CHOLESTEROL NANOPARTICLES TO RESCUE HUNTINGTON'S DISEASE PHENOTYPE

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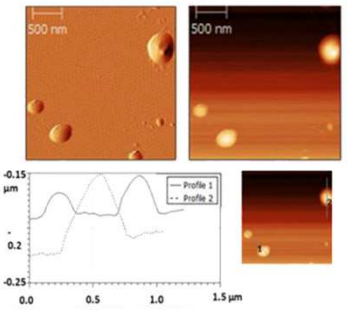
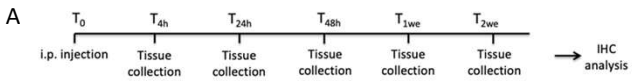
Background Huntington's disease (HD) is a neurodegenerative genetic disorder caused by a mutation in the gene coding for the huntingtin protein (HTT), which results in **cognitive and motor impairments**. At cellular level, mutant HTT produces several neurological effects including **decreased brain cholesterol** (Chol) biosynthesis, which has been demonstrated to be detrimental for neuronal function. Thus, Chol restoration may be a promising approach for HD treatment, but, since Chol is unable to cross the **Blood-Brain Barrier** (BBB), it is crucial to develop improved Chol delivery strategies.

Nanoparticle formulation A novel, **hybrid formulation of PLGA and Chol**, in which Chol is both the therapeutic molecule and a structural element, was successfully produced using a nanoprecipitation method (Fig 1). To target the brain, **targeting peptide *g7*** was chemically bond to PLGA before formulating into NPs. PGLA-g7 NPs were previously demonstrated to be able to cross the Blood Brain Barrier and to release their payload into brain parenchyma.



Average Size	PDI	Zeta Potential	Chol Content
240 nm	0,24	-34 mV	30 µg /100µg NPs

Fig. 1 Microscopic characterization of MIX NPs samples. Combination of AFM and TEM of the sample; left: AFM images (error signal, topographical images with the related analyses of profiles) are reported- right: STEM images and related magnification. Table: average characteristics of MIX NPs.



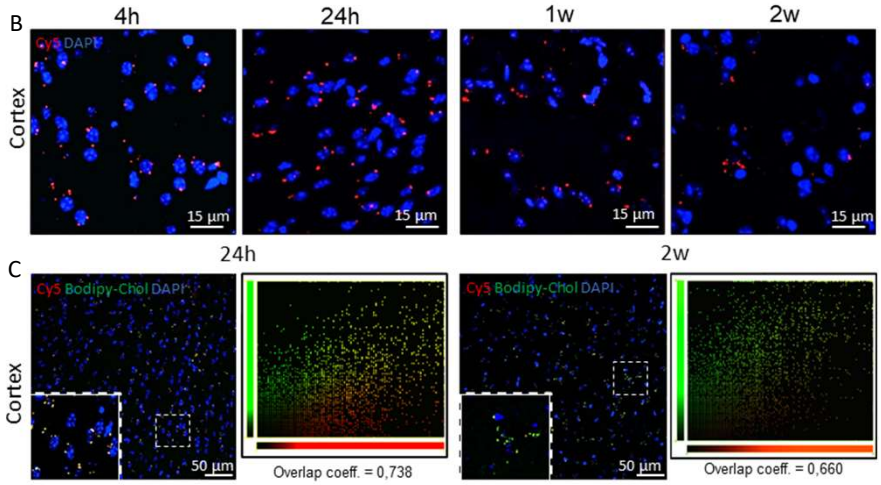
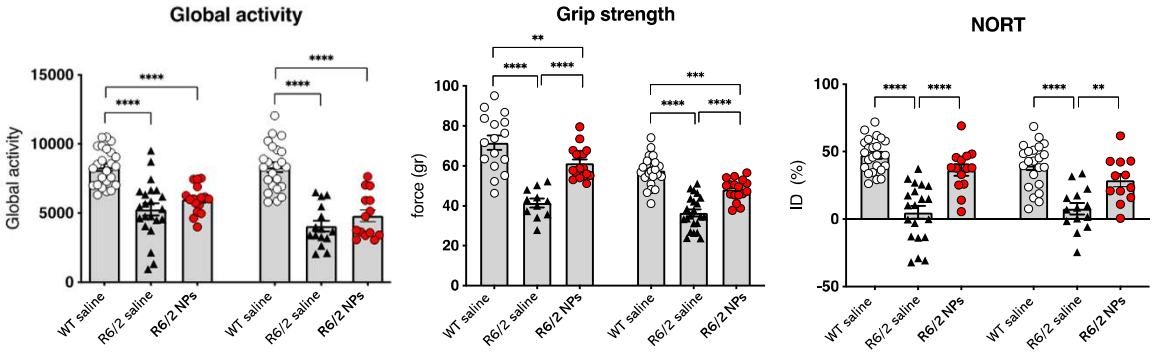


