3 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 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 (SIRP α -Fc-CD40L) and SL-279252 (PD1-Fc-OX40L) are two bi-functional fusion proteins in phase I clinical trials (NCT04406623 and NCT03894618). The binding interaction between a biologic compound and its targets (CD47 or PD-L1) typically does not stimulate migration from the blood, allowing direct measurement of drug and target. Evaluation of RO for immune agonists (CD40 and OX40) is challenging because agonists can stimulate lymphocytes 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).

Abstract 3 Figure 1 CD4⁺ OX40⁺ T cells and SL-279252

Methods To assess full receptor engagement of CD40 or OX40, the formula shown in figure 1b was derived. The formula captures 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.

Results Within 2 hours of SL-172154 infusion at 1 mg/kg (n=3), CD40⁺ B cell counts decreased from a pre-dose average by 87%. Of the CD40⁺ B cells remaining in the blood, ~95% were bound with SL-172154. CD40⁺ B cell counts recovered in the blood by the next dose, and as counts increased so did the proportion of cells that were bound with SL-172154. Similarly, within 2 hours of SL-279252 infusion at 1 mg/kg (n=10), CD4⁺OX40⁺ T cell counts decreased from a pre-dose average by 41.5% (range 0 – 70%). Of the CD4⁺OX40⁺ cells remaining in the blood, ~32% were bound with SL-279252. CD4⁺OX40⁺ cells returned to pre-treatment numbers over a 7-day interval and nearly all cells remained bound with SL-279252. Taken together, these data demonstrate that a large proportion of CD40⁺ (SL-172154) or OX40⁺ (SL-279252) cells bind drug immediately post infusion, migrate from the blood, and slowly return to the blood with drug still bound to the cell surface.

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 derive 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.

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Ethics Approval This study is being conducted in full conformity with the Declaration of Helsinki and was approved by all IRBs/ethics committees from each clinical site participating in the study. Specific approval numbers can be provided upon request.

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