Title: On the role of PEG-lipids in the development of Lipid Nanoparticles for siRNA delivery

Presenting Author: Teresa Ferrillo, University of Naples Federico II, Napoli, Italy
Co-Authors: Paola De Cicco, Amico Rebecca, Claudia Conte, Francesca Borrelli, Fabiana Quaglia, University of Naples Federico II, Napoli, Italy

Introduction: It is well known that PEG-lipids represent a pivotal component of Lipid Nanoparticles for RNA delivery (LNPs), since the affect key properties such as size, polydispersity, encapsulation efficiency, biodistribution and apparent pKa [1]. Neverheless, some PEG-lipids, which have short alkyl/acyl chain lenghts can detach from LNPs in the blood [2] favouring the absorption of specific proteins, such as APO-E, and their accumulation in the liver.
The aim of the study is to employ an LNP formulation for siRNA delivery combining two different PEG-lipids (a detachable DMG-PEG_{2k} and a persistent DSPE-PEG_{2k}) and evaluate through orthogonal techniques how composition affects LNP physical-chemical properties, biological identity, and transfection efficiency.

Methods: siRNA-loaded LNPs containing DMG-PEG_{2000} (LNP_{DMG}), DSPE-PEG_{2000} (LNP_{DSPE}), and a combination of both ($LNP_{DMG/DSPE}$) (1.5% of total lipids) together with Cholesterol, DSPC, and DLin-MC3-DMA were prepared by the ethanol injection method and were fully characterized for their colloidal properties, encapsulation efficiency and apparent pKa. LNPs behaviour in protein solution (HSA), human plasma and cell culture medium for transfection (DMEM-FBS^{+}) was studied by DLS. Subsequently, the transfection efficiency of siRNALuc-LNPs were evaluated in a colon cancer cell line (HCT116).

Results: All the formulations exhibit similar colloidal properties (Size: ~120 nm, PdI: ~0.18 and Z-Potential: ~ -3 mV) and siRNA encapsulation efficiency (~90%). LNPs were stable in PBS at room temperature, 4°C and 37°C for extended time and increased their size in protein-rich media showing a tendency to aggregation in DMEM-FBS^{+} (48 h, 37 °C). All the formulations were uptaken in HCT116 cells, underwent endosomal escape, and effectively downregulated Luc expression until 72 h even if the LNP_{DSPE} and $LNP_{DMG/DSPE}$ containing the long alkyl chain PEG-lipid require longer times for an efficient transfection.

Conclusions: The results show that the Pegylated lipid type, besides providing colloidal stability, affect the apparent pKa and the biological behaviour of LNPs leading to differences in the transfection efficiency over time. Other studies are ongoing to investigate the impact of short PEG-lipid detachment process on the biological efficiency of LNPs and how this process can be exploited to facilitate the precision targeting of LNPs avoiding liver accumulation.

Acknowledgments: We acknowledge the grant CN00000041 “National Center for Gene Therapy and Drugs based on RNA Technology” (concession number 1035 of 17 June 2022-PNRR MUR - M4C2 - Investment 1.4 Call "National Centers”, financed by EU- NextGenerationEU), project code E63C22000940007.

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Presenter biography: Teresa Ferrillo is a PhD Student in “RNA Therapeutics and gene Therapy” at the University of Naples Federico II, working in the field of LNPs for RNA precision delivery.