Summary

In the last decades, personalized medicine represents one of the safest and most effective solution for the treatment of individual patients or of groups with similar characteristics, according to specific health requirements. The application of nanotechnologies to this field is encountering an exponential increase due to their intrinsic features such as size, charge, morphology, composition, and surface chemistry, that can be finely tuned to obtain smart and/or biomimetic nanostructured materials. The smartness of the nanodevice lies in the ability to deliver a specific compound, in a spatial and temporal controlled way, to the target site. The biomimetism combines synthetic and biological nanomaterials, mimicking a natural mechanism, trying to overcome the hurdles associated with the delivery process, and developing high-performance imaging or therapeutic nanotools for diagnosis and therapy.

In this context, this Ph.D. Thesis focuses on extracellular vesicles (EVs) and on their use as native and engineered nanotechnological platform. EVs are lipid nanovesicles, naturally secreted by cells, that mirror the lipid and protein membrane's composition and cargo of the parental cell, and they specifically interact with close or distant target cells. Thanks to their unique characteristics, EVs are suitable candidates for biomedical applications in diagnosis, therapy, and theranostics in oncology, immunotherapy, and regenerative medicine. Furthermore, the use of autologous EVs for diagnosis and therapy should be considered one of the most promising clinical applications of the personalized nanomedicine approach.

In this Thesis, EVs' intrinsic physical characteristics, biocompatibility, homing, and targeting capabilities, with or without post isolation engineering, and an *ad hoc* storage protocol were investigated.

All the experiments were performed in an *in vitro* model constituted by B-lymphocytes as healthy control cell line and source of EVs, by a cancerous target (Burkitt lymphoma cells, Daudi), and by a negative tumoral control (acute myeloid leukemia cells, HL60).

The two different mechanisms of intercellular trafficking, homing and targeting, of lymphocytes-derived EVs were evaluated *in vitro*. To enhance the vesicles' internalization inside the target cells, an active targeting strategy was implemented through a surface biofunctionalization. Since Daudi cells overexpressed the CD20 antigen, EVs' membranes were decorated with anti-CD20 monoclonal antibodies. This surface functionalization enhanced the internalization in CD20 positive cancer cells if compared to healthy lymphocytes and to CD20 negative cancer cells, HL60.

Then, EVs were further engineered by loading a therapeutically active core. Zinc oxide nanocrystals (ZnO NCs) were selected as therapeutic nanoparticle for their intrinsic cytotoxic, optical and stimuli-responsive properties, general safety and biodegradability, to load inside EVs obtaining a hybrid nanotool, called TrojaNanoHorse (TNH).

These two engineering steps, i.e. the loading with ZnO NCs and the surface functionalization with anti-CD20 targeting monoclonal antibodies, were then combined for the creation of a hybrid smart nanotheranostic device for lymphoma *in vitro* treatment, called TNH^{CD20}.

Furthermore, since one of the major issues for the EVs-based systems clinical translation from bench to bedside is their conservation, a specific method to maintain the stability and healthiness of the product was studied. Besides deep cold storage at -80 °C, that required a very expensive ultra-low temperature freezer and the maintenance of the cold chain during transportation, freeze-drying demonstrated good results. Since EVs were composed by a phospholipid bilayer surrounding an aqueous core, carefulness must be applied in the choice of the protocol and the formulation, to avoid mechanical, osmotic, and drying stresses that can affect the EVs' morphology and bio-functionality.

The results presented in this Ph.D. Thesis represent a proof of concept for the development of a new class of EVs-based smart hybrid nanotools. The use of EVs to deliver a wide range of therapeutic cargoes to the selected target with the high specificity, provided by the surface functionalization with disease-specific targeting ligands, is a promising approach in the field of personalized medicine. The just proposed techniques, in future, will pave the way for the direct isolation of EVs from patients, not only for diagnostic purposes, but also to re-engineer them with a wide range of targeting and/or loading molecules to be used for biomedical applications.