Development of mesothelin-based nano vaccines for pancreatic cancer treatment

Sana Sayedipour [1], Timo Schomann[1,2], Christina Eich[1*], Luis J. Cruz [1*]

[1]Translational Nanobiomaterials and Imaging, Department of Radiology, Leiden University Medical Centre (LUMC), 2333 ZA Leiden, Netherlands

[2]Percuros B.V., Leiden, the Netherlands

Introduction and background

Pancreatic ductal adenocarcinoma (PDAC) is an aggressive tumor with high mortality. Due to lack of early detection markers, the prognosis of patients with advanced pancreatic cancer is enormously poor, Also, the location of the tumor in the retroperitoneum limits the options for local therapy and only a few standard treatments are available. Immunotherapy has led to a fundamental shift in the treatment of several advanced cancers. However, its efficacy in PDAC in terms of clinical benefit is limited, as PDAC is considered as a cold tumor with low immunogenicity. Peptide vaccines represent an important type of cancer vaccine that can induce strong anti-tumour immune responses by improving stimulation of T-cell immunity. Targeted delivery of nanoparticles (NPs) carrying vaccine components to dendritic cells (DCs) is a promising strategy to initiate antigen-specific immune responses. To improve the efficacy of cancer vaccines, we aimed to modulate the suppressive tumor microenvironment with poly (I:C), Resiquimod (R848) immune modulators and mesothelin (MSLN) peptide. Mesothelin expression is mostly limited to tumors in adult mammals. Because of its differential overexpression and its possible involvement in cancer development and progression, mesothelin is an emerging as potential therapeutic target for PDAC. Here we designed 4 different MSLN peptide to search their therapeutic efficiently as target in PDAC vaccin.

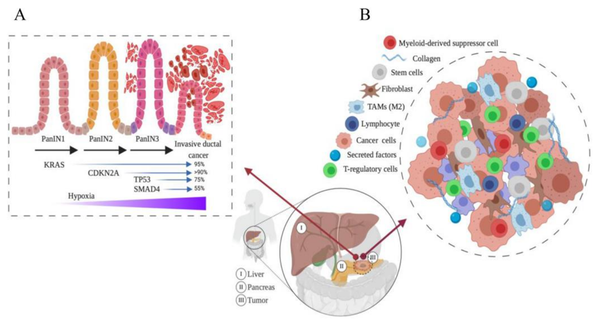


Figure 1. Pancreatic ductal adenocarcinoma (PDAC) stages and tumor microenvironments (TME). A) Different stages of PDAC, and the expression of oncogenes at each stage are shown. B) The complexity of TME components that attenuate cytotoxic drug penetration is shown.

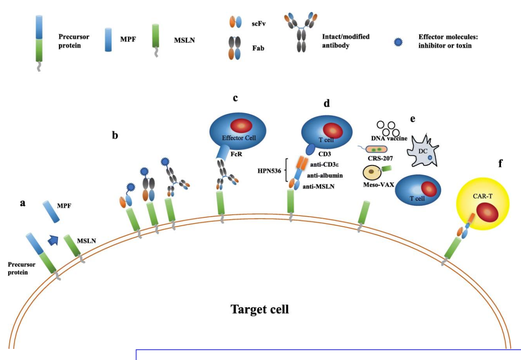


Figure 2. MSLN-targeted therapy strategies. **a**, the precursor protein is cleaved into two products, i.e. soluble protein MPF and GPI-anchored membrane protein MSLN; **b**, anti-MSLN antibody derived scFv, Fab, or intact/modified antibody are conjugated with the effector molecules (inhibitor or toxin) and induce cell death after binding to tumor cells; **c**, the binding of amatuximab to MSLN expressed on tumor cell membrane leads to ADCC; **d**, HPN536 directs T cells to kill tumor cells expressing MSLN; **e**, cancer vaccines arouse tumor specific immune response; **f**, the T cells are engineering to express CAR and redirected to tumor cells

