Small activating RNA-mediated induction of HBG via liposome delivery for *in vivo* treatment of sickle cell disease and beta-thalassemia

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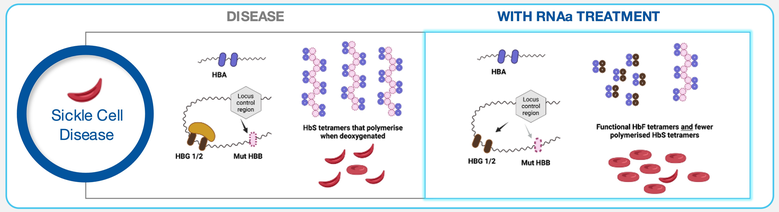
Background

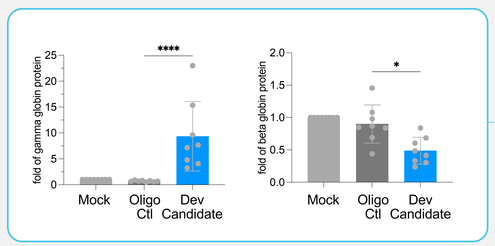
- Hemoglobinopathies affect 7% of the world's population, with forms such as Sickle Cell Disease (SCD) affecting 300,000 newborns / year[1].
- Upregulation of fetal haemoglobin (HbF) is a clinically-validated approach to treat beta-hemoglobinopathies[2].
- saRNAs have been developed by MiNA therapeutics as a specific method of transcriptional activation of target genes[3,4,5].
- Efficient *in vivo* delivery may expand the potential of nucleic acid therapeutics to treat beta-haemoglobinopathies, which would be favourable as it would not require myeloablative preconditioning or autologous cell engineering required by other SCD therapeutics[3].
- NOV340 is an amphoteric liposome containing non-immunogenic lipid components with a demonstrated NOAEL of 18mg/kg in NHPs.
- NOV340-formulated small activating RNA (saRNA) against the *CEBPA* gene (MTL-CEBPA) has proven to be safe and well-tolerated treatment in over 130 patients when delivered intravenously (IV) with repeat dosing for over 1 year; clinical PD seen in human monocytes[4,5] (Plummer *et al.,* Cell Reports Medicine, *In Press*).
- We previously demonstrated efficient *in vivo* delivery of an saRNA payload formulated in NOV340 to approximately 60% of erythroid progenitors in the bone marrow (*Keystone Delivery of Oligonucleotides Meeting 2024, Abstract #2015*).
- Here, we describe the discovery and characterisation of MTL-HBG, an saRNA targeting *HBG* encapsulated in NOV340, as a development candidate for SCD.



HBG saRNA mediated transcriptional upregulation of gamma globin genes and protein; gamma globin replaces mutant beta globin within haemoglobin tetramers, diluting the impact of HbS, reducing sickling, and ameliorating symptoms in patients with Sickle Cell Disease.

saRNA Upregulates Gamma Globin and Downregulates Beta Globin

- The saRNA development candidate MT011391 induces persistent gamma globin protein induction in human primary erythroid progenitor cells (average=9.4X, range=3.3 to 23X fold), and decreases beta globin protein (average=0.49X, range=0.23 to 0.83X)

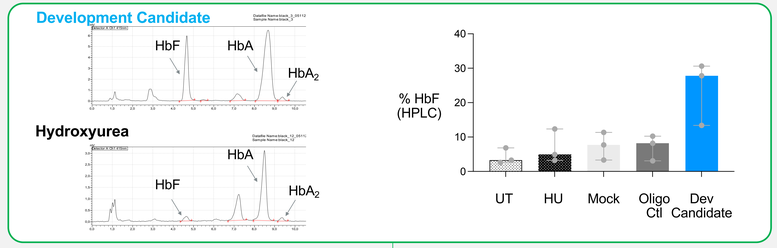


Activity of candidate saRNAs were evaluated in erythroid progenitor cells derived from primary human bone-marrow isolated CD34+ cells using a two-phase culture system (expansion followed by erythroid differentiation). Cells were nucleofected with 2μM saRNA at day 1 of differentiation. Protein for gamma and beta globin was assessed after 7 days by digital Western blot. Negative controls included a nucleofection only control (mock) and an oligo with no human genome homology. Data represent eight independent donors. Significance determined with Kruskal-Wallis test.

