

## **Targeting the CD105/Endoglin Pathway in Pulmonary Fibrosis via Inhalable siRNA Delivered by pH-Responsive Lipid@PLGA hybrid Nanoparticles**

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CD105/endoglin, a TGF- $\beta$  co-receptor, was recently demonstrated to be overexpressed on extracellular vesicles from IPF patients, identifying it as a novel fibrosis marker. Despite its therapeutic potential, effective delivery strategies for CD105 downregulation remain limited, underscoring the need for tailored carriers.

With this idea in mind, the general aim of this work is to harness a siRNA against CD105/endoglin (siCD105) for local treatment of IPF through biodegradable hybrid nanoparticles comprising a poly(lactic-co-glycolic acid) (PLGA) core engineered on the surface with lipids (Lipid@PLGA hNPs), especially designed to deliver the RNA payload in the deep airways.<sup>2,3</sup> Here, we evaluate if the addition of ionizable lipids may contribute also to the endosomal escape of the RNA entrapped in hNPs. The role played by ionizable lipids in escaping the endosome has been exploited in the engineering of lipid nanoparticles, first in Onpattro and then in the SARS-CoV-2 mRNA-based vaccines.<sup>4</sup>

iLipid@PLGA hNPs loaded with siRNA against CD105/endoglin were produced by either conventional emulsion-solvent diffusion technique or microfluidic to improve production scalability and throughput. Through an in-depth formulation study, hNPs able to meet critical quality attributes for inhalation were produced and fully characterized showing a spherical shape, size < 200 nm, PDI ~0.150. The pH responsivity was confirmed by the surface charge at physiological and acidic pH, reproducing the endo/lysosomal compartment. In vitro aerosolization studies demonstrated the ability of the optimized siCD105-loaded hNPs to deposit in the deep airways, while the ability to cross the mucus barrier after landing, was evaluated through a combination of DLS measurements and Small Angle X-Ray Scattering analysis. Data confirmed the potential of hNPs as carriers for pulmonary delivery and prompted an in-depth investigation of their therapeutic effectiveness in vitro and in vivo.

A proof of principle for CD105 silencing was established in both normal and diseased fibroblast cell lines, as well as in LPS-stimulated A549 lung epithelial cells, demonstrating downregulation of endoglin, fibronectin, and  $\alpha$ -SMA levels following transfection with siCD105-loaded hNPs, as assessed by flow cytometry. Furthermore, in vivo studies using a bleomycin-induced murine model of pulmonary fibrosis confirmed the effective downregulation of CD105 after intratracheal administration. These findings validate CD105 as a promising therapeutic target and underscore the potential of the hNPs iLipid@PLGA platform for pulmonary delivery.

[1] d’Alessandro, M. et al., *Int. J. Mol. Sci.*, 24(4), 4071, (2023).

[2] D’Angelo, I. et al., *J Aerosol Med Pulm Drug Deliv.*, 31(3), 170–181, (2018).

[3] Conte, G., et al. *ACS Applied Materials and Interfaces*, 14(6), 7565–7578, (2021).

[4] Schlich et al. *Bioeng Transl Med.* 6:e10213 (2021).