Fabrication of PLGA- CS nanovaccines

Here, poly Lactic-co-Glycolic Acid (PLGA) NPs were synthesized in an oil/water emulsion, using a solvent evaporation-extraction method. Briefly, 200 mg of PLGA powder was dissolved in 3 mL of DCM. Depending on the NP, the following was added: 8 mg of pIC, and/or 4 mg of R848 and/or 250 µg of MSLN. The prepared solution was then added dropwise to 40 mL of aqueous 2.5% (w/v) PVA and emulsified for 120 s using a sonicator (250 W; Sonifier 250; Branson, Danbury, USA). Next, the emulsion was gently poured to a beaker previously prepared containing 40 mg of Chitosan (CS) oligosaccharide lactate dissolved in 20 mL of water, homogenized for 30 min by sonication, followed by overnight at 4 C on a magnetic stirrer. Following NP collection by ultracentrifugation and lyophilization, the concentration of the NPs constituents was determined by reverse phase high-performance liquid chromatography (RP-HPLC), as described elsewhere.

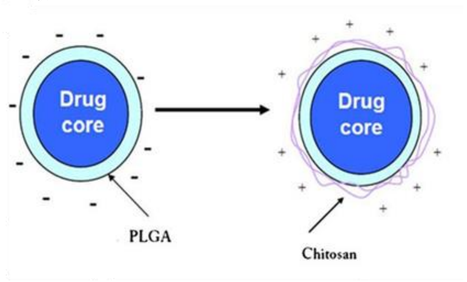
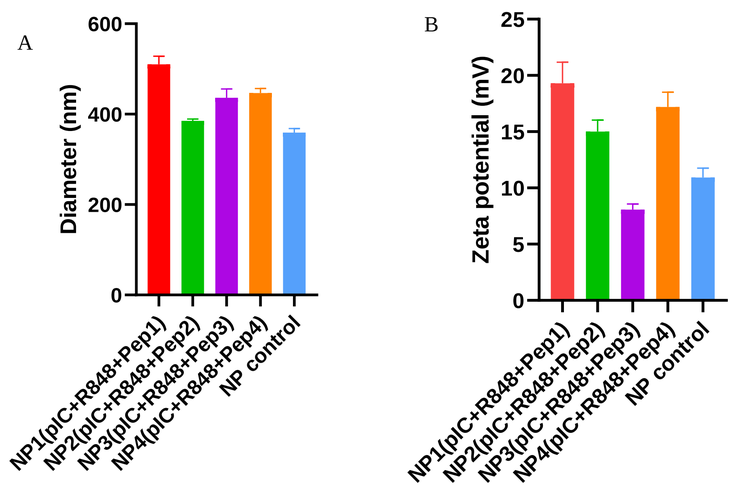


Figure 3. PLGA- CS NPs. Immunomodulators and target peptide encapsulated inside PLGA core and surrounded with positive charge chitosan oligosaccharide lactate.

Characterization of empty and loaded PLGA-CS NPs

The 5 different NPs were characterized for average size, polydispersity index and surface charge (zeta-potential) by dynamic light scattering. Briefly, 50 µg of NP sample in 1 mL of ultrapure MilliQ H_2O were measured for size using a Zetasizer (Nano ZS, Malvern Ltd., UK) and a similar sample was analyzed for surface charge by laser Doppler electrophoresis on the same device. The average size was approximately 350 nm and differed depending on the cargo (A). The average ζ potential was positive(B). TEM analysis revealed that the NPs were all spherical with a smooth surface, with PLGA core and Chitosan in surface, and uniform sizes (C).



C

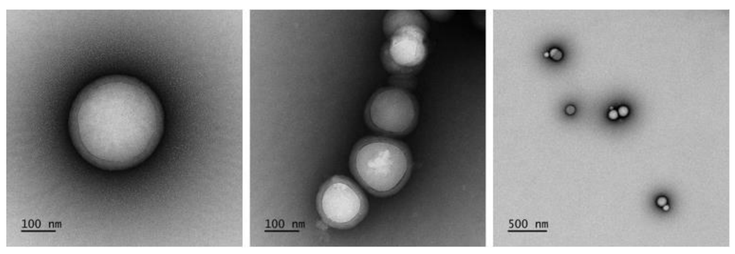


Figure 4 . (A-B) Particle size and zeta potential of the NP1, NP2, NP3, NP4, NP Control, (C) TEM images of PLGA-CS nanoparticle

Release kinetics

We measured the release kinetics of encapsulated components from the NPs over time. To this end, the NPs were dissolved in PBS and kept at 37° C in a thermo-shaker at a constant shaking velocity for 30 days. After 12 days, approximately 50% of pIC was released, 30% of peptide and 22% of R848, respectively. The release profile of pIC was the most rapid, compared to the other drugs, due to its high hydrophilicity. The other encapsulated compounds showed a typical drug profile release from the PLGA. These results suggest that the NPs drug release was slow, and of sustained manner.

