1359 NTX-0471, ENGINEERED MULTIVALENT SIRPA AND BISPECIFIC SIRPA-ANTICCR4 MOLECULES DEMONSTRATE SUPERIOR ACTIVITY PROVIDING PATH FOR MRNA EXPRESSED *IN-VIVO* BIOLOGICS

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Background A transformative approach to medicine involves expressing complex and therapeutically relevant biologics utilizing patients' own body and tissues via mRNA administration. Here, we engineered complex molecules targeting CD47, as multivalent SIRPa-Fc fusion proteins or bi-specifics targeting both CD47 and CCR4 molecules. CD47 is a widely expressed transmembrane glycoprotein that sends 'do not eat me' signals to macrophages by binding to signal regulatory protein alpha (SIRPa). With more than 50 ongoing clinical trials, anemia is the most common adverse effect due to the expression of CD47 on the Red Blood Cells (RBCs).[1] We show that *in-vitro* activity, selectivity, and *in-vivo* efficacy could be significantly improved either by increasing the valency or by targeting dual antigens via mRNA produced proteins.

Methods The SIRPa N-terminal IgV domain was genetically fused to IgG1 Fc to generate bivalent, tetravalent, hexavalent, and octavalent molecules as well as bispecific molecules by combining with anti-CCR4 binding domain (figure 1). We produced, characterized, and compared these molecules with the bivalent SIRPa Wild Type-Fc and SIRPa High Affinity (HA)-Fc[2] proteins that are similar to the molecules currently in clinical trials. Specifically developed western and mass spectrometry methods were employed to assess the purity of the *in-vivo* expressed proteins following IV administration of mRNA in Nutshell® nanoparticles. Antibody-dependent cellular cytotoxicity (ADCC) assay as well as Raji xenograft mouse model was utilized to demonstrate the activity and efficacy.

Results We observed that the activity of octavalent SIRPa WT was similar to that of the affinity improved (>50000-fold) SIRPa HA[2], however, it showed low to no binding to RBCs *in-vitro* potentially mitigating anemia risk (figure 2). Further mechanistic studies demonstrated a direct correlation between valency and target density, suggesting a role for the avidity effect. The observed activity could be further improved with addition of aCCR4 Fab, leading to >1000-fold improvement in EC50. Administering mice with formulated mRNAs encoding the tetravalent, octavalent, and bispecific molecules resulted in robust protein expression (~10–100ug/ml) with high purity/homogeneity similar to or better than the DNA expressed proteins. Further, the engineered molecules fully eradicated established subcutaneous tumors in the Raji xenograft mouse model (figure 3).

Conclusions We demonstrate that significant enhancement in therapeutic window and efficacy could be achieved by engineering complex multivalent and bispecific molecules. Further, systemic delivery of mRNA encoding these complex molecules avoids the need to produce and retain stability using the traditional cell line development paradigm which is often challenging.

