken together, these observations pointed to shifts in the memory (CD45RO/CD197) and proliferation (ki67) states of T cells resultant to SL-279242 administration lasting multiple days post infusion.

## Conclusions

- Administration of SL-172154 (CD40) and SL-279252 (OX40) stimulated rapid egress of target cells from the blood.
- An integrated assessment, termed 'receptor engagement', was developed to integrate RO both on circulating cells and those that rapidly marginated.
- When CD40$^+$ or OX40$^+$ cells returned to the blood, they remained drug-bound, indicating that the compounds may piggy-back on target cells into tissues.
- SL-279252 (OX40) adminstration led to phenotypic changes in T cell populations lasting at least 5 days post infusion.