



The 66th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

113. SICKLE CELL DISEASE, SICKLE CELL TRAIT, AND OTHER HEMOGLOBINOPATHIES, EXCLUDING THALASSEMIA: BASIC AND TRANSLATIONAL**Cereblon-Based Compounds for Induction of HbF**

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Fetal hemoglobin (HbF) induction is a well-known strategy for the treatment of hemoglobinopathies such as Sickle Cell Disease (SCD) and Thalassemia, worldwide life-threatening conditions. Increased levels of HbF are able to prevent HbS polymerization and mitigate disease manifestations, resulting in lower morbidity and mortality in SCD. Nowadays there are only four drugs approved by the FDA for SCD treatment: Hydroxyurea, voxelotor, L-glutamine and crizanlizumab, while the search for new effective and safe treatments are scarce. Controlling the expression of the gamma-globin gene by inhibiting or degrading epigenetic targets has been shown to be promising for HbF induction, and histone deacetylases (HDACs 1 and 2) stand out as potential targets.

Targeted protein degradation (TPD) was explored in this work with the synthesis of new cereblon-based molecules able to anchor to cereblon and HDACs 1 and 2 simultaneously, leading to their dose dependent decrease via polyubiquitination and proteasome degradation. Six new cereblon derivatives containing a HDAC inhibitor subunit were designed, synthesized and evaluated in HCT-116 cells for HDAC 1, 2 and 3 degradation via quantitative western blot at concentrations ranging from 0.01 to 10 μ M. The promising results showed that three of these compounds (ARP-26, ARP-37 and ARP-49) were able to degrade HDAC-1 more selectively, when compared to HDAC-2 and HDAC-3, achieving DC_{50} values of 2.5 μ M. A derivative with no ability to bind to cereblon was also synthesized resulting in no degradation of HDACs 1-3, suggesting that these compounds act via cereblon binding. ARP-49 was then selected for further studies in HUDEP-2 cell culture at concentrations of 500 and 750nM, and incubation time of 72 and 96h. After the treatment, cells were stained with HbF-antibody and the percentage of HbF positive cells was assessed by flow cytometry. The quantification of HBG1/2 mRNA was obtained by qPCR. HUDEP-2 cells exhibit a basal level of HbF positive cells lower than 1%, then the results obtained from our compounds were normalized based on the control. At 72h, the concentrations of 500nM and 750nM were able to significantly increase the HBG1/2 mRNA levels by 26.9-fold and 35.5-fold, respectively (CTRL = 0.027 ± 0.006 arbitrary units (a.u.) vs 500nM = 0.73 ± 0.25 a.u. and 750nM = 0.96 ± 0.11 a.u., $p < 0.0001$, $n=4$). At 96h, both concentrations were also able to significantly induce the expression of the HBG1/2 genes by 34-fold and 44.5-fold, respectively (CTRL = 0.034 ± 0.012 a.u. vs 500nM = 1.12 ± 0.35 a.u. and 750nM = 1.52 ± 0.37 a.u., $p < 0.0001$, $n=4$). HbF-positive cells at 72h presented a significantly increase of 7.5-fold and 9-fold at 500 and 750nM, respectively (CTRL = 0.58 ± 0.15 % vs 500nM = 4.43 ± 0.88 % and 750nM = 5.29 ± 1.52 %, $p < 0.0001$, $n=4$), while at 96h the increase was 8.37-fold and 10.6-fold, respectively (CTRL = 0.65 ± 0.15 % vs 500nM = 5.44 ± 0.79 % and 750nM = 6.89 ± 1.05 %, $p < 0.0001$, $n=4$). HUDEP-2 cells were also treated with HU at 100 μ M, reaching its highest level of HBG1/2 expression at 72h (10-fold), when compared to control, and an increase in the HbF-positive cells population by 8.5-fold and 8.9-fold at 72h and 96h, respectively (CTRL 72h = 0.66 ± 0.23 % vs HU 72h = 5.65 ± 0.59 %, CTRL 96h = 0.64 ± 0.15 % vs HU 96h = 5.73 ± 0.52 %; $p < 0.0001$, $n=2$).

Compounds obtained in this study present a degradation selectivity towards HDAC-1 with no previous description in the literature. Furthermore, the ability to significantly induce HBG1/2 expression in HUDEP-2 cells was superior to the one seen for the standard SCD treatment, HU, with levels of HBG1/2 expression 2.6-fold higher (500nM, 72h), at a concentration 200-

fold lower. These findings support that ARP-49 shows promising potential for further pre-clinical studies for increasing the production of HbF.

Disclosures No relevant conflicts of interest to declare.

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