NOV340 liposome encapsulating nucleic acid payload achieves efficient biodistribution to erythroid progenitor cells

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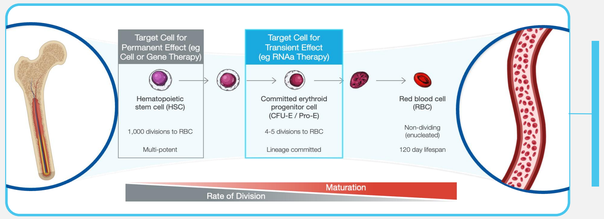
Background

- 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 well-validated approach to treat beta-hemoglobinopathies[2].
- Evidence from allogeneic bone marrow transplant suggests inducing sufficient expression of HbF in **at least 20% of committed erythroid progenitors (ErP)** is predicted to translate into a transformational SCD therapy[3,4].
- Efficient *in vivo* delivery may expand the potential of nucleic acid therapeutics to beta-hemoglobinopathies which would be beneficial as they do not require myeloablative preconditioning or autologous cell engineering required by other SCD therapeutics[5].
- Efficient in vivo delivery would allow the development of RNA activation (RNAa) using small activating RNA (saRNA) against HbF for the treatment of beta-hemoglobinopathies.



Analysis of the differentiation of ErPs identifies that optimal pharmacology with RNAa requires delivery to committed ErP cells such as CFU-E and ProE cells. These target cell will differentiate into RBCs within the pharmacodynamic window of RNAa against HbF allowing for restoration of pathology in these cells.

Clinical Proof of Concept for Liposome-formulated saRNA

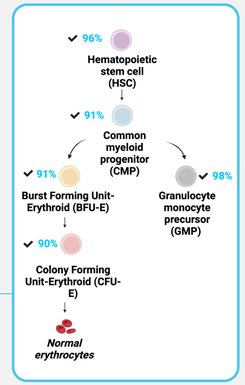
- saRNAs have been developed by MiNA therapeutics as a specific method of RNAa potentiating the transcription of target genes[6].
- NOV340 is an amphoteric liposome containing non-immunogenic lipid components with a demonstrated NOAEL of 18mg/kg in NHPs.
- Efficient uptake in myeloid cells without sequestration in hepatocytes has been achieved.
- NOV340 formulated saRNA against the CEBPA gene (MTL-CEBPA) has proven to be safe and well-tolerated treatment in over 130 patients when delivered IV, repeat dosing for in excess of 1 year has been demonstrated to be well-tolerated.
- Clinical PD data demonstrates target engagement in monocytes.

References: 1 - PMID: 37331373, 2 - PMID: 23103089, 3 - PMID: 28887325, 4 - PMID:2729929, 5 - PMID: 9028341, 6 - PMID: 28882451

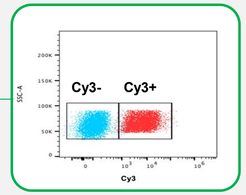
Human – *In Vitro* Delivery to Bone Marrow Derived Erythroid Progenitor Cells

- Incubating healthy donor blood with NOV340-saCEBPA-Cy3 confirmed tropism to myeloid cells. Further *in vitro* work with bone marrow-derived (BM) CD34⁺ cells indicated delivery to different stages of erythroid differentiation, with >90% labelling of committed ErP cells, and burst forming unit-erythroid (B/CFU-E) cells.

Summary of Cy3+ cell biodistribution to populations of ErPs after 24hr treatment. Two different flow cytometry panels were designed to study delivery efficiency into human bone marrow stem and progenitor cells from 3 different donors using NOV340-saCEBPA-Cy3. % refers to the median percentage of Cy3 positive cells across key populations.



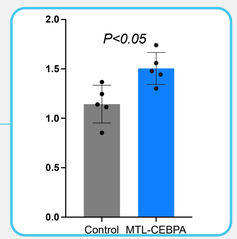
Representative FACS plot showing cells treated with 35 ug/ml NOV340-Fluc (non-targeting control) and 35 ug/ml NOV340-saCEBPA-Cy3 for 24 hours in one donor. Blue cells – NOV340-Fluc (not conjugated to Cy3). Red cells – represent samples treated with NOV340-CEBPA-Cy3.



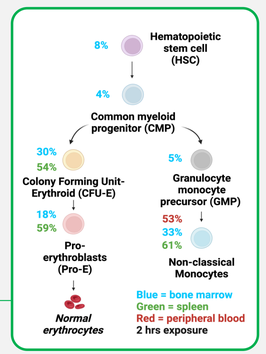
Mice – *In Vivo* Delivery to Bone Marrow Resident Erythroid Progenitor Cells

- Single tail vein IV administration of NOV340-saCEBPA-Cy3 provides *in vivo* evidence of delivery to erythroid progenitor cells and nuclear localisation of delivered saRNA in the bone marrow. NOV340-saCEBPA demonstrates prolonged PD in mice.

