Advancing a new nanomedicine for Fabry disease treatment towards clinical translation

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Introduction

Despite advances in the development of new orphan medicines, the number of available therapies for rare diseases remains low. As few as 6% of rare diseases have an approved treatment option and if so, they come with limited effectiveness and high cost. This work addresses the need of cost-effective treatments for Fabry disease (FD), one of the most frequent Lysosomal Storage Disorders (LSD). Mutations in the alpha-galactosidase (GLA) gene, lead to the malfunction or absence of the GLA enzyme and the accumulation of globotriaosylceramide (Gb3) and similar glycosphingolipids in the lysosomes throughout the body, provoking multisystemic clinical symptoms. Of note, endothelial cells are thought to play a central role in the pathophysiology of the disease [1].

The Enzyme Replacement Therapy (ERT), which consists of the intravenous administration of an active recombinant version of the defective enzyme, is the main treatment option for patients with LSDs. However, ERT present limitations due to: (i) poor biodistribution, (ii) incapacity of enzymes to cross the Blood Brain Barrier (BBB); (iii) rapid enzyme degradation and short plasma half-life; and (iv) high immunogenicity. Consequently, frequent dosing is required (every other week) resulting in high-cost treatments.

With the aim of developing more effective ERT for FD, a nanoliposomal formulation that delivers recombinant GLA selectively to endothelial cells has been developed in the frame of the EU H2020 Smart4Fabry Project. The currently granted EU H2020 Phoenix Project pursues the transfer of this new nanomedicine to clinical phase, covering the GMP clinical lot production.

Methods

GLA-loaded nanoliposomes (nanoGLA) functionalized with Arginine-Glycine-Aspartic acid (RGD) peptide were prepared by a one-step, green, and easy scalable method based on compressed CO2, named DELOS-susp, followed by a tangential flow filtration (TFF) procedure [2].

The relevant physicochemical/biological properties critical to product quality were identified and extensively characterized combining different techniques (e.g. DLS, cryoTEM, HPLC, 4-MUG assay). A deep optimization work based on the Quality by Design (QbD) approach was carried out to obtain a nanoformulation with optimal characteristics to scale-up and perform preclinical testing [3].

The efficacy of nanoGLA to metabolize the Gb3 accumulations was compared to the non-nanoformulated GLA, both in vitro and in vivo. In vitro, the ability of nanoGLA to reduce (hydrolyse) the Gb3 substrate was measured by using Nitrobenzoxadiazole (NBD)-Gb3 fluorescent substrate in Mouse Aortic Endothelial Cells (MAEC) derived from Fabry KO mice. This assay measures the ability of the enzyme to internalise in cells, reach the lysosomes and hydrolyse the substrate of the GLA enzyme, offering a rapid and clear assay to test both the internalisation and the efficacy of the tested compound. The in vivo efficacy of the nanoGLA was tested in Fabry KO mice [4] by comparing the Gb3 levels in animals treated with intravenous (IV) administrations of single or repeated doses of nanoGLA, free (non-entrapped) GLA or the commercialized Replagal® at 1 mg/kg. A pharmacokinetic study was also completed in rats, where plasma levels of the total GLA were assessed following IV administration of nanoGLA.

Results

After a deep nanoformulation's optimization process, small, uniform, and mainly unilamellar GLA-loaded RGD-targeted nanoliposomes were successfully prepared by DELOS-susp, with high enzyme entrapment efficiency (>90%), enhanced enzymatic activity, and superior efficacy in cell culture [4]. Moreover, this nanoGLA has provided in vivo

evidence supporting an increase in GLA activity in plasma and higher Gb3 clearance in liver, spleen, lung, heart, kidney, skin and even brain, compared to the non-nanoformulated enzymes (included the commercially available agalsidase alpha or Replagal®) [5]. These results were consistent with an extended half-life compared to the free GLA formulation in a pharmacokinetic study in rats.

Conclusions

DELOS-susp has emerged as a robust manufacturing technology for the preparation of GLA-loaded RGD-targeted nanoliposomes with the consistent quality for further regulatory purposes. This new liposomal formulation of GLA improves the ERT efficacy in preclinical models of FD. Based on the potential clinically relevant advantage of the nanoGLA versus authorized ERTs, the EMA has granted the Orphan Drug Designation [6]. This designation has important implications for the translation of the new therapeutic product from bench to bedside.

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Figures

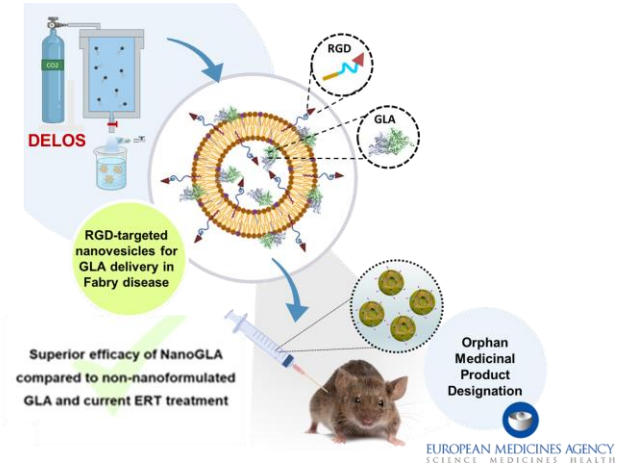

Figure 1. Graphical summary of the milestones achieved with the GLA-loaded nanoliposomes functionalized with the RGD peptide.