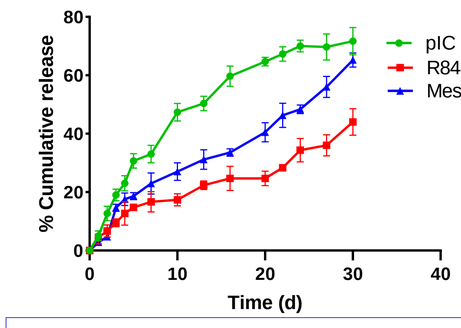


Figure 5. NP release kinetics of encapsulated drugs at 37° C in PBS, and kept in a thermo-shaker at a constant shaking velocity. n = 3 from one representative experiment.

Cell Cytotoxicity

The MTS assay showed that all NPs formulations were non-cytotoxic to the cells at the studied time points. PLGA-NPs are present in several FDA-approved drug formulations, and in recent years many studies have shown that they are highly biocompatible and suitable for *in vitro* and *in vivo* treatment and imaging.

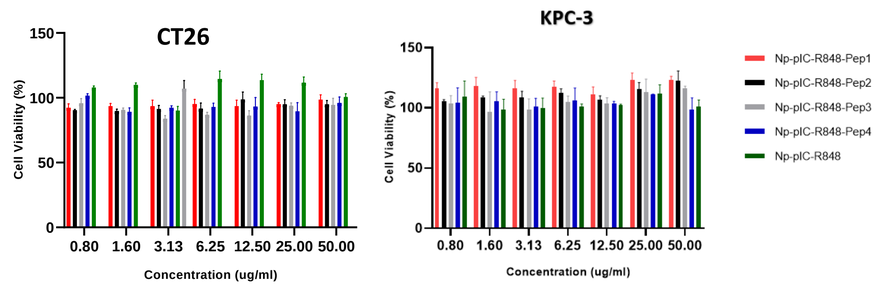


Figure 6. *In vitro* cell viability of KPC-3 and CT26 tumor cells after treatment with NP (pIC+R848+MSLN)

Dendritic cell maturation assay

Dendritic cell (DC) activation and maturation were assessed based on upregulation of CD86 D1 cells (murine DCs). Briefly, DCs were incubated with different formulations of NPs. As control, cells were incubated with soluble pIC, R848 and MSLN, matching the concentrations encapsulated in the NPs core. The solutions were then distributed into 96-well plates and sequentially diluted, after which 5x104 D1 cells were added to each well and allowed to incubate for 48 hours at 37º C in 5% CO2 and 100% Humidity. The cells were used to analyze the CD86 expression LSR-II laser flow cytometer and analyzed with FlowJo software. Activation of DCs measured by CD86 expression upon 48 hours, showed significant increase in CD86 expression when treated with NP (pI

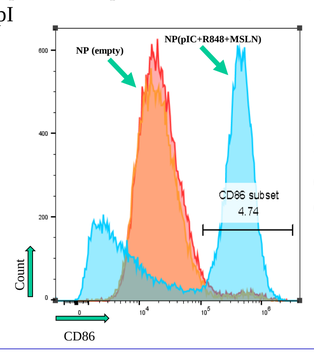


Figure 7. Activation of DCs measured by CD86 expression upon 48 hours incubation with NP(pIC+R848+MSLN) and NP(empty), is shown. The cells were pooled from n = 3 from each condition, one representative out of three independent experiments.

Conclusion

Overall, these findings support the idea that therapeutic cancer vaccines can be potentiated by combined NP-mediated co-delivery of poly (I:C), R848 and MSLN, which indicates that a more favourable milieu for cancer fighting immune cells is created. Based on these *in vitro* findings, we designed animal experiments for further investigation of potential MSLN-based nano vaccine in a pancreatic tumor mouse model.

Acknowledgment

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 861190 (PAVE).

Funded by the EU
This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 861190
EU Framework Program
HORIZON 2020
Contact Us
Zernikedreef 8
2333CL Leiden
The Netherlands
Contact