The NAD project: Nanoparticles for therapy and diagnosis of Alzheimer's Disease
Preliminary results with nanoliposomes
Francesca Re¹, Marco Gobbi¹, Mario Salmoña², Massimo Masserini¹
¹DIMS, Univ. Milano-Bicocca, 20052 Monza and ²Mario Negri Institute, 20100 Milano, Italy
massimo.masserini@unimib.it

ABSTRACT SUMMARY
NAD is a multidisciplinary project with the goal of early diagnosis and effective treatment of Alzheimer disease. The research, started on September 1, 2008, is a large-scale program with a duration of 5 years funded by the European Union 7a Framework Program and carried out by 19 European partners. Within the present investigation are reported the preliminary results of a research aiming to create liposomes able to effectively interact with Abeta, the peptide that accumulates in the brain of Alzheimer patients.

INTRODUCTION
Recent statistics indicate that 24.3 million people worldwide are affected by dementia with 4.6 million new cases per year (one new case every 7 seconds). In Europe there are 5 million cases of dementia, 3 million of which are classified as Alzheimer. Given the continuing increase in life expectancy, these numbers are expected to rise dramatically. In 2040, cases are expected to double in Western Europe and to triple in Eastern Europe. Despite great progress in the scientific field, which has made possible the understanding of the molecular bases of the disease, there has been little progress in diagnosis and therapy. Production and accumulation of beta-amyloid peptide (Ab), a fragment of the Amyloid Precursor Protein (APP) plays a central role in the onset and development of Alzheimer Disease. Fragments of beta-amyloid aggregate in the brain to form oligomers, fibrils and plaques that induce a progressive degeneration of neurons. The goal of NAD, developed in the field of nanotechnologies, is to create nanoparticles (NPs) able: i) to bind Abeta, ii) to cross the blood-brain barrier, iii) to deliver molecules able to image amyloid aggregates in animal models of the disease (transgenic mice). 3 types of nanoparticles will be used in the NAD Project: polymeric (PNP), solid-lipid (SLN) and liposomal (LIP). Within the present investigation we describe the preliminary results concerning the preparation of nanoliposomes able to specifically interact with Ab.

EXPERIMENTAL METHODS
Recombinant human Ab 1-42 was purchased by Sigma-Aldrich (Italy). Nanoliposomes were prepared by extrusion through polycarbonate filters with pores of 100nm diameter and were composed of cholesterol/sphingomyelin (1/1) and glycerophospholipids or glycosphingolipids (gangliosides). The interaction between nanoliposomes (3mM) and Ab (0.5µM) after incubation at 37°C for 90 min was assessed separating bound and unbound Ab by ultracentrifugation in a discontinuous sucrose density gradient followed by ELISA assay. Surface Plasmon Resonance (SPR, Biacore) experiments were performed with a ProteOn using GLC sensor chip, to which Abeta was immobilized. Liposomes were injected at rate
of 30 μL/min for 5 min, at different concentrations.

RESULTS
Our results provide evidence that the lipid composition strongly affects the ability of nanoliposomes to bind the Aβ peptide. In fact, sucrose gradient centrifugation experiments showed that nanoliposomes composed of Chol/Sm/ anionic phospholipids (derivatives of phosphatidic acid) display a better ability to bind Aβ (Figure 1) in comparison with zwitterionic phospholipids or sphingolipids. The ability of nanoliposomes to bind Aβ peptide was also dependent on the proportion of embedded anionic phospholipid/ganglioside tested, in the range 5 to 40% molar. The best performance was attained at 5% molar. Liposomes containing anionic phospholipid displayed a Kd value in the nanomolar order of magnitude against values in the micromolar order for ganglioside GM1, as assessed by SPR experiments.

CONCLUSION
The results suggest that liposomes containing phosphatidic acid derivatives display a very strong interaction with Abeta peptide, and therefore will be utilized in further experiments in vitro, using biological fluids and cellular models, and then in vivo on animal models of Alzheimer disease. Liposomes with an high affinity towards human Abeta, identified within the present investigation, have been patented.

REFERENCES

![Graph showing Aβ1-42 binding to liposomes](image)

Figure 1. Quantification of the proportion of Aβ1-42 binding to liposomes.
Bound Aβ1-42 after incubation with liposomes composed of Chol/Sm (1:1 molar ratio) and of Chol/Sm/GM1 or glycerophospholipid at 20% molar ratio was assessed by ultracentrifugation on a discontinuous sucrose density gradient and ELISA assay (see text for details). Error bars are means ± S.D. (n=3).

Chol = cholesterol; Sm = sphingomyelin; PI = phosphatidylinositol; CL = cardiolipin; PA = phosphatidic acid; PE = phosphatidylethanolamine; PS = phosphatidylserine; PC = palmitoyl-oleoyl-phosphatidylcholine; GM1 = GM1 ganglioside; DPPG= dipalmitoylphosphatidylglycerol.