Title: Innovative and smart functionalisable polymeric Nanoparticles for the delivery of Nucleic Acid and chemotherapeutic in combination for tumor solid treatment.

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Introduction: Cancer remains a significant threat to human health. While numerous therapies have been developed to combat the disease. Traditional treatments such as chemotherapy and radiotherapy have a lot of side effects like an high recidivism and a multi-drug resistance (MDR) that strongly limit the effectiveness of a treatment. Gene therapy is an emerging therapeutic approach that offers improved targeting and reduced side effects. In the last year , mRNA targeted therapeutic approaches through nanotechnology have been identified as promising tools to fight cancer, especially in combination with chemotherapeutics, since it is possible to selectively deliver multiple drugs through the same nanocarrier, thus modulating their pharmacokinetic as well as obtaining a safe and more pronounced pharmacological effect. In particular this project is focused on the fabrication of polymeric nanoparticles (NPs), starting from innovative and smart functional polymers to overcome the extra- and intracellular barriers to reach selectively the tumor site. To this purpose a panel of polymeric nanoparticles comprising of amphiphilic copolymers based on inulin (INU), polyethyleneimine (PEI) and aliphatic polyesters such as Polylactic acid (PLA)and polycaprolactone (PCL) were developed. Inulin will be selected as natural polyethylene glycol (PEG) alternative, having the advantages to be natural, non-immunogenic and functionalisable along its entire length. The polietilenimmine associated to the system could help the entrapment of the mRNA and also to overcome the endosomal escape. The NPs offering an approach to encapsulating and delivering Nucleic Acids in combination with chemotherapy like Doxorubicin or Docetaxel.

Methods: Inulin_PEI_PLA_NPs (IPP_NPs)entrapping siRNA and Docetaxel were prepared by the emulsion/solvent diffusion technique. NPs was produced and fully characterized in terms of size (i.e., hydrodynamic diameter or D_H), polydispersity index (PDI) and ζ-potential. To obtain detailed information about the architecture of the NPs, specific analytical assays were performed. Indirectly siRNA quantification were performed using Quant-it™ RiboGreen RNA Assay , The Docetaxel has been quantified with an HPLC method. Freeze drying studies were carried out with Hydroxypropyl-β-cyclodextrin in different polymer/cryoprotectant ratio (w/w). The release studies were carried out under sinc conditions in PBS pH 7.4

Results: All the formulations exhibited similar colloidal properties (Size: ~180 nm, PdI: ~0.16, and Z-Potential: ~ +68 mV) , siRNA entrapment efficiency (~60%), Docetaxel entrapment efficiency (~100%). IPP_NPs were stable at room temperature. “Preliminary results showed that the release of siRNA reaches 60% after 7 days.” All formulations subjected to the freeze-drying process preserve the structural properties.

Conclusion: Overall, results highlight the potential of the developed nanoparticles to successfully deliver of RNA and prompting toward further investigation.