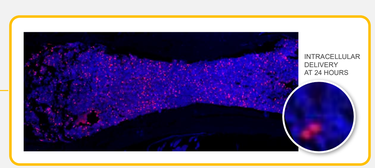
qPCR analysis of CEBPA mRNA levels in bulk, unsorted bone marrow cells extracted from the femur 4 weeks after single IV administration by tail vein injection of 4mg/kg MTL-CEBPA. Functional pharmacodynamic activity was observed at additional timepoints during an 8 week study.



Summary of the in vivo biodistribution of 4mg/kg NOV340-CEBPA-Cy3 in C57/BL6J mice after single IV administration by tail vein injection. % refers to the median percentage of Cy3 positive cells across key populations in the bone marrow, spleen and blood after 2 hours. 10-15 mice across one or two independent experiments.

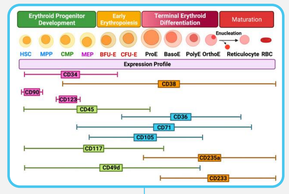


Representative image of mouse hind leg bone 24 hours after IV delivery of 4mg/kg NOV340-CEBPA-Cy3. Bones were sectioned from FFPE blocks for direct detection of Cy3 fluorescence. Whole femur bones with inset magnified image demonstrating nuclear delivery of CEBPA-Cy3 saRNA. Whole femur image shown at 2X magnification

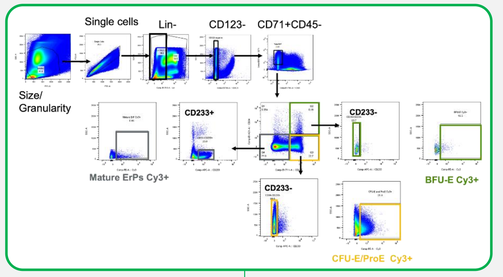


NHP – *In Vivo* Delivery to Bone Marrow Resident Erythroid Progenitor Cells

- In order to characterise delivery to specific subsets of ErPs the following flow cytometry panels and gating strategies were employed.

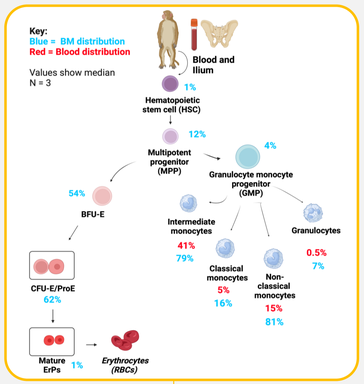


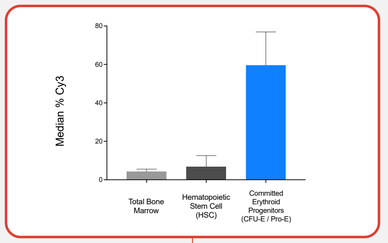
Schematic representation of differential expression patterns of marker proteins on different cellular subsets during differentiation of ErPs. From PMID: 37172755



Gating strategy for ErP subset characterisation. Cells were firstly gated based on side and forward scatters (SSC-A and FSC-A), followed by doublet (FSC-A and FSC-H) and lineage positive cell exclusion. Next, CD123- cells were selected. CD71+CD45- cells were then gated for the expression of CD117 and CD34. Each subset was gated for the expression of CD233. BFU-E subset was identified as CD117+CD34+CD233- (green). CFU-E/ProE cells were identified as CD117+CD34-CD233- population (yellow). Mature ErPs were identified as CD117-CD34-CD233+ population (dark grey). Cy3 fluorescence of these populations was then analysed.

- Single saphenous vein IV administration of NOV340-saCEBPA-Cy3 provides *in vivo* evidence of delivery to erythroid progenitor cells in NHPs. Equivalent delivery was observed in ErPs and monocytes, clinical PD readouts has been demonstrated with MTL-CEBPA in monocytes.





Delivery of NOV340-CEBPA-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 demonstrated.

Summary of the in vivo biodistribution of 4mg/kg NOV340-CEBPA-Cy3 in Cynomolgus Macaques after single IV administration by saphenous vein injection. % refers to the median percentage of Cy3 positive cells across key populations in the bone marrow, and blood after 24 hours. Combined data from 3 independent repeats.

Conclusions

saRNA can be delivered to therapeutically relevant ErP cells *in vivo* using a clinically established liposomal formulation. Rodent data demonstrates PD response. NHP data demonstrates delivery to greater than 60% of committed ErPs, and equivalence to monocytes in which clinical PD has been demonstrated. This delivery data provides foundations for the development of transformational RNAa therapeutics to treat beta-hemoglobinopathies. saRNA therapeutics have the potential to be a simpler treatment paradigm with lower treatment burden for patients when compared to *ex vivo* cell and gene therapy approaches.