References: 1 - PMID: 37331373, 2 - PMID: 23103089, 3 - PMID:34407972, 4 - PMID: 28882451, 5 – PMID: 32357963, 6 – PMID: 24222332, 7 – PMID: 30092854, 8 – PMID: 34139105, 9 – PMID: 9028341

Development Candidate Has Superior Induction of % HbF Compared with Hydroxyurea

- saRNA development candidate MT011391 is superior in inducing HbF (average=3.6X, range 2.7-4.4X), as measured by HPLC, to that achieved with hydroxyurea (HU) (average 1.5X, range 1.2-1.8X). The level of induction achieved exceeds the clinical protective threshold (20%).

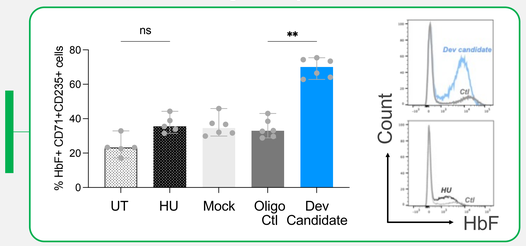


% HbF induction was measured by HPLC in expanded primary human erythroid progenitor cells after 7 days of differentiation and treatment with either 10μM hydroxyurea or nucleofection with 2μM saRNA, with appropriate controls. Representative HPLC histograms for one donor treated with either saRNA or HU (left). Data represent three independent donors (right).

Development Candidate Induces Pancellular HbF

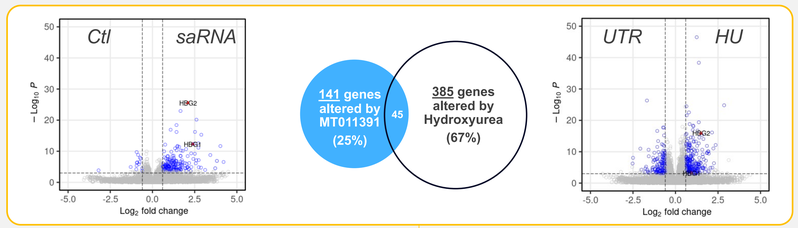
- Pancellular HbF is a key attribute for maximum therapeutic effect of HbF induction[6]. The saRNA development candidate MT011391 induces pancellular HbF that persists for at least 7 days in ErPs. Hydroxyurea also increases HbF, albeit non-significantly.

HbF expression was measured at a single cell level using antibody clone 2D12 in bone marrow-derived differentiated primary erythroid cells, 7 days after treatment with HU or nucleofection with saRNA. Data are representative of six independent donors (left). Representative histograms from one donor (right). Significance determined with Kruskal-Wallis test.



RNA-Seq Demonstrates On-target Specificity

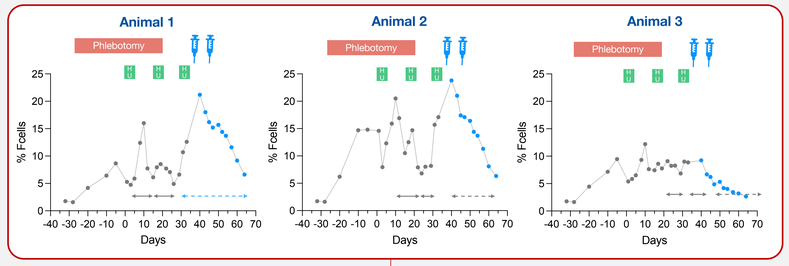
- Hybridisation-dependent *in silico* prediction within 3'UTR regions in the transcriptome indicates MT011391 has no off-target hits with 2 or less mismatches outside the seed region and 5 hits with 3 mismatches out of the seed region.
- Experimentally, fewer genes were differentially expressed between MT011391 and oligo ctl, compared to between HU and untreated.