REFERENCES

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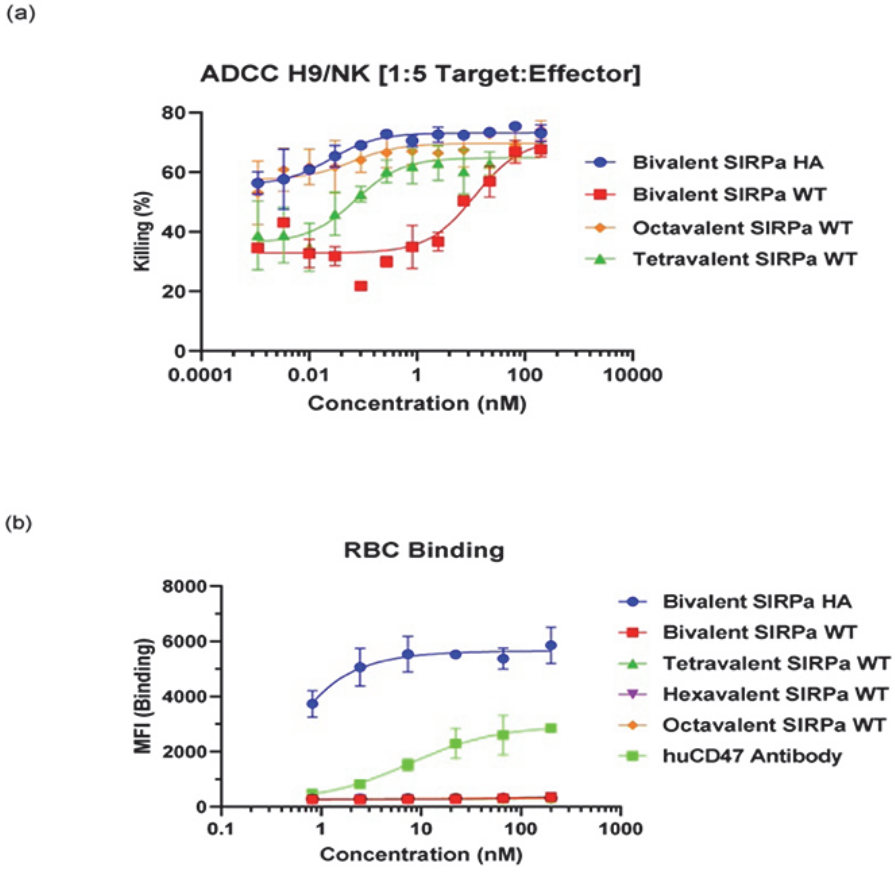
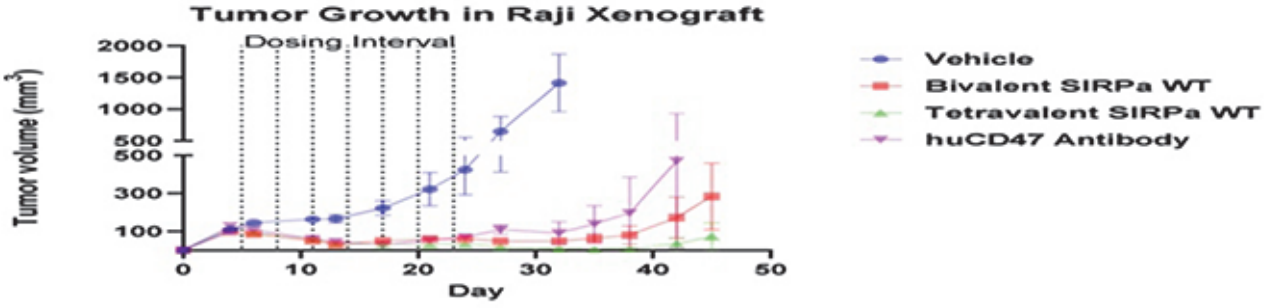
Abstract 1359 Figure 1 Schematics showing examples of engineered multivalent and bispecific molecules. The N-terminal domain of the human SIRPa wild type (WT), which binds to the CD47, is fused to human IgG1 Fc to generate bivalent, tetravalent, hexavalent, and octavalent molecules. For the multivalent bispecific molecules, the Fab sequence derived from the Mogamulizumab (anti CCR4) was utilized. The asymmetric bispecific molecule has knob-hole mutations at the Fc, leading to predominantly heterodimeric architecture



Abstract 1359 Figure 2 (A) ADCC killing assay comparing the novel tetravalent and octavalent proteins to the bivalent SIRPa High Affinity (HA)[2] and WT molecules. The higher valency of the octavalent design leads to activity similar to that of the affinity enhanced SIRPa (HA). However, unlike the bivalent SIRPa HA, the octavalent shows no or little binding to the RBC (B), which helps to widen the therapeutic window



Abstract 1359 Figure 3 Tetravalent SIRPa demonstrates efficacy in the Raji xenograft mouse tumor model. 10e6 Raji cells implanted in CB17 SCID mice and allowed to grow 100–150mm^3. 100ug of protein administered intravenously every 3 days (Q3D) for a total of 7 doses for each non-vehicle arm. Anti huCD47 antibody is used as a positive control. All molecules have huFc lgG1 isotype

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