mRNA-LNP *ex vivo* interactions with human whole blood

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As structure-function relationships for mRNA-LNPs remain unpredictable, screening for mRNA-LNP performance and effects in biological systems is an inherent component of mRNA-LNP development. Although these in vitro and in vivo screening efforts are indispensable, clinical failure rates, as for any other pharmaceutical, are expected to be high also for mRNA-LNPs.

To evaluate the utility of human blood in mRNA-LNP development, we exposed heparinized fresh human blood from healthy donors (n=6) to mRNA-LNPs and assessed the interactions between mRNA-LNPs and cells (monocytes, neutrophils, T-cells, NK-cells).

In line with expectations, we observed (using flow-cytometry) that monocytes incorporated the highest levels of mRNA-LNPs, while T-cells were associated with minimal levels of mRNA-LNP interactions. Furthermore, all cells continued to take-up material as a function of time during 2 hours of incubation. Due to the large individual variation, the detection of significant effects is challenging. However, by designing a mini-library of mRNA-LNPs in which we systematically varied lipid composition, significant effects can be detected. For example, and also in line with expectations, replacing cholesterol by β-sitosterol significantly increased mRNA-LNP uptake in monocytes, while replacing PEG2000-DMG by PEG2000-DSPE significantly reduced cellular uptake. Cytokine induction is currently being assessed on serum samples stored after the human blood incubation with mRNA-LNPs, using multiplex assays.

We showed that the human blood model can provide useful information on mRNA-LNP interactions with blood cells. We demonstrate it is feasible to deal with large individual variation through the design of mRNA-LNP mini-libraries with systematic variation of their composition. This approach showed that significant effects of individual lipids on mRNA-LNP uptake by different blood cells can be obtained using only 6 donors. We hypothesize that with such an approach, human blood assays can be a useful complementary tool in mRNA-LNP screening and development pipelines.