SIMPLER IS BETTER: DEVELOPMENT OF SINGLE-COMPONENT CHOLESTEROL-BASED NANOMEDICINE FOR PRECISION THERAPY IN HUNTINGTON'S DISEASE

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Huntington's Disease

- Rare, genetic, autosomal dominant, adult-onset neurodegenerative disorder
- HD is associated to motor, cognitive, and psychiatric symptoms
- There's no cure for Huntington's disease. Medication and therapies can help treat some of the symptoms
- HD brain shows a cholesterol (Chol) deficiency

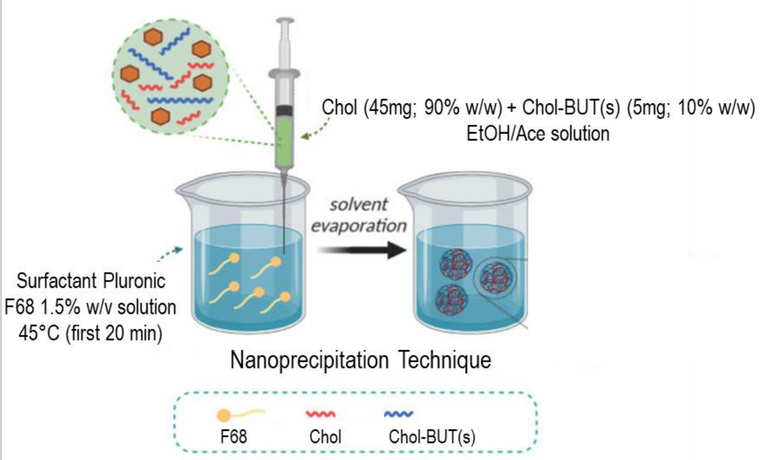
Gene Therapy to increase the expression of key Chol biosynthesis genes

Chol Supply → Nanomedicines (NMeds) for brain delivery of Chol

Free Chol does not cross the Blood-Brain Barrier (BBB)

Optimization of NMeds

- Modified Nanoprecipitation method
- FDA-approved, safe, biocompatible materials
- Ligands conjugated to the PLGA or Chol
- Optimized technological variables (Organic phase solvent, Surfactant concentration in aqueous phase, Aqueous phase temperature, Amount of targeting ligand, Novel ligands derived from fatty acids)



Conclusion

We developed **safe, efficient, single-component, and easy-to-produce advanced nanosystems** that demonstrated complete recovery in HD animals

We demonstrated the ability of **two novel ligands** to allow the passage of NMeds **across the BBB**

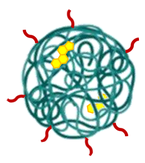
Current and Future work

1. **IN VIVO EFFICACY: Behavioral tests** on animal models to assess the phenotype rescue with novel ligand-modified Chol NMeds
2. **SCALE-UP:** Production of these NMeds with the **microfluidic technique** to narrow the gap towards the industry
3. **GENE THERAPY:** Optimization of the **loading of mRNA** for the simultaneous delivery of therapeutic genetic material and Chol to HD brain

← Increasing cholesterol content

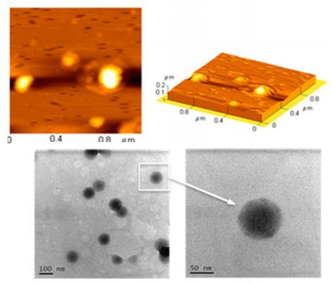
2015: Chol 1.0 — PLGA NMeds loaded with Chol – g7

