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POSTER ABSTRACTS

801.GENE THERAPIES

Phenotypic HSPC Rescue By RNA Lipid Nanoparticles in a Murine Model of Fanconi Anemia

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Background: Fanconi Anemia (FA) is a recessively heritable multisystem disorder that manifests with bone marrow failure in early childhood. Current gene therapy trials for FA rely on transplantation of *ex vivo* transduced autologous hematopoietic stem cells (HSCs). This approach is limited by poor mobilization efficiency and a depleted HSC pool in FA patients. The recent use of mRNA encapsulated within lipid nanoparticles (LNP ^{mRNA}) for cancer immunotherapy and vaccines has created an interest in adapting mRNA delivery for protein replacement therapies. Phase 1/2 trials have demonstrated both safety and efficacy for *in vivo* treatment of multiple monogenic metabolic disorders. We hypothesized that LNP ^{Fancc} could be developed as an *in vivo* protein replacement therapy for FA.
Methods: Custom mRNA transcripts modified with N1-methylpseudouridine and packaged into LNPs (diameter 80 ± 5nm; PDI 0.01), their performance was tested *ex vivo* and *in vivo*. LNP ^{mRNA} were delivered either intrafemorally or by tail vein injection into *Fancc* $^{-/-}$ mice, a mouse model of FA or Ai14 mice, a Cre-sensitive reporter mouse model. We also tested LNP performance using *ex vivo* expanded hematopoietic stem and progenitor cell (HSPC) populations and fresh CD150 $^{+}$CD48 $^{-}$Lin $^{-}$Sca1 $^{+}$ckit $^{+}$ (long-term hematopoietic stem cells; LT-HSC) cells obtained from wildtype (WT, *Fancc* $^{+/+}$) and knockout (KO, *Fancc* $^{-/-}$) mice. LNP formulations delivered mRNA transcripts of the fluorescent reporter mCherry (LNP ^{mCherry}), cre-recombinase (LNP ^{Cre}), luciferase bioluminescent reporter gene (LNP ^{Luc}) or a truncated murine *FANCC* gene (LNP ^{Fancc}).
Results: To characterize access to the bone marrow compartment *in vivo*, we delivered LNP ^{mCherry} or LNP ^{Cre} via systemic (intravenous) and direct (intraosseous) routes to healthy WT and Ai14 mice. Using LNP ^{Cre}, we achieve up to 92.6% ± 5% tdTomato reporter expression in Lin $^{-}$Sca1 $^{+}$ckit $^{+}$ (LSK) cells 5 days following *intraosseous* delivery. Consistent with reports by others, we demonstrate lower average transfection rate of 24.8% ± 15.9% fluorescent positivity rates in LSK cells after intraosseous LNP ^{mCherry} delivery, and 9.5% ± 6.7% after intravenous delivery. mCherry fluorescent signal was detectable by FACS for up to 72 hours following LNP ^{mCherry} injection. We reasoned that meaningful FA HSC correction would require more extended protein expression. Circular mRNA shows improved biostability and durability of expression. Comparing both linear and circular mRNA performance, we observed that circularized LNP ^{Luc} is expressed for up to 7 days post *ex vivo* exposure in LT-HSC populations as compared to 3 days when using linear LNP ^{Luc}. Functionally, *ex vivo* LNP ^{Fancc} treatment improves proliferation rates and Mitomycin C (MMC) resistance in colony forming unit (CFU) assays of *Fancc* $^{-/-}$HSPC. Here, LNP ^{Fancc} treated HSPC demonstrate improved MMC survival rates reaching 37.2% ± 10.5% up from 16.1% ± 8.2%. *Ex vivo* expanded *Fancc* $^{-/-}$ HSPC demonstrate an even greater benefit from LNP ^{Fancc} treatment, with CFU survival rates increasing to 62.7% from a baseline of 3.3%. Finally, we demonstrate improved repopulation of LNP ^{Fancc} treated HSPC 2 weeks after intravenous delivery to myeloablated recipients, measuring up to 73.9% donor chimerism after treatment with circular LNP ^{Fancc} compared to 50% among untreated (no LNP) controls.
Conclusion: Our studies show that LNP ^{Fancc} can rescue *Fancc* $^{-/-}$ HSPC *in vitro*, and ongoing experiments reveal improved repopulation *in vivo*. Along with evidence for efficient *in vivo* delivery to the bone marrow, our data support the use of LNP ^{mRNA} as a potential *in vivo* treatment of bone marrow failure in FA patients.

Disclosures Rivella: *GSK:* Consultancy, Ended employment in the past 24 months; *Disc Medicine:* Membership on an entity's Board of Directors or advisory committees; *Vifor:* Membership on an entity's Board of Directors or advisory committees; *Meira GTx:* Membership on an entity's Board of Directors or advisory committees; *Ionis Pharmaceuticals:* Membership on an entity's Board of Directors or advisory committees; *BMS:* Consultancy, Ended employment in the past 24 months; *Incyte:* Consultancy,

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