Adult-onset leukoencephalopathy with axonal spheroids and pigmented glia (ALSP) is a rare, autosomal dominant disorder that is caused by loss of function mutations in the gene encoding for CSF1R (see poster from Papapetropoulos et al.). These mutations result in microglial dysfunction and cell death. CSF1R and TREM2 share a common convergent signaling pathway through phosphorylation of SYK.

**Hypothesis:** Agonism of TREM2 signaling will compensate for CSF1R loss of function in ALSP.

**Objects:**
- Model ALSP in vitro utilizing healthy, human monocyte derived macrophages (MDM) and induced pluripotent stem cell-derived microglia (iMGL).
- Model CSF1R haplosufficiency via pharmacological inhibition (PLX5622) or CSF1R ligand withdrawal.
- Determine ability of VGL101, a TREM2 agonist, to ameliorate effects of CSF1R dysfunction in these models.

**MATERIALS AND METHODS**

**Materials and Methods**

**MIMM:** Peritoneal Myeloid monocytic neurons (PMN) or CD+16monocytes isolated from human hMDM. Cells were incubated at 25,000 cells/ml in 96 well plates coated with 0.2, 1, 10 µg/ml of VGL101 or IgG matched control, at 1:5 PMX5622 was immediately added to the cultures incubated for 72-96 hours. Cells were imaged for 3 days using a IncuCyte S1 analyzer and assessed changes in morphology/phagocytosis. Cell viability was measured using the Promega CellTiter Glo assay.

VGL101 cell microglia from PDCm were thawed on Matrigel-coated 6 well plates for 3 days in complex media. MIMM were then plated into 96 well plates, pre-coated with VGL101 of IgG matched control at 10,000 cells/dish in duplicate. Complex media “withdrawal media” comprised of complete media without any growth factors or bFGF media containing withdrawal media with supplementation of only CMF and IL-13 PMX5622 was added to cells in complex media after 24 hours after plating. After another 24 hours, apoptosis was assessed via Annexin V-FITC. Cell viability was measured at 5 independent replicates using CellTiter Glo.

VGL101 agonist was performed using T Cell Enriched Phospho-SYK (SYK (S252/S253) Alpha-Dia kit according to manufacturer’s instructions. MIMM were treated with half-log concentrations of VGL101 over 48 hours after 6 days of differentiation, and MIMM were treated for 5 minutes, 2 days post-to-plating in 96 well plates.

cSF1R/TREM2: For cSF1R assay, cell culture plates were pre-coated with VGL101 or human IgG matched control. Cell microglia were then added into the plates pre-coated with VGL101 or IgG matched control for 24 hrs. For cTREM2 assay antibody was added into up to 3 independent concentrations. Soluble cSF1R and soluble TREM2 levels were measured using Abscon CSF1R and TREM2 ELISA kits, respectively, per manufacturer protocol.

**RESULTS**

**Figure 1:** VGL101 is a Noncompetitive Agonist of Phospho-SYK in Both MIMM and iMGL

**Possible Mechanism:** VGL101 rescued disease phenotypes in both cell types and in two models. VGL101 treatment induced phosphorylation of SYK in both models, in line with Schlepckow et al. (2014) and Schlepckow et al. (2016). Together, these data demonstrate that TREM2 agonism is a viable mechanism to compensate for CSF1R dysfunction in vitro, supporting further evaluation of VGL101 as a viable therapeutic for ALSP.

**REFERENCES**