NanoVaccin - Nanovaccines based on Synthetic Outer Membrane Vesicles (S-OMV) for nosocomial infection preventions

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Project's objectives and research hypotheses. **Nosocomial infection** are solely bacterial infections transmitted in a hospital or another health care facility. The incidence rate of nosocomial infection is more than the 5% of hospitalized patients. The nosocomial infection is more frequently found in the area of lower respiratory, urinary tract, surgical wound with an incidence of more than 50% of the total infections. Gram-negative bacteria (GN) often cause these infections. Unfortunately, the use of antibiotics produces antibiotic resistant microorganisms, which may change the frequency of pathogen for nosocomial infection. Protecting patients trough vaccinations against bacterial infections is mandatory.[1] The aim of vaccination is to induce protective immunity of the population's individuals against diseases. This is more important in a pandemic disease scenario that overfloods and weakens any national health system.[2,3] In this frame, different research groups obtained interesting results in using **Outer Membrane Vesicles** (OMV, Figure 1A,B).[4] OMVs are released from the outer membrane of gram negative bacteria and consist of a phospholipid (PL) bilayer that contains outer membrane proteins, lipopolysaccharide (LPS) and periplasmic constituents playing the role of a "*Troy-horse*" weakening cells and promoting the infection (Figure 1D). OMV are considered today attractive candidates for vaccine delivery platforms, but they are not easy to produce, characterize and formulate.[5–8] In most of gram-negative bacteria, toxic lipo-polysaccharides (LPS) constitute the 75% of OMV.[9] The immune elicitation power of toxic LPS resides on Lipid A that acts in mammals as potent stimulator of the innate immune system either through a Toll-like receptor, the TRL4 [10] or through intracellular receptors. [11,12] This makes Lipid A, a very interesting candidate for vaccine development, i.e. as immune-stimulant, adjuvant or suppressive agent. Many scholars have hypotized the formulation of OMV based vaccines against GN bacteria.[3] The main characteristic of OMV is their physical similarity with a simplified version of the cell membrane in the form of giant vesicles (GV, Figure 1C).[13] A recent study carried out by Michele Fiore (MF) has shown that it is possible to combine phospholipids and synthetic glycolipids to form synthetic OMV (S-OMV) with relevant and promising biological activity as **vaccine carriers.**[14] A few progresses were obtained before the COVID-19 sanitary emergence thanks to the support of an M2 accorded to MF from the scientific advice of ICBMS. The aim of this project is to prepare phospholipid based S-OMVs from combination of naturally occurring and synthetic immuno-stimulants [9,14,15] such as LPS and LPS mimics (Figure 2). Synthesized as glycolipids, LPS mimics with controlled toxicity and enhanced immunity response will be used to formulate synthetic OMVs. The main objective will be to work on both aspects: (1) the preparation of potential antibacterial carriers by combination of natural occurring Lipid A and phospholipids and (2) preparation of synthetic vaccine prototypes made of a combination of synthetic Lipid A, glycolipids and phospholipids (Figure 2A and 2B). Genuine samples of Lipid A extracted from different GN have been delivered to CO2Glyco thanks to an ongoing collaboration with Prof. Antonio Molinaro of the University of Naples "Federico II" (Figure 2A). For the formulation of S-OMVs, MF and his team have developed a new methodology based on the use of glass supported giant vesicles (g-MSGVs) as reactors

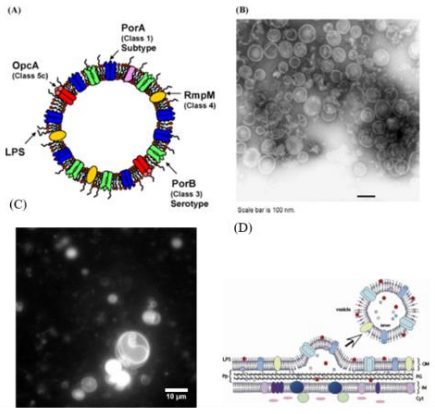

Figure 1. (A) Schematic model of the OMV entity showing the main constituents of MenBvac: outer membrane proteins, porins, LPS. (B) Electron micrograph of outer membrane vesicles of *N. meningitidis* (bar scale 100 nm), (C) Multilamellar GVs obtained by the "gentle hydration method"; (D) the generation of an OMVs from bacterial membranes.

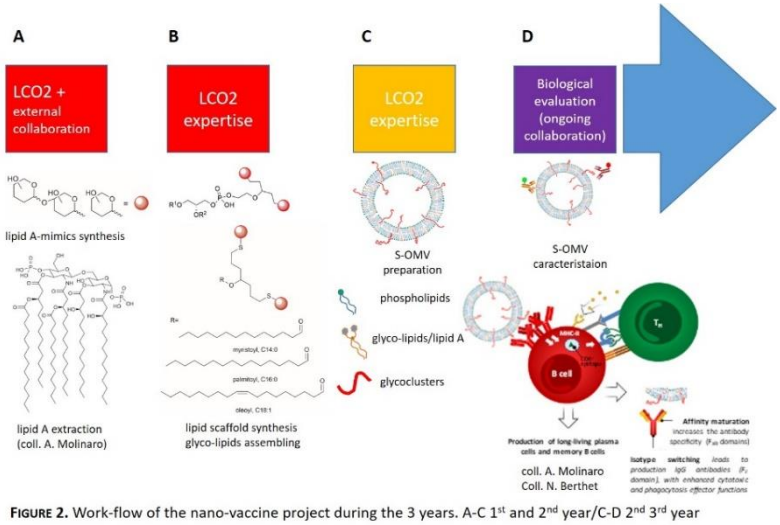

Figure 2. Work-flow of the nano-vaccine project during the 3 years. A-C 1st and 2nd year/C-D 2nd 3rd year

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for the production of giant vesicles with precise concentration of amphiphiles, thus, any glycolipids (Figure 2C).[16–18] In this frame, a scientific consortium between Michele Fiore (CO2Glyco) and another ICBMS team with expertise in glycolipids and synthetic phospholipids will produce advantages for the success of the project. Research groups need to have experiences and expertizes in synthesis of modified carbohydrates,[19–23] and glycolipids synthesis[14,24–26] for medicinal chemistry purposes. The CO2Glyco team, in addition, have a strong expertise in the preparation and purification of vesicles and their use in systems chemistry and synthetic biology (Figure 2C).[27,28] The biological characterization and biological tests for the nano-vaccines developed during the research program proposed for this PhD will be carried out thanks to the long-lasting external collaborations with the research group of Prof. Olivier Renaudet and Dr. Nathalie Berthet at the University of Grenoble Rhone-Alpes (Figure 2D).[14,19,29–33]

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