Fig. 2 A Schematic representation of the biodistribution study timeline. **B** Confocal images of the cortex at different timepoints after MIX NPs injection. Cy5 (red) is encapsulated in NPs, DAPI stains for cell nuclei. **C** Confocal images of the cortex analyzing colocalization of Cy5 labelled NPs and encapsulated Bodipy-Cholesterol in R6/2 mice. DAPI stains for cell nuclei.

Biodistribution studies 7-weeks old wild type mice (WT) were treated with a single I.P. injection of MIX-NPs and sacrificed at different time point (4h, 24h, 1w, 2w) to verify that g7-NPs-chol are uptaken in vivo. By analyzing the distribution of NPs (red dots), we found that **they cross the BBB** and are detectable in the brain starting from 4h after the I.P. injection.

Cholesterol Release To analyze the Chol release over time, 7 weeks old WT and R6/2 mice were treated with a single I.P. injection of Cy5 labelled MIX-NPs and loaded with fluorescent Bodipy-Cholesterol, and sacrificed at different time point (24h and 2 weeks after the I.P.). By analysing the distribution of MIX-NPs (red dots) and the distribution of Bodipy-Chol (green signal), we found that 24 h after a single I.P., NPs and Bodipy-Chol co-localized in brain cells. However, starting from 1 week after the injection, red and green signals were no longer co-localizing, meaning that **cholesterol was released and nanoparticles were degraded**, as indicated by the overlapping coefficient.



IN BRIEF

- Huntington's Disease related cognitive and motor impairment is linked to the **reduction of Cholesterol synthesis** in the brain.
- We developed **novel hybrid nanoparticles** using a simple nanoprecipitation method, with FDA-approved PLGA, Cholesterol, and the brain targeting peptide g7.
- These NPs are not only able to reach and **cross the BBB**, which is impossible for free Cholesterol, but also to slowly release their payload upon polymer degradation.
- Following a 4-week-administration pattern, we performed behavioral tests on wild type and HD model mice. Although no significant difference was found in the global activity, the treatment with NPs was able **to restore muscle strength and cognitive deficit** similar to wild type level.

Behavioral studies To study the effects of exogenous cholesterol supplementation, WT and R6/2 were treated with I.P. injections of saline or MIX-NPs twice a week, from pre-symptomatic stages (5 weeks of age) to late-symptomatic stages (9 weeks of age) of the disease.

The **activity cage** test was used to analyze mice locomotion activity. At 9 and 11 weeks of age mice were placed for one hour into a squared arena surrounded by infrared detectors. Starting from 9 weeks, R6/2 mice typically showed impairment in global activity compared to WT, indicating severely impaired motor function. At both the analyzed time point, cholesterol **supplementation has not improved motor performance** of cholesterol-treated mice.

The **grip strength** test allows to study neuromuscular functions and strength by determining the force developed by mouse when the operator tries to pull it out of a grid. R6/2 mice muscular strength is reduced from 9 weeks of age and **it is completely rescued by cholesterol treatment** at both 9 and 11 weeks of age.

Novel Object Recognition Test was used at 9 and 11 weeks of age to evaluate the effect of cholesterol supplementation on cognitive function. As expected, the DI of R6/2 mice was decreased at 9 weeks of age compared to WT, but the delivery of **cholesterol completely rescued the cognitive defects**.

Futher studies Further behavioral studies with different NPs administration pattern are now ongoing, in order to assess intensity and duration of phenotypical rescue of HD. Moreover, another trial is planned to be performed using a different HD animal model, i.e. Q175 mice.

References: Belletti, Daniela, Andreas Martin Grabrucker, Francesca Pederzoli, Isabel Menrath, Maria Angela Vandelli, Giovanni Tosi, Thomas Jason Duskey, Flavio Forni, e Barbara Ruozi. «Hybrid Nanoparticles as a New Technological Approach to Enhance the Delivery of Cholesterol into the Brain». *International Journal of Pharmaceutics* 543, n. 1–2 (maggio 2018): 300–310. https://doi.org/10.1016/j.ijpharm.2018.03.061.

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