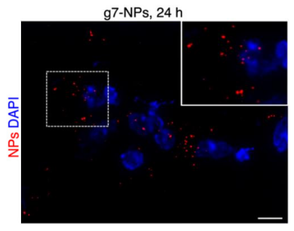
PLGA NMeds

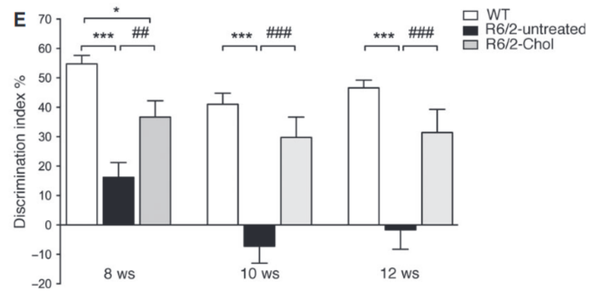


- Polymeric NMeds of PLGA
- Loaded with **1-10% Chol**
- BBB targeted with **g7 peptide**

Size: ~ 230 nm	PDI < 0.3	Z-Pot ~ -30 mV



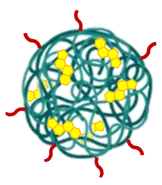




g7 allowed brain permeation and NMed accumulation up to 2 weeks, leading to positive behavioral effects

2018: Chol 2.0 — Hybrid Chol-PLGA NMeds – g7

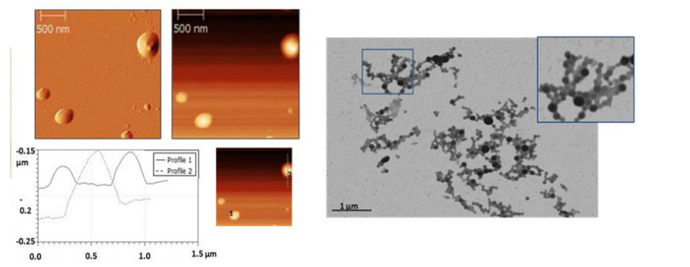
Hyb NMeds



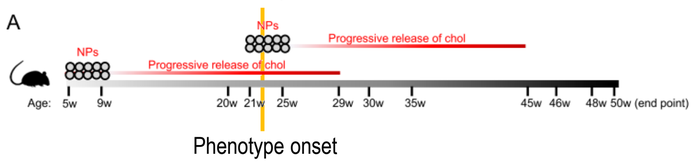
Hybrid matrix composed of
- 50% Chol
- 50% poly (lactic-co-glycolic) acid
- BBB targeting ligand: **g7 peptide**

- **Chol is both the active drug AND structural part of the NMeds**

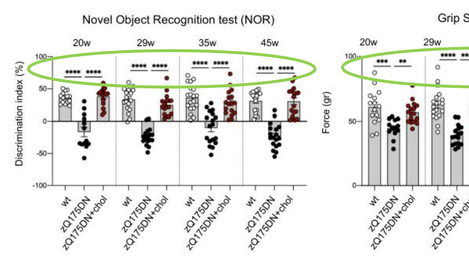
Size: ~ 250 nm	PDI < 0.3	Z-Pot ~ -25 mV



***In vivo*: Q175 HD animals**



Complete and long-lasting rescue of motor and cognitive symptoms with two administration cycles

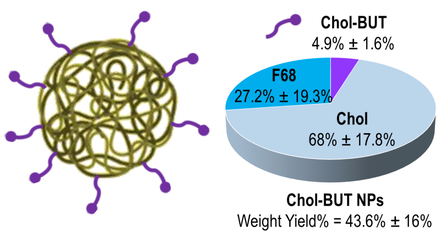


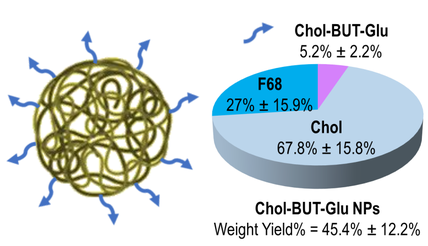
2023: Chol 3.0 — Pure Chol NMeds – new ligands

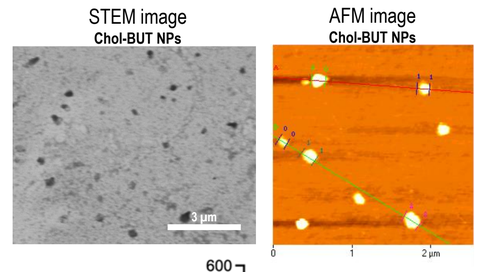
100% Chol NMeds

Engineered with **two <u>novel ligands</u>** for BBB crossing

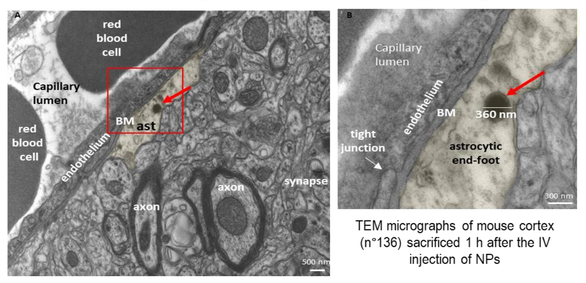
Size: ~ 230 nm	PDI < 0.2	Z-Pot ~ -20 mV



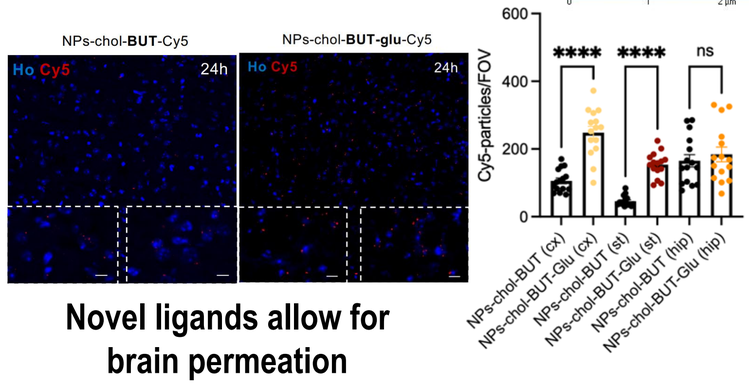




New Ligands for BBB targeting



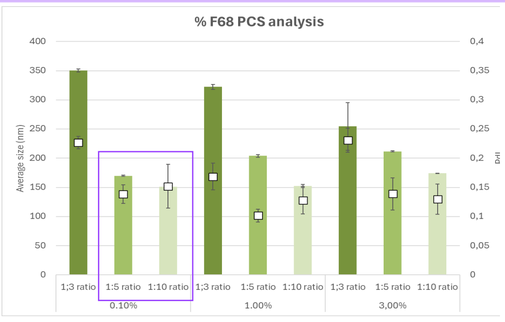
TEM micrographs of mouse cortex (n°136) sacrificed 1 h after the IV injection of NPs



Novel ligands allow for brain permeation

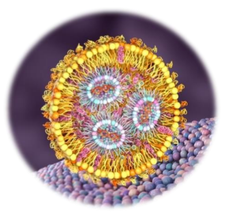
2024: scale-up — Microfluidic production

Industrial Scale-up



Continuous and reproducible production of NMeds with the microfluidic technique in **GMP conditions**

By optimizing the matrix of Chol NMeds we can <u>load genetic material</u> for **non viral-gene therapy**



Chol NMeds, with BBB targeting ligands, loaded with genetic materials could **both meet the Chol deficiency and correct the genetic aetiology of HD**

Patent: Nanoparticles for transport across the blood-brain barrier/E. Cattaneo, M. Valenza, G. Birolini, G. Tosi, B. Ruozi, F. Forni, P. Linciano, M.A. Vandelli – PCT/IB2021/059750
Telethon project grant number GMR22T1079 "Deciphering Cholesterol efficacy on cognition in prodromal Huntington's disease" - PRIN 2023 "Nanomedicine And Brain Diseases: Tailoring The Design And Application In Neurodegenerative Diseases"