**Nanoparticles-mediated enzyme replacement therapy rescues GALC enzymatic activity in the brain of a mouse model for Globoid cell leukodystrophy**

Ambra Del Grosso1, Marianna Galliani 1,2, Lucia Angella 1, Melissa Santi 1,2, Ilaria Tonazzini1, Gabriele Parlanti 1, Giovanni Signore 1,2,3 and Marco Cecchini1.

1NEST, Isituto Nanoscienze-CNR and Scuola Normale Superiore, Piazza San Silvestro 12, 56127 Pisa, Italy

2Center of Nanotechnology Innovation@NEST, Piazza San Silvestro 12, 56127 Pisa, Italy

3Fondazione Pisana per la Scienza ONLUS, 56021 Pisa, Italy

Lysosomal storage disorders (LSDs) are a large group of metabolic diseases, individually rare but collectively common (1:5,000 live births). Usually, they result from an enzyme deficiency within lysosomes, which ultimately causes accumulation of undegraded substrates. The most clinically applied method to treat LSDs is the systemic administration of the missing enzyme. This approach, however, is not effective in the case of LSDs that involve the central nervous system (CNS); the presence of the blood brain barrier (BBB), in fact, forbids translocation of big molecules into the brain. Here, a new enzyme delivery system based on the encapsulation of cross-linked enzyme aggregates (CLEAs) into poly-(lactide-co-glycolide) (PLGA) nanoparticles (NPs) functionalized with brain targeting peptides (Ang2, g7 or Tf2) is demonstrated for Globoid cell leukodystrophy (or Krabbe disease KD; OMIM #245200), an inherited neurodegenerative LSD caused by the genetic deficiency of the enzyme galactosylceramidase (GALC; EC 3.2.1.46). We firstly synthesize and characterize Ang2, g7 and Tf2-targeted GALC CLEA NPs. Then, we study NP cell uptake and trafficking, assessing their capability to reinstate enzymatic activity *in vitro*. Finally, we successfully test our NP formulations in the Twitcher mouse, the spontaneous murine model of KD. We report enzymatic activity measurements in the nervous system and in typical accumulation districts upon NP intraperitoneal injections, demonstrating GALC activity recovery in the brain up to the level of unaffected control mice. In addition, the presence of targeted NPs in the brain was confirmed by confocal microscopy. Taken together, these results open new therapeutic perspectives for KD, and for all LSDs with major involvement of the CNS.