# (PF1259) TARGETING DCN1 OVERCOMES DELETERIOUS EFFECTS OF BROAD NEDDYLATION INHIBITION AND ENABLES FETAL HEMOGLOBIN INDUCTION IN SICKLE CELL DISEASE

**Topic:** 27. Sickle cell disease

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## Background

Sickle cell disease (SCD) is a genetic disorder caused by a point mutation in the beta-globin gene characterized by hemolysis, vaso-occlusive crisis (VOC), and pain. Human genetic studies and therapeutic approaches have demonstrated that increasing fetal hemoglobin (HbF) reduces VOCs and ameliorates SCD progression. The standard of care, hydroxyurea, induces HbF, but has side effects such as myelosuppression and genotoxicity with high variability in patient responses. Recent approval of an autologous cell-based gene therapy, targeting the BCL11A enhancer, has demonstrated the curative potential of HbF in SCD. However, this therapy remains limited due to inaccessibility and high cost and risk of toxicity. Unmet need in SCD remains high, with a strong interest in oral HbF inducers potentially overcoming access challenges.

## Results

We dissected human erythropoiesis using single cell transcriptomics and derived cellular trajectories with discovery of neddylation as a regulator of HbF. Pan-neddylation inhibition using MLN4924 or knockout of ubiquitin conjugating enzyme E2M (UBE2M) or Cullin 3 (CUL3) induced HbF accompanied with cytotoxicity. Using single cell transcriptomics, we identified that pan-neddylation inhibition, though efficacious in inducing HBG, also caused erythropoiesis to skew toward an aberrant cell state. We therefore investigated CUL3 regulation to preserve robust HbF induction avoiding lineage skewing.
We explored a neddylation protein-protein interaction sub-network pruned of nodes with significant impact on cell viability using the cancer Dependency Map CRISPR viability dataset. This revealed Defective in Cullin Neddylation 1 (DCN1), a scaffold protein bridging UBC12 and CUL3 facilitating neddylation. Genetic knockout and pharmacological inhibition of DCN1 in CD34 cells, induced both HBG and HbF. Pharmacologic inhibition of DCN1 led to a 2.5-fold increase in HBG expression (EC50 = 17.5nM)25% HbF by HPLC, and a 3-fold increase in HBG/HBB ($p<0.001$). Mechanistic deconvolution revealed that DCN1 inhibition selectively induces HBG via globin switching through partial suppression of CUL3 neddylation, without impairment of erythropoiesis. DCN1 inhibition resulted in differential regulation, higher chromatin accessibility and transcriptional activity at the HBG loci, via known activators such as NFYA. These observations provide the first evidence linking DCN1 inhibition and globin locus regulation.
Using structure guided chemistry, we generated a first-in-class DCN1 inhibitor, CLY-124. In NBSGW mice engrafted with human CD34 cells, CLY-124 administration led to a 4 -fold induction in HBG and up to 30% HBG over total globin gene expression ($p<0.0001$), 2-fold greater than hydroxyurea and approaching levels reported for BCL11A enhancer knockout. In addition, DCN1 inhibition combined with hydroxyurea induced >25% HbF *in vitro* and a robust synergistic induction *in vivo*.

**Summary/Conclusion**

In summary, we identified DCN1 as a novel target for the treatment of SCD, and discovered a first-in-class inhibitor, CLY-124, that induces HbF without cytotoxicity as monotherapy and shows synergy with hydroxyurea. At pharmacologically active doses in preclinical studies, CLY-124 exhibited a favorable safety profile with no observed toxicities or cytopenias. CLY-124 is being evaluated in a first-in-human study assessing safety, pharmacokinetics, and HbF induction in healthy volunteers and participants with SCD.