Off-target profile of saRNA development candidate, MT011391, was determined by nucleofection of HUDEP2 cells with 2μM MT011391 or Ctl for 72 hours, followed by RNA-Seq (left). HUDEP2 cells were treated with 10μM HU every 24h for 72h, then compared to untreated (UTR) by RNA-Seq (right). Lines; +/- 0.6 Log2FC, P value 0.001. Genes in blue; adjusted P < 0.01 and +/- 0.6 Log2FC. The overlap of genes significantly altered by MT011391 (cf. Ctl) and HU (cf. UTR) is shown by Venn diagram (middle).

Pharmacodynamic Activity of Development Candidate Demonstrated in NHP

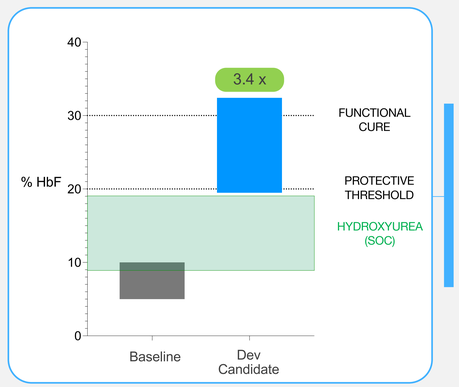
- Pharmacodynamic activity of MTL-HBG was assessed in non-human primates (NHP) that had previously undergone phlebotomy and HU treatment to induce F cells (pre-saRNA %F cell levels are lower in this model than seen in SCD patients). Pharmacodynamic activity was observed following treatment with MTL-HBG. The third animal had a muted response of HbF induction to phlebotomy, HU, and MTL-HBG.



MT011391 was formulated in NOV340 liposomes and evaluated in pilot study of previously phlebotomised Cynomologus Macaques after pre-treatment with hydroxyurea (n=3)[7]. Two doses of MTL-HBG were administered 3 days and 10 days post hydroxyurea. Animals were dosed with 100 mg/kg of hydroxyurea daily for 5 days orally, and MTL-HBG at 10 mg/kg (of saRNA), intravenously. Red dots indicate pre-treatment period, blue dots indicate saRNA treatment period.

Quantitative Systems Pharmacology Predicts Best-in-Class Activity

- A quantitative systems pharmacology model[8] was used to predict the activity of MTL-HBG in SCD patients. The model predicts an average 3.4X increase in %HbF post treatment, implying that almost all patients with at least 5% baseline HbF can reach %HbF levels that exceed the clinical protective level of 20%.



Delivery efficiency of NOV340-saRNA-Cy3 to target ErP cells in Cynomolgus Macaque bone marrow after IV injection is in excess of the predicted threshold needed for a transformational therapeutic for haemoglobinopathies. This delivery percentage approximates that observed in monocytes, in which clinical PD has been previously demonstrated. Simulated levels of HbF protein in blood assuming Q4W i.v. administration of 4 mg/kg in SCD patients. Simulation based on delivery to 40% of BFU-E and CFU-E. Seven-fold increase in HBG; 30% reduction in HBB. An average adult response to hydroxyurea is shown in green (1.8X[9]).

Conclusions

saRNA can be delivered to therapeutically relevant ErP cells *in vivo* using a clinically established liposomal formulation, NOV340. NHP data demonstrates delivery to greater than 60% of committed ErPs and a PD response. The development candidate induces persistent, pancellular levels of %HbF that exceed the protective threshold of 20%. The compound is potent, does not induce cytotoxicity, and has a clean off-target profile. saRNA therapeutics have the potential to be a transformational *in vivo* treatment for Sickle